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## Biotreatment of industrial tannery wastewater using *Botryosphaeria rhodina*

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**Abstract:** The treatment of a tannery wastewater was performed on the laboratory scale using the ascomyceteous fungus *Botryosphaeria rhodina* MAMB-05, a ligninolytic and a constitutive producer of laccases (EC: 1.10.3.2). The wastewater samples were collected in the retanning and dyeing steps and presented high values of chemical oxygen demand, *COD* ( $15,023 \pm 60.0$  mg L<sup>-1</sup>), fifth-day biochemical oxygen demand, *BOD*<sub>5</sub> ( $4374 \pm 0.1$  mg L<sup>-1</sup>), total solids ( $28500 \pm 2.0$  mg L<sup>-1</sup>), total organic carbon, *TOC* ( $4685$  mg L<sup>-1</sup>), and chloride ion concentration ( $2911 \pm 0.3$  mg L<sup>-1</sup>). The fungus was inoculated and after five days under agitation at 180 rpm at 28 °C, the *COD* was reduced by 91 %. The total organic carbon also decreased from 4685 to 375.0 mg L<sup>-1</sup> and the turbidity from 331.0 to 6.5 NTU, indicating that the biological treatment was efficient as the fungus consumed almost all the organic compounds present in the wastewater. It was not necessary to add an additional carbon source for the treatment, indicating that the concentration of organic compounds presented in the tannery wastewater effluent were sufficient for microorganism growth, during which the *COD* and *TOC* were reduced by about 91 and 93 %, respectively.

**Keywords:** *Botryosphaeria rhodina* MAMB-05; wastewater treatment; tannery industry; *COD*.

### INTRODUCTION

The tannery industry represents an important sector in the economy of many countries. On the other hand, depending on the leather process, it generates large quantities of wastewater with ammonium, sulfates, surfactants, acids, dyes, sulfonated oils and organic substances, including natural or synthetic tannins. These chemical substances are applied to transform the animal skin into products with

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great capacities for dyeing, as well as to increase the mechanical and hydrothermal resistance. Considering that the greater part of these organic compounds are resistant to conventional chemical and biological treatments, the wastes discharged into natural waters increase environmental pollution and the health risks. The treatment of this type of wastewater is very complex mainly because of the variety of chemical products added in different concentrations.<sup>1-4</sup>

The fact that environmental legislation is very strict in almost all countries that are leather producers, a great deal of effort has been made to develop treatments for and remediation of the contaminated environments. Several methods have been described in the scientific literature, such as direct recycling,<sup>5</sup> coagulation,<sup>6</sup> flocculation,<sup>7</sup> chemical precipitation,<sup>5,8</sup> ion-exchange,<sup>9</sup> adsorption,<sup>10</sup> biological treatment,<sup>11-13</sup> electrochemical treatment,<sup>14,15</sup> membrane separation,<sup>16-18</sup> thermal techniques<sup>9,19</sup> and others. Physical chemistry processes, such as coagulation/flocculation, adsorption and membrane separation, have been the most utilized for the removal of colored effluents. However, these treatments do not solve the problem because of the transfer of contaminants from one phase to another. However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive.

A study of some biotechnological processes showed that ascomycetes and basidiomycetes are efficient at degrading a large variety of dyes, phenolic and non-phenolic compounds, demonstrating a high potential for environmental bioremediation.<sup>20</sup> The fungus *Botryosphaeria rhodina* MAMB-05 is ligninolytic and a constitutive producer of laccases (EC: 1.10.3.2).<sup>21, 22</sup> These enzymes are multi-copper oxidases, in which the copper atoms participate in the active site and in catalytic reactions. Laccases are also involved in lignin degradation,<sup>21,23</sup> and in the decolorization of effluents.<sup>24</sup> Microorganisms that are laccase producers have potential to be used in bioremediation processes and biodegradation of xenobiotic compounds.<sup>25</sup> The technological importance of laccases is correlated with its catalytic capacity to oxidize a large number of phenolic and non-phenolic compounds, as well as some other aromatic and non-aromatic compounds when these are oxidized in the presence of mediators.<sup>24,26,27</sup>

This work describes the biological treatment of effluent from a tannery collected in the retanning and dyeing steps, employing the fungus *Botryosphaeria rhodina* MAMB-05, with the objective to decrease the *COD* and remove organic compounds.

