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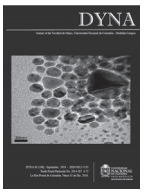

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Use of white rot fungi in the degradation of an azo dye from the textile industry

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Abstract
Textile industry effluents—a complex mix of chemicals, among which colorants are of particular concern—impose great environmental challenges. In this study, a full 2^3 factorial design was used for determining the best conditions for the degradation of textile dye Basic Red 46 under solid state fermentation (SSF). Three white rot fungi *Trametes versicolor, Pleurotus ostreatus,* and *Pleurotus pulmonarius* were used in the fermentation process. A maximum degradation percentage of 63.0% was achieved at 17 days of incubation with *T. versicolor* under a moisture content of 90%, carbon to nitrogen ratio of 12: 1, and at 20°C. *P. ostreatus* and *P. pulmonarius* reached a maximum degradation percentage of 69.3% and 63.1%, respectively, after 25 days of fermentation. The scale-up of the fermentation process using *T. versicolo*r led to a degradation percentage of 45.7% after 30 days of incubation. Additionally, the enzyme activity of laccase, manganese peroxidase and lignin peroxidase was measured. The results indicate that SSF offers a satisfactory degradation, whose efficiency depends on the optimization of process conditions.

Keywords: Solid-state fermentation; white-rot fungi; basic red 46; banana peel.

Utilización de hongos de la podredumbre blanca en la degradación de un colorante tipo azo de la industria textil

Resumen
Los efluentes de la industria textil imponen grandes retos ambientales. Estos son una mezcla compleja de productos químicos, donde colorantes son de particular interés. En este estudio, se implementó un diseño factorial completo 2^3 para establecer las condiciones más apropiadas para la degradación del rojo básico 46 bajo fermentación en estado sólido, FES. Tres hongos de podredumbre blanca *Trametes versicolor, Pleurotus ostreatus y Pleurotus pulmonarius* fueron utilizados en el proceso de fermentación. Se alcanzó una degradación máxima de 63.0% a los 17 días de incubación con *T. versicolor* con una humedad del 90%, proporción carbono:nitrógeno de 12:1 y a 20°C. *P. ostreatus y P. pulmonarius* alcanzaron porcentajes máximos de degradación del 69.3% y 63.1% respectivamente luego de 25 días de fermentación. El escalado del proceso con *T. versicolor* condujo a una degradación del 45.7% a los 30 días de incubación. Adicionalmente, se cuantificó la actividad de las enzimas lacasa, manganeso peroxidasa y lignina peroxidasa. Los resultados señalan que la FES ofrece una degradación satisfactoria, cuya eficiencia depende de la optimización de las condiciones del proceso.

Palabras clave: Fermentación en estado sólido; hongos de podredumbre blanca; rojo básico 46; cascara de banano.

1. Introduction

The textile and clothing industry is one of the main pillars of the Colombian economy. It generates more than 800,000 jobs directly and indirectly and it accounts for about 12.1% of the national industrial production, 6.0% of total exports

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and 13.3% of non-traditional products sales [1,2]. Colombian textiles are recognized by high quality, color, and design. Such characteristics have allowed its success in highly competitive markets like the United States, the European Union, and the Andean Community, among others [1].

Textile effluents are usually made up of acids, bases, salts, oils, fats, surfactants and various types of dyes. Concentration of such pollutants is highly variable since it depends on the stage of the process and type of fabric. The variability of effluent composition and extreme temperatures and pH, make textile industry effluents difficult to treat [3]. The dying stage, in particular, has the greatest negative environmental impact. It uses large amounts of water; it is energy intensive, and releases highly toxic dyes into surface water [4].

It is estimated that 10 to 14% of the colorants used in the dyeing and finishing processes are discarded in effluents [5]. Unfortunately, their presence, even in minimal concentrations of 1.0 ppm, is sufficient to cause the aesthetic deterioration of the environment [6]. The most dramatic impact of their presence is the negative effect on photosynthetic processes. They reflect solar radiation reducing the self-regeneration of water resources. They also increase the biological oxygen demand (BOD) and chemical oxygen demand (COD) [1, 7] that could lead to anoxic conditions affecting the whole aquatic ecosystem [8].

