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Fungal Treatment For Recalcitrant Compounds Removal In Raw Leachate And Synthetic Mixtures

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Abstract: Recalcitrant compounds represent one of the major problems in wastewater treatment since biological processes, based on bacterial degradation, are not suitable for their removal. Recently, the capability of white-rot fungi (WRF) in transforming recalcitrant pollutants generated a significant interest among bio-based industries.
This study focused on the treatment of 3 effluents with the white-rot fungus *Bjerkandera adusta* MUT 2295 in batch tests. The fungal strain *B. adusta* MUT 2295 was selected during a previous decolourisation experiment due to its ability to act towards a raw leachate sample (Italy). Treatment efficiency of *B. adusta* was evaluated on a) landfill leachate deriving from Brady Road landfill in Canada and b) two solutions containing synthetic recalcitrant compounds prepared with 1) tannic and 2) humic acid. Different parameters such as the pH of the treated effluent, its chemical oxygen demand (COD) and glucose consumption of *B. adusta* during the treatment were monitored for 10 days of fungal treatment. COD removal was up to 48%, 61% and 48% in in raw leachate and the two synthetic solutions containing tannic and humic acids. Moreover, leachate color removal between 25% and 49% was achieved after 1 week of treatment. Results obtained encourage further investigations on the use of the selected white-rot fungus as potentially suitable for the treatment of the tested recalcitrant compounds.

Keywords: landfill leachate, recalcitrant compounds, white-rot fungi

INTRODUCTION

The concept of recalcitrant compounds was introduced to define structurally novel and naturally occurring compounds resistant to microbial attack and persistent in the environment for extended periods (Alexander 1965). Consequently, these compounds represent a serious concern in wastewater treatment processes since the state of the art technologies for their removal are, in general, complex and not sustainable in terms of costs (Prieto-Rodríguez *et al*., 2012; Kamaruddin *et al.*, 2014). The search for alternative, efficient and green technologies led to an increasing interest in biological processes that are typically implemented at low operating costs. In particular, within biological processes, the white-rot fungi (WRF) and their extracellular enzymes have been regarded with increasing interest in terms of hazardous and recalcitrant pollutants removal (Kalčikova *et al.*, 2014).

Pollutants biodegradation capacity of WRF is correlated with their ability to secrete extracellular enzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP) or laccases (Schoemaker 1990), which are involved in lignin and lignocellulosic substrates degradation (Wesenberg *et al*., 2003).

Due to the high organic content and complex composition, the decontamination of landfill leachate requires innovative and sustainable technologies, among which the fungal-based one shows promising result (Kamaruddin *et al.*, 2014). Treatments involving fungi may be more beneficial than those using bacteria since they offers an easier degradation of high molecular mass organic pollutants and a higher rate of COD reduction in extreme environments (Ellouze *et al*., 2008).

However, at the moment, only a limited number of studies are available in the literature about the use of WRF in landfill leachate treatment. Saetang & Babel (2012) revealed that *Trametes versicolor* BCC 8725 could remove 78% color, 68% BOD_5 and 57% COD from leachate sample within 15 days of incubation. Ellouze *et. al* (2008) detected COD removal efficiencies for *Phanerochaete chrysosporium, Trametes trogii* and *Lentinus tigrinus* of 68%, 79% and 90%, respectively with a two-fold dilution of leachate. COD removals were accompanied by a significant enzyme secretion and a high reduction in the toxicity expressed as percentage of *Vibrio fischeri* bioluminescence inhibition (% BI < 20%).

Data from the literature reported *Bjerkandera adusta* as effective in the degradation and detoxification of a wide variety of wastewaters. Indeed, Anastasi *et al.* (2010) reported the capability of *B. adusta* to completely remove the color of several dyes and three simulated wastewaters, reducing the toxicity as well. Spina *et al.* (2012) tested *B. adusta* degradative potential towards real industrial effluents achieving 75% color removal and 90% COD reduction in textile and pharmaceutical wastewaters, respectively.

In the present study, the fungal strain *Bjerkandera adusta* (MUT 2295), previously selected for its decolourisation capability on landfill leachate Italy, is used for the treatment of 3 effluent samples, including raw landfill leachate (Canada) and two synthetic wastewaters containing recalcitrant compounds prepared respectively with 1) tannic acid and 2) humic acid. The synthetic compounds were selected as components of the recalcitrant fraction of landfill leachate (humic acid) and textile industry wastewater (tannin). The efficiency of the treatment on the diverse recalcitrant compound solutions was evaluated through batch tests.