#### EXPERIMENTAL

All the reagents used in this work were of analytical grade: citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>) 99 %, zinc sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) 99 %, ammonium iron(II) sulfate dodecahydrate (Fe(NH<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O) 98 %, potassium bromide (KBr) 98 %, sodium chloride (NaCl) 98 %, sodium molybdate dihydrate (Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O) 84 % and sodium bicarbonate (NaHCO<sub>3</sub>) 98 %

were purchased from Synth; potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) 98 %, ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) 98 %, calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) 99 % were obtained from Nuclear; boric acid ( $\text{H}_3\text{BO}_3$ ) 99 %; potassium chloride (KCl) 98 %; magnesium chloride ( $\text{MgCl}_2$ ) 98 % from Merck; sodium citrate dihydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) 99 %; anhydrous D(+)-glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) 99 % were purchased from Biotec; copper(II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) 98 % from Quimibras; magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) 98 % from Chemco; manganese(II) sulfate monohydrate ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ) 98 %, from CAAL, agar from Biobras.

The crude effluent generated from the retanning step was collected in a leather company located in the north of the Paraná State, in the south of Brazil. The effluent was maintained at 4 °C in a cold room, and after being homogenized vigorously, was filtered to remove all suspended solids. Then, the filtrate was diluted with distilled water in two proportions (1:2 and 1:10) before inoculation with the fungus. The effluent characteristics without previous treatment are presented in Table I.

TABLE I. Characterization of the crude retanning and dyeing step effluent

Property	Value
pH	3.5±0.7
COD / mg L <sup>-1</sup>	15,023±60.0
BOD <sub>5</sub> / mg L <sup>-1</sup>	4,374±0.1
BOD <sub>5</sub> /COD	0.29
TOC / mg L <sup>-1</sup>	4,685
Total solids, mg L <sup>-1</sup>	28,500±2.0
Fixed total solids, mg L <sup>-1</sup>	17,983±2.0
Volatile total solids, mg L <sup>-1</sup>	10,517±4.0
Chloride, mg L <sup>-1</sup>	2,911±0.3
Turbidity, NTU	331±0.02
Chromium content, mg L <sup>-1</sup>	198±0.5
Ammonium nitrogen content, mg L <sup>-1</sup>	376±0.1

The inoculum was prepared by transferring the fungus *B. rhodina* MAMB-05 from the agar medium to Petri dishes containing 30 mL of solid VGA medium (minimum Vogel salts medium, 1 % (w/v) glucose and 2 % (w/v) agar) in a laminar film hood (Veco, model MM200600). These plates were incubated for 5 days at 28±2 °C in a BOD type incubator (Nova Ética 411D). The effluent was sterilized for 20 min in an autoclave (Fabbe Primar, model 103) and the pH was adjusted to 7.0. The treatment was developed in Erlenmeyer flasks (125 mL) containing 25 mL of effluent with or without the addition of minimum Vogel salts medium and 1 % (w/v) glucose. Four plugs (0.7 cm diameter) of solid VGA culture medium covered with *B. rhodina* hyphae were inoculated into each Erlenmeyer flask. All flasks were incubated at 28±2 °C on a rotary bench shaker (Cientec, model CT712) under agitation at 180 rpm for 5 days. All assay procedures were developed in triplicate and the results correspond to the average and standard deviation. After five days, the effluent treatments were centrifuged (Boeco, model U-32R) at 7000 rpm at -4 °C for 15 min. The supernatants were filtered through glass wool and used for analytical determinations. The fungal biomass produced by *B. rhodina* MAMB-05 was determined by gravimetric measurements (Fanem, model 315-SE) after drying at 70 °C to constant weight.