Act number 3930 of 2010 in Colombian law regulates water quality standards and parameters for industrial and domestic effluents. The absence, in both the current and preceding act (Act number 1594 of 1984), of the amount of dyes permitted in industrial effluents, has led to the appearance of colored bodies of water emerging around the country. This is as a recurring and disturbing phenomenon in large cities such as Medellín.

Physicochemical treatment technologies such as activated carbon adsorption, flocculation, chemical oxidation, ozonation and filtration, among others, exhibit satisfactory decoloration efficiency. However, their use is restricted due to high costs, incomplete removal of pollutant, and sludge and the generation of toxic byproducts. Therefore, it is important to develop alternative treatment methodologies [9-11]. Between these methods, adsorption with agricultural wastes has emerged as a promising strategy; nevertheless, its study has been primarily limited to metals [12,13].

Biological strategies have been implemented as a result of the limitations associated with the traditional methods and the need to solve the persisting environmental problem. These methodologies use the microorganism's metabolic potential for transforming the pollutants into smaller molecules or for affecting the functional groups involved in their toxicity [14]. In the present study, a novel "mixed" or "combined" strategy is employed for treating a simplified simulated textile effluent. It combines a physiochemical and a biological process. The first step consists of removing dissolved dye from solution without breaking the molecule. In the following step, solid state fermentation (SSF) is used to mineralize or partially degrade the previously adsorbed pollutant.

SSF is characterized by the growth of microorganisms on solid substrates in the absence of free water (low water activity). In this case, the residue-dye complex was used as a source of support and nutrition for the microorganism [15-19]. Previous studies demonstrated the efficiency of the methodology by reaching degradation percentages higher than 90% under the best conditions during the fermentation process [20]. Contaminant degradation is attributed to the action of various enzymes produced by the microorganism.

White rot fungi (WRF) are proven the most efficient microorganisms in the treatment of xenobiotic molecules such as synthetic dyes [21]. Their metabolic capacity to mineralize complex polymers, even like lignin, is attributed to the secretion of non-specific and non-stereoselective enzymes. Laccases (EC 1.10.3.2), manganese peroxidases (EC 1.11.1.13) and lignin peroxidases (EC 1.11.1.14) stand out among the biological catalyzers [22] and they have been able to degrade recalcitrant compounds under conditions similar to the natural habitat of WRF, which are recreated under SSF conditions [23].

Banana peel (BP) was selected as an adsorbent and subsequent substrate in SSF because it allowed us to simulate the natural environment of WRF. Additionally, the lignin, cellulose and hemicellulose content of BP [24] seem to be appropriate for enhancing the production of ligninolytic enzymes [25-27]. Moreover, BP also represents an alternative, highly available, low-cost agricultural residue. On the other hand, basic red 46 (BR46) was selected because it is an azo dye widely used in the textile industry and it is characterized as a highly recalcitrant xenobiotic [28].

In the present study, the most appropriate conditions for the biodegradation of the synthetic dye BR46 under SSF were evaluated. Residues from the banana industry were implemented as substrates. *P. ostreatus*, *P. pulmonarius*, and *T. versicolor* were used to inoculate the fermentation.

2. Materials and methods

2.1. Microorganisms and culture conditions

Three WRF were evaluated, *P. ostreatus*, *P. pulmonarius* and *T. versicolor.* Microorganisms were obtained from Plant Tissue Laboratory at the University of Antioquia, Medellín, Colombia. They were preserved in Petri dishes with PDA agar at 4 °C and subcultured every two months. Before each fermentation, 1.2 cm disks containing the microorganism were subcultured and incubated at 28 °C for a period of 5 to 7 days. For degradation of RB46, small discs (diameter 1.2 cm) were taken from the edge of fungal growth zone for ensuring the exponential growth.

2.2. Adsorbent pretreatment and adsorption of BR46

Banana Peel was acquired in one of the dining halls at the Universidad Nacional de Colombia –Medellín Campus. It was washed, dried at 100 °C, and ground in an Ika mill. Then, it was sieved and particles ranging in size from 300 to 500 µm were chosen for performing the SSF experiments. To neutralize organic acids, BP was washed again with a KOH solution (83.17 mM) for 60 minutes [27,29]. The BR46 dye CI 110 825, was purchased from a local company.