METHODS

Chemicals

All chemicals used in this study were of analytical grade and purchased from VWR Canada.

Fungal strain

Bjerkandera adusta MUT 2295 was obtained from the *Mycotheca Universitatis Taurinensis Collection* (MUT), University of Turin. This fungal strain was selected out of 12 strains due to its capability to treat raw leachates (Italy). Strain selection was performed through a biodegradation experiment in which decolourisation potential was used as main criteria.

Target effluents

The efficiency of the treatment with *B. adusta* was tested on a raw leachate collected from Brady Road landfill (Winnipeg, Canada) and two synthetic recalcitrant compound solutions prepared with 1) tannic acid (1.3 g/l) and 2) humic acid (1.5 g/l). Details of the raw leachate chemical characterization are reported in Table 1.

Parameter	pH	Ammonia (mg/l)	Phosphorus (mg/l)	COD (mg/l)	BOD_5 (mg/l)
Value	7.61	704	1.62	1636	150

Table1 Chemical characterization of the Brady Road landfill leachate

The two types of synthetic solutions were prepared simulating raw leachate in terms of organic load. Initial COD values ranged between 1630 and 1740 mg/l. Glucose 1 g/l was added to the three effluents as fungal co-substrate for growth.The COD values of the two syntetic wastewater after glucose addition ranged between 2545 and 2780 mg/l. The pH was adjusted to 4.5 using 10% sulfuric acid.

Fungal cultivation

B.adusta was cultured on Malt Extract Agar (MEA, 20 g/l glucose, 20 g/l malt extract, 20 g/l agar, 2 g/l peptone) at 25°C for one week (Figure 1a). After the cultivation, *B.adusta* was homogenized under sterile conditions, with sterile saline (9 g/l NaCl) (and inoculated into 1L flasks containing glucose and yeast extract liquid media (GLY, 5 g/l glucose; 1.9 g/l yeast extract) and 2 cm^3 polyurethane foam cubes (PUF). Flasks were incubated in agitation for one week in order to enable the immobilization of the fungus into the cubes (Figure 1c). After 7 days, the cubes were removed and added to 500 ml flasks containing target recalcitrant compounds.

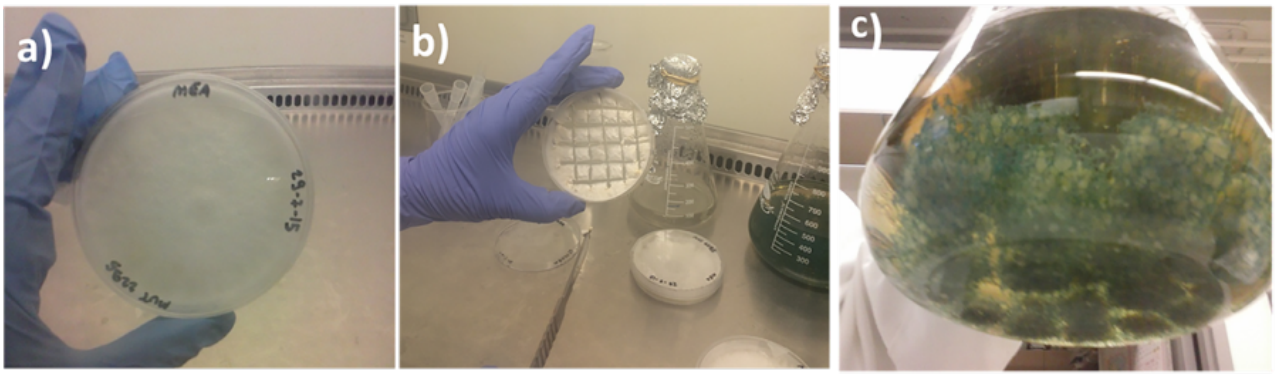


Figure 1 Fungal cultivation and immobilization on PUF

Parameters for the evaluation of treatments efficiency

A panel of parameters was used to evaluate the efficiency of the treatment. In particular, effluent pH, COD removal, decolourisation, glucose consumption, enzymatic activities (MnP and laccases) were measured for 10 days at 24 h intervals . Trials were triplicated including the same number of unseeded controls, without fungal inoculum.