All the effluent analyses (*COD*, *BOD*<sub>5</sub>, total solids, turbidity, ammonium nitrogen) before and after the biological treatment step were performed following the methods described by the standard methods for the examination of water and wastewater.<sup>28</sup> Total organic carbon (*TOC*) was analyzed by the combustion method in a Shimadzu model *TOC 5000 A* analyzer. Chlorides were determined by the Mohr method<sup>29</sup> and the turbidity was measured in a turbidimeter (Hach, model 2100P). The chromium concentration was determined by atomic absorption spectrometry (Shimadzu, model 6601F).

### RESULTS AND DISCUSSION

Animal skin is transformed to leather through three operations: tanning, re-tanning and dyeing, and painting. The effluent of each step is mixed in a later step to produce a homogenized effluent, characterized as recalcitrant, which is highly pollutant. The physico-chemical characteristics of the retanning and dyeing effluent before the biological treatment are given in Table I. The effluent studied herein presented high values of *COD* (15023±60.0 mg L<sup>-1</sup>), *BOD*<sub>5</sub> (4374±0.1 mg L<sup>-1</sup>), total solids (28500±2.0 mg L<sup>-1</sup>), *TOC* (4685 mg L<sup>-1</sup>) and chloride ion concentration (2911±0.3 mg L<sup>-1</sup>), due to the presence of chemical products such as dyes, synthetic and vegetable tannins, surfactants, chromium and aluminum salts, organic acids, proteins, *etc.* The *COD*, *BOD*<sub>5</sub>, and *TOC* values of this retanning and dyeing effluent without any treatment were, at least, 100 fold higher than those described by Schrank *et al.*,<sup>1,2</sup> Preethi *et al.*,<sup>4</sup> Garrote *et al.*,<sup>30</sup> Dantas *et al.*<sup>31</sup> and Sauer *et al.*,<sup>32</sup> as shown in Table II.

TABLE II. Some tannery wastewater chemical treatments described in the scientific literature

Process	<i>COD</i> / mg L <sup>-1</sup>		<i>BOD</i> <sub>5</sub> / mg L <sup>-1</sup>		<i>TOC</i> / mg L <sup>-1</sup>		pH	Reference
	Before	After	Before	After	Before	After		
H <sub>2</sub> O <sub>2</sub> /Fe <sup>2+</sup> and H <sub>2</sub> O <sub>2</sub> /Fe <sup>2+</sup> /UV	1803	180	106	n.d.	n.d.	n.d.	2.5	31
H <sub>2</sub> O <sub>2</sub> /Fe <sup>2+</sup> (100 mg L <sup>-1</sup> /100 mg L <sup>-1</sup> )	130	48	47	29	45	28	3.5	1,2
H <sub>2</sub> O <sub>2</sub> /UV (1020 mg L <sup>-1</sup> )	130	n.d.	47	15	45	19	3.0	
Electro-fenton	2810	1686	910	n.d.	n.d.	n.d.	7.2	3
O <sub>3</sub>	2365	2095	1010	660	820	720	11.0	1,2
O <sub>3</sub> /UV	2365	1860	1010	645	820	710	11.0	
O <sub>3</sub> /O <sub>2</sub>	5000	4000	300	195	n.d.	n.d.	11.0	4
TiO <sub>2</sub> /UV	2365	2295	1010	1010	820	740	11.0	1,2
FeCl <sub>3</sub> /Ca(OH) <sub>2</sub>	3192	445	506	n.d.	n.d.	n.d.	8.8	30
TiO <sub>2</sub> /UV	801	626	349.1	26.9	n.d.	n.d.	8.7	32