The adsorption was performed in 500 mL beakers at room

temperature and 150 rpm for 3 h in a Heidolph Unimax 1010 shaker; 500 mg of BP were added to 250 mL of BR46 solution at 800 ppm. A preliminary analysis of the influence of pH on dye removal showed that a pH greater than 7.0 favors adsorption. Therefore, pH=7.0 was selected for avoiding the costs of adding an alkali. The concentration of the dissolved dye was determined using an UV-Vis Lambda 35 spectrophotometer, Perkin Elmer. Absorbance before and after the adsorption were determined at the wavelength of maximum absorption (λmax = 531 nm). The amount of dye impregnated in the substrate or removal percentage, %R, was calculated using Equation 1, where c_0 and c_f correspond to the initial and final concentrations of BR46, respectively.

$$\%R = \frac{c_0 - c_f}{c_0} \times 100 \qquad (1)$$

2.3. Determination of the best conditions of degradation in 50 mL erlenmeyer flasks

For each microorganism, we evaluated the effect of temperature (20 and 28 °C), ATRO moisture (90 and 142), and carbon nitrogen ratio C:N (12:1 and 20:1) in the degradation of BR46 dye adsorbed on BP. A randomization scheme was implemented in a randomized full block factorial design with four replicates resulting in a total of eight treatments, whose characteristics are detailed in Table 1.

Fifty mL Erlenmeyer flasks were used with 140 mg of colored solid substrate (BP-BR46) that were inoculated with three disks (1.2 cm in diameter) of the respective fungus grown on PDA agar.

The ATRO moisture (water percentage in the dry matter of the substrate) was reached using a nutrient solution at the beginning of the fermentation and afterwards regulated by adding sterile distilled water. The solution contained NH_4Cl 0.35 g/L, $(NH_4)_2SO_4$ 1.40 g/L, KH_2PO_4 2.00 g/L, $CaCl_2$ 0.30 g/L, $MgSO_4$ 0.30 g/L, $FeSO_4$ 5.00 mg/L, $MnSO_4$ 1.60 g/L, $ZnSO_4$ 1.40 mg/L, $CuSO_4$ 44.70 mg/L and peptone 1.00 g/L. Moisture was determined by gravimetry on a moisture balance. The C: N ratio was adjusted taking into account the results of the bromatological analysis of the BP, adding the required amount of glucose in the nutrient solution. Compositional analysis was performed at the Laboratory of Chemical and Bromatological Analysis at the Universidad Nacional de Colombia – Medellín Campus.

2.4. Optimization of the BR46 desorption

BR46 dye desorption was initially evaluated using different mixtures of polar and nonpolar solutions, finding that the combination of acetone-hydrochloric acid gave the best results. This mixture was optimized through a Box-Behnken design, in four blocks, where the concentration of acetone (84, 86 and 88% v/v), contact time (13, 16 and 19 h), and solid support dosage varied (80, 140 and 200 mg), reaching a maximum desorption of 99.5% with an acetone concentration of 86% v / v in 0.2 M HCl, a contact time of 16 hours and a dosage of 140 mg [30].

2.5. Degradation kinetics curves

BR46 dye degradation in the SSF process was quantified comparing the amount of desorbed dye in the control sample (substrate without the action of the microorganism) with the amount of desorbed dye in each test sample. The degradation was monitored every five days and from the tenth day, every three days for a total period of 30 days. Equation 2 was used to establish the degradation percentage of the samples at a specific time.

Table 1.
Characteristics of the SSF for the three fungal species evaluated in 50 mL Erlenmeyer flask

Treatment	Temperature (° C)	C:N	ATRO moisture
1	20	12	90
2	20	12	142
3	20	20	90
4	20	20	142
5	28	12	90
6	28	12	142
7	28	20	90
8	28	20	142

Source: The authors

$$\%Degradation = \frac{(m_i - m_r)}{m_i} \times 100 \qquad (2)$$

Where:
m_i: mass of the desorbed dye in the control sample [mg]
m_r: residual mass of desorbed dye in the study sample [mg]

Since the desorption achieved was close to 100%, no correction factor for the terms involved in equation 2 was implemented.

2.6. Evaluation of the degradation process in 100 mL erlenmeyer flasks

This preliminary scale-up of the SSF process was conducted with the fungal species that presented the best adaptation and growth on the solid substrate in the experiments carried out in 50 mL Erlenmeyer flasks, and under the conditions that allowed obtaining the highest degradation percentage.