COD was measured according to Standard Methods for Examination of Water and Wastewater 20th edition Section 5220, Hach Spectrophotometric procedure (DR2800 manual). The decolourisation percentage (DP) in 1) raw leachate and 2) humic acid solution was determined spectrophotometrically as the decrease of the spectrum area in the visible range (380-760 nm) with respect to the abiotic control. Decolourisation in tannic acid solution was measured spectrophotometrically as the decrease of the spectrum area in UV range (200-380 nm) with respect to the abiotic control. Glucose consumption was measured according to the reducing sugars protocol (Miller 1959).

MnP activity was measured at 25 °C, monitoring the oxidation at 590 nm of dimethylaminobenzoic acid/3-methyl-2-benzothiazoline hydrazone hydrocloride (DMAB/MBTH), in 0.1 M succinate lactate buffer pH 4.5 (Vyas *et al*., 1994). Laccase activity was assayed at 25 °C, monitoring the oxidation at 420 nm of 2,2'-azinobis (3 ethylbenzothiazoline-6-sulfonic acid) (ABTS), in 0.1 M sodium citrate buffer, pH 3 (Niku-Paavola *et al*., 1988).

Statistical analysis

All the data were elaborated with the aid of one-way ANOVA, and the means were separated by Bonferroni multiple-comparison test ($P \leq 0.05$) using the specific software Statgraphics 6.1 (Statistical Graphics Corp., USA).

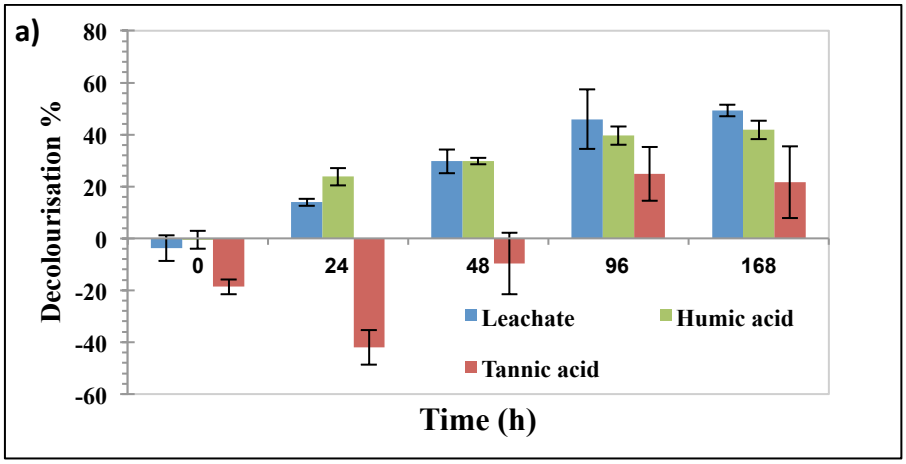
RESULTS AND DISCUSSION

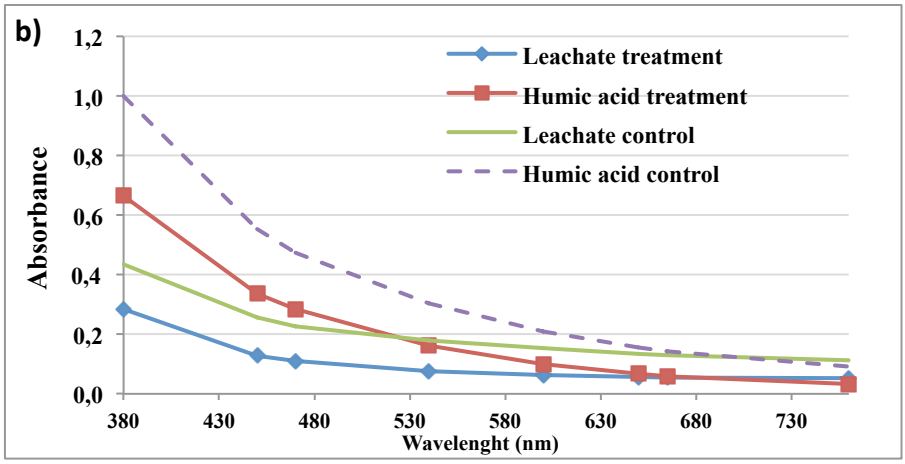
Color removal

B.adusta decolorized raw leachate, humic acid and tannic acid solution up to 49%, 42% and 25%, respectively. Decolourisation results of one week of fungal treatment are showed in Figure 2.