The *COD*, *BOD*<sub>5</sub> and *TOC* values found in the scientific literature before and after different chemical treatments, converted to the same units (mg L<sup>-1</sup>) for comparison, are given in Table II. Usually, the papers did not mention the process step from where the effluents were collected. Comparing these values, discrepancies can be observed among the literature values and those obtained in this study, which can be attributed to the effluent, collected after different steps du-

ring leather production. This information is important because, depending on the process step, the composition and volume percentages can vary considerably, as described by Cassano *et al.*<sup>33</sup> In this paper, the authors emphasized the high variation of *COD* values between the chromium tanning phase (from 400 to 800 mg L<sup>-1</sup>) and the dyeing, fat liquoring and retanning steps (from 15000 to 75000 mg L<sup>-1</sup>). Comparing the results presented in Table II before and after different chemical treatments, it is possible to observe that some treatments were more efficient than others and some had no efficiency. This also can be explained by the composition of the effluent in leather industries, which varies depending on the leather treatment step.<sup>33</sup>

Most of the biological treatments of tannery effluents described in the literature used bacteria,<sup>34–36</sup> and a few used fungi. Srivastava and Thakur<sup>37</sup> isolated fungal strains from tannery effluents, and optimized some parameters of the treatment process to remove chromium with a strain of *Aspergillus* sp, which removed 70 % of the chromium after 3 days in the bioreactor. To the best of our knowledge, this is the first report that describes the employment of the ascomycete fungus *B. rhodina* MAMB-05 to treat tannery effluent. Fungal biomass is a useful parameter to evaluate the toxicity of wastewater;<sup>21,23</sup> thus in this work, it was considered the mycelium biomass produced by *B. rhodina* MAMB-05 with and without addition of minimum Vogel salts medium and/or glucose as a carbon source, at an initial pH of 7.0 was considered at a 5-day cultivation at 28 °C with effluent dilutions 1:2 and 1:10, Table III.

TABLE III. Biomass produced by *Botryosphaeria rhodina* with and without addition of nutrients and carbon source using 7.0 as initial pH

Treatment	Dilution	Biomass content, mg L <sup>-1</sup>	COD <sup>a</sup> / mg L <sup>-1</sup>	COD RD reduct %	pH <sub>final</sub>
Bot <sup>b</sup>	1:2	0.402±0.003	9126±91.0	39.3	6.6
Bot	1:10	0.621±0.002	5422±46.0	64.1	6.7
Bot+Glc <sup>c</sup>	1:2	0.585±0.010	22546±67.0	–	6.7
Bot+Glc	1:10	3.402±0.005	9705±81.0	39.6	6.8
Bot+Vogel <sup>d</sup>	1:2	0.953±0.006	3341± 60.0	77.8	6.8
Bot+Vogel	1:10	1.265±0.008	1305±144.0	91.3	6.7
Bot+Vogel+Glc	1:2	0.998±0.002	4300±60.0	71.4	6.8
Bot+Vogel+Glc	1:10	8.198±0.030	3123±133.0	79.2	6.9

<sup>a</sup>Initial *COD* was 15023±60.0 mg L<sup>-1</sup>; <sup>b</sup>*Botryosphaeria rhodina* MAMB-05; <sup>c</sup>glucose (10.0 g L<sup>-1</sup>);

<sup>d</sup>Vogel minimal salts medium

*B. rhodina* MAMB-05 did not grow in the crude effluent and it was necessary to dilute it 1:2 and 1:10. As shown in Table III, the effluent pH had decreased from 7.0 to within the range 6.9 to 6.6 after 5 days, and it was dependent on the effluent dilution. When the effluent was diluted 1:2 without any addition, a *COD* reduction of 39 % was registered, whereby 0.40 mg L<sup>-1</sup> of fungal bio-

mass was produced. However, the biomass increased to  $0.62 \text{ mg L}^{-1}$  when the effluent was diluted 1:10 and the *COD* reduction was 64 %. These results indicate that there were sufficient organic compounds in this tannery effluent to promote fungal growth after effluent dilution to decrease its toxicity.