Thus, 2,500 mg of BP were used. They were contained in a 100 mL Erlenmeyer flask, and were inoculated with five agar disks, 1.2 cm in diameter. The assembly included a total of 20 Erlenmeyer flasks. Ten experimental units were selected for determining the degradation percentage at 15 and 30 days of fermentation. Since the growth of the fungus is not homogeneous on the colored solid substrate, three samples of 140 mg were taken randomly from each experimental unit. They were used to perform the desorption process in triplicate and for the respective analysis.

2.7. Evaluation of the enzyme activity

In order to determine the ligninolytic activity, an enzymatic extraction was conducted by using previously reported protocols [14,25]. The experimental setup was similar to that described for the previous test of the scale-up in 100 mL Erlenmeyer flasks in terms of dosage and fermentation times.

For the extraction, 20 mL of acetate buffer (50 mM, pH = 5.5) were added to each experimental unit. These were stirred at 160 rpm, ensuring the agglomerate disintegration of the

fermented substrate, in an ice bath for one hour and centrifuged at 6,000 rpm for five minutes. The supernatant was used to determine the nonspecific enzymatic activity of the selected fungal species.

Laccase (Lac) activity was established using 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 2 mM as substrate and sodium citrate buffer (0.1 M, pH = 3.0), by measuring the increase in absorbance at 420 nm (ε_{420} = 36.00 $mM^{-1}cm^{-1}$) [31,32]. Manganese peroxidase (MnP) activity was determined using 0.5 mM $MnSO_4$ and 0.1 mM H_2O_2 in sodium malonate buffer (50 mM, pH = 4.5), measuring the increase in absorbance at 270 nm (ε_{270} = 11.59 $mM^{-1}cm^{-1}$) [31,32]. Lignin peroxidase (LiP) activity was determined using 2.0 mM veratryl alcohol as substrate, and 0.5 mM H_2O_2 in sodium tartrate buffer (50 mM, pH = 2.5), by measuring the increase in absorbance at 310 nm (ε_{310} = 9.30 $mM^{-1}cm^{-1}$) [31,33]. The enzyme activity is reported as enzyme units (U) per gram of solid substrate, gss, defined as the amount of enzyme required to produce 1.0 µmol product/min [32,33] or, in parallel, the amount necessary to oxidize a 1.0 µmol substrate/minutes [31].

2.8. Statistical analysis

All experimental trials were assessed with 95% confidence. To compare the results an analysis of variance was conducted (ANOVA), followed by the Scheffe's test. To determine the validity of the variance analysis, Shapiro-Wilk and Levene tests were performed with a confidence level of 99%, verifying the criteria of normality and homoscedasticity, respectively. To ensure independence, the sample selection and treatments were applied randomly. All results are reported as the average of measurements plus or minus (±) the standard deviation.

3. Results and discussion

3.1. Adsorbent pretreatment and BR46 adsorption

A sufficient amount of BP was prepared to carry out the BR46 adsorption process. Thus, with the conditions previously described concerning BP dosage, initial amount of dye, pH value, temperature and contact time, it was proceeded to quantify the concentration of the remnant BR46 in the solution. A solution of 40 ppm was obtained at the end of the process, which is equivalent to 95% removal. This high percentage of removal suggests that BP represents a suitable adsorbent material for its incorporation as a solid support in SSF processes aimed at treating colored effluents.

3.2. Determination of the best conditions of degradation in 50 mL erlenmeyer flasks

The results of BR46 degradation due to the action of the fungal species *P. ostreatus, P. pulmonarius and T. versicolor* are presented in Table 2. The eight treatments evaluated are shown in descending order of the mean percentage of degradation.

Treatments classified with the same letter for each microorganism exhibit no significant statistical difference. These results are derived from an ANOVA analysis with a confidence level of 95%.

The results suggest that the dye degradation process with *P. ostreatus* and *P. pulmonarius* worked best at the lowest temperature (treatments 1-4). In particular, a maximum degradation of 72.87% with *P. ostreatus* under treatment 1 was reached, which corresponds to a temperature of 20 °C, moisture content of 90% and C:N ratio of 12:1. That is, it includes the lowest levels for each factor, resulting in a decrease in the amount of glucose (lower C:N ratio) and in the volume of water (low moisture). For the above reasons, the conditions of this treatment were selected for constructing the kinetic curves.

It is noteworthy that under the same conditions, the highest percentage of RB46 degradation with *P. pulmonarius* was 63.38%, which is satisfactory but about 10 percentage