In the case of raw leachate and humic acid solution, it is important to notice that these effluents were dark colored and that the experiment was performed without diluting the samples. Figure 2b showed the spectrum area reduction of the two dark colored effluents towards the respective unseeded controls after one week of treatment.

On the contrary, tannic acid solution was initially almost colorless. After a first increase in the spectrum area within the first 48 hours of treatment, in the following days of the experiment, a reduction up to 25% was observed. In Figure 2b the spectra of the trials of tannic acid solution inoculated with *B. adusta* and the respective abiotic controls are showed. In this case, the reduction of the spectrum area and the evident flattening of the treatments spectrum shape were presumably related to a degradative process operated by *B. adusta*.





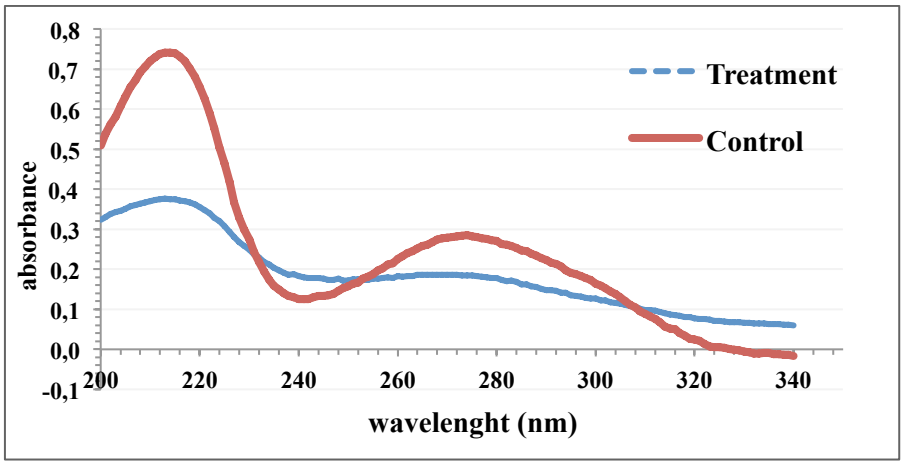


Figure 2 a) Effluents decolourisation percentages in one week of treatment. b) Spectrum area reduction in raw leachate and humic acid after 168 h and c) spectrum area reduction in tannic acid after 96 h. Decolourisation values are given as the average among triplicates with DS (+/-). A negative data should be considered as an increase and not a reduction of the parameter.

COD removal

Results of COD removal in the three effluents are reported in Figure 3a. Data showed a reduction of 48%, 61% and 48% in raw leachate, tannic acid and humic acid, respectively, in 10 days of fungal treatment.

As glucose represented about 36-39% of effluent's COD, assuming its complete depletion, the detected COD removal percentages were higher than glucose consumption in all the samples inoculated with *B. adusta*. It is possible to observe a clear pattern in the two synthetic solutions where the difference in COD removal between treatments and the respective unseeded controls reached 56% and 33% in tannic acid and humic acid solution, respectively.

On the contrary, the percentage of COD removal in raw leachate was the same in treatments and controls. This result could be related to the presence of autochthonous microorganisms capable of removing a certain amount of recalcitrant compounds from the leachate itself (Anastasi *et al*. 2010). Although there was a lack of evident COD decrease in raw leachate attributable to the presence of the fungus, the treatment could have led to different rearrangement of recalcitrant compounds chemical structure, enhancing their bioavailability for other organisms. Hence additional parameters as biological oxygen demand (BOD) could provide a deeper understanding of the process occurred during fungal treatment.

The maximum COD removal was achieved in tannic acid, resulting in a final COD value of 995 mg/l, much lower than the initial one of 2780 mg/l, providing further evidence of the presence of a degradative process due to the treatment and confirming the results of the spectrum analysis (Figure 3b).

The decrease of COD detected in humic acid solution treatment was presumably due to glucose consumption since the difference in the removal between treatments and controls (33%) is lower compared to the organic load represented by glucose. However, as previously hypothesized concerning raw leachate, possible rearrangements of recalcitrant compounds chemical structure due to the treatment with *B. adusta* can not be excluded.