When glucose 1 % (w/v) was added to the effluent in both the previously mentioned dilutions, the production of biomass increased to  $0.58 \text{ mg L}^{-1}$  and the *COD* did not decrease using the dilution 1:2. Moreover the biomass increased to  $3.4 \text{ mg L}^{-1}$  when the dilution was increased to 1:10 and the *COD* was the same as that obtained without glucose (39 %), using the dilution 1:2 (Table III). The addition of glucose was important for *B. rhodina* MAMB-05 growth, which can be explained by the high biomass production; it was observed that the effluent visually became more viscous. This increase in viscosity is due to *B. rhodina* MAMB-05 producing a  $\beta$ -(1 $\rightarrow$ 3);(1 $\rightarrow$ 6)-D-glucan named botryosphaeran,<sup>38</sup> that could explain the lower *COD* reduction.

The addition of minimum Vogel medium to the effluent at the same dilution confirmed that the fungus grew better when the effluent was diluted 1:10, producing  $1.26 \text{ mg L}^{-1}$  of biomass. Under these conditions, the *COD* reduction was the highest (91 %). The minimum Vogel salts medium contains microelements and the trace elements that are important for fungal growth in conjunction with the organic compounds present in the tannery effluent, which simultaneously aided in the effluent treatment. However, the simultaneous addition of Vogel salts and glucose confirmed that it is more important to increase the biomass production ( $8.1 \text{ mg L}^{-1}$ ), mainly when the effluent was diluted 1:10, once the *COD* had decreased to 79 %.

Consequently, some physico-chemical parameters of the effluent were determined using the dilution 1:10, because this presented the best biological treatment results in terms of *COD* reduction. Hence, after the treatment, the chromium concentration had decreased from 198.0 to  $1.2 \text{ mg L}^{-1}$ , the turbidity from 331.0 to 6.5 NTU and the *TOC* to 93 % ( $338 \text{ mg L}^{-1}$ ), indicating that the fungus was efficient in leather wastewater treatment.

#### CONCLUSIONS

The ascomycete *B. rhodina* MAMB-05 was able to grow and treat the tannery effluent more efficiently when it was diluted 1:10 and minimum Vogel salts medium added. No extra carbon source was necessary, indicating that the concentration of organic compounds present in the tannery wastewater effluent were sufficient for microorganism growth and to decrease the *COD* and *TOC* values by around 91 and 93 %, respectively.

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## ИЗВОД

БИОТРЕТМАН ОТПАДНЕ ВОДЕ ИНДУСТРИЈСКЕ ШТАВИОНИЦЕ КОРИШЋЕЊЕМ  
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Третман отпадних вода штавионице изведен је у лабораторијским условима помоћу аскомицетне гљивице *Botryosphaeria rhodina* MAMB-05, која је лигнолитична и конститутивни произвођач лаказа (ЕС: 1.10.3.2). Узорци отпадне воде су сакупљани у фазама задржавања и бојења, а садржавали су високе вредности *COD* ( $15023 \pm 60 \text{ mg L}^{-1}$ ), *BOD* ( $4374 \pm 0,1 \text{ mg L}^{-1}$ ), укупно чврсте материје ( $28500 \pm 2 \text{ mg L}^{-1}$ ), *TOC* ( $4685 \text{ mg L}^{-1}$ ), и хлоридних јонова ( $2911 \pm 0,3 \text{ mg L}^{-1}$ ). Гљивица је инокулисана и након пет дана, уз мешање на  $180 \text{ o min}^{-1}$  и  $28 \text{ }^\circ\text{C}$ , *COD* је смањен за 91 %. Укупни органски угљеник је такође смањен од  $4685$  на  $375 \text{ mg L}^{-1}$ , а мутноћа од  $331$  на  $6,5 \text{ NTU}$ , што указује на то да је биолошки третман био ефикасан, јер је гљивица конзумирала готово сва органска једињења присутна у отпадној води. Није био потребан никакав додатни извор угљеника у третману, што значи да је концентрација органских једињења присутних у отпадној води штавионице била довољна за раст микроорганизма и за смањење *COD* и *TOC* концентрација за око 91, односно 93 %.

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