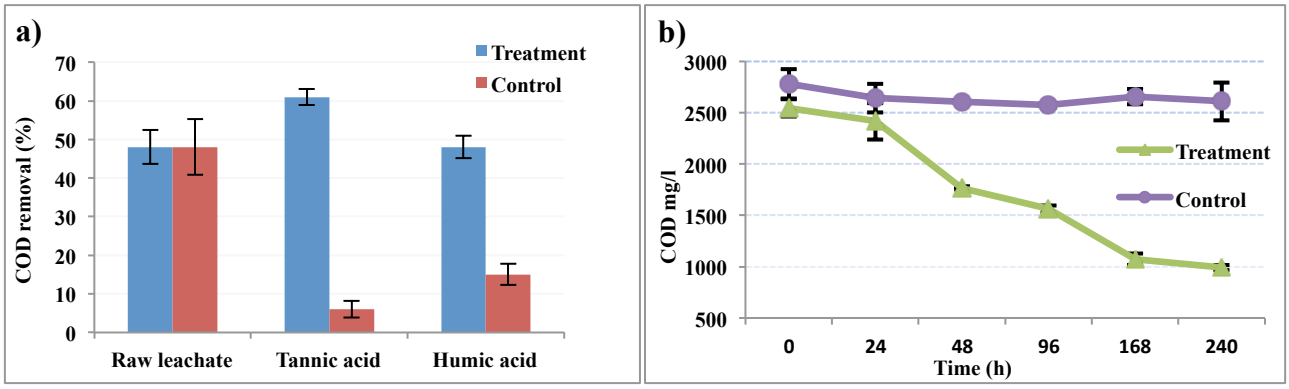


Figure 3 a) COD removal percentages in the effluents and b) COD removal in tannic acid as mg/l. Values are given as the average among triplicates (+/-) DS.

Enzymatic activities

Further evidence of the presence of a degradative process related to the presence of *B.adusta* has been provided by the results of the enzymatic activities (data not shown). Indeed MnP activity was detected in all the treatments, confirming that *B. adusta* was metabolically active for all the duration of the experiment.

The maximum value was reached in the tannic acid solution after 72 hours with 8.9 ± 2.3 U/l, in correspondence to the beginning of spectrum area reduction. The maximum values of MnP achieved in raw leachate and humic acid were respectively 1.92 ± 1.4 U/l and 5.6 ± 1.5 U/l.

These results confirm the vitality of *B. adusta* in the growth condition reported and are positively correlated to COD and color removals observed. In fact previous studies reported peroxidases as the major enzymes involved in the decolourisation of leachate (Tigini *et al.*, 2013).

Laccases activity was not detected in the tested conditions. This result is consistent with literature data since *B. adusta* is well known to produce peroxidases (Anastasi *et al*., 2010).

pH values

In the trials inoculated with *B. adusta*, effluents pH values ranged between 4.6 and 6.2. In the unseeded controls, the pH was between 4.4 and 4.9 (Figure4).

Since many enzymes, including peroxidases, have a pH optimum among 5 and 6, the values observed in the treatments were compatible with fungal active metabolism.

The increasing trend of treatments pH values could suggest that *B. adusta* buffered the effluents as similar as possible to the optimum enzymatic range of the enzymes involved in the process as has already been reported by Kaushik & Malik (2009).

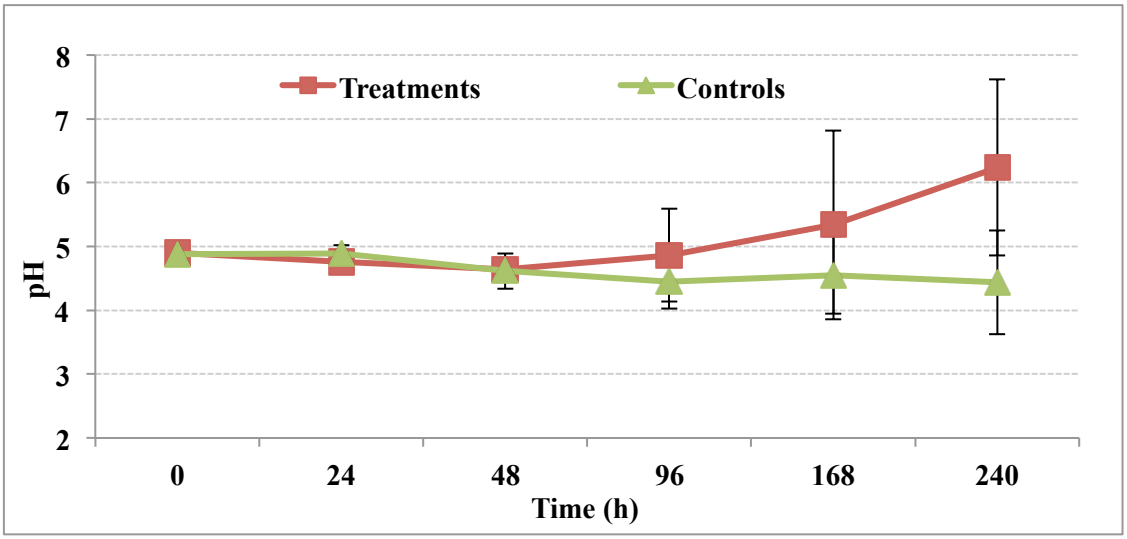


Figure 4 Effluents pH values during the treatment given as the average among trials with standard deviations (DS) (+/-)

Glucose consumption

Glucose consumption percentages detected in the treatment ranged between 54 and 63% (Figure5). Results showed low percentages of glucose consumption in both synthetic solutions controls, resulting in 2 and 13% in humic acid and tannic acid solutions, respectively. On the contrary, 65% of glucose consumption was detected in raw leachate control, suggesting the presence of autochthonous microorganisms capable of using glucose as carbon source.

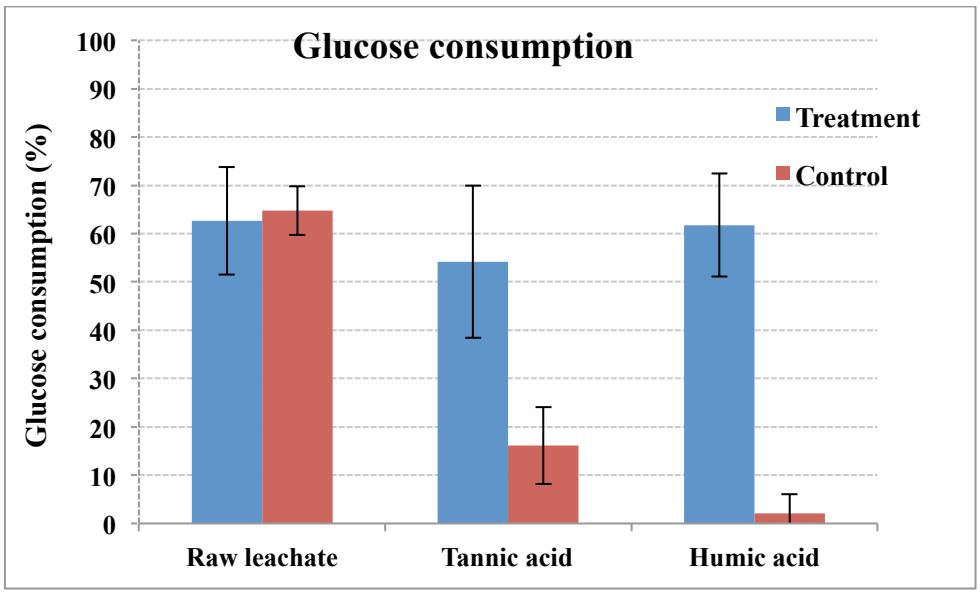


Figure 5 Glucose consumption measured against the different effluents in one week of treatment. Values are given as the average among triplicates with DS (+/-).

CONCLUSION

Bjerkandera adusta MUT2295 was able to survive for the duration of the experiment, overcoming the harsh conditions in terms of toxicity and autochthonous microorganisms' competition of the tested recalcitrant compounds solutions. The treatment with the selected strain resulted in the

decrease of several parameters such as COD and color. The process, positively correlated with the quantification of enzymatic activity (MnP), that confirmed the active metabolism of *B. adusta*. These results encourage further evaluation of the selected fungus for the treatment of the tested recalcitrant compounds. A panel of analysis would provide a deeper understanding of the degradative mechanism performed by *B.adusta,* confirming or rejecting preliminary results achieved with this strain.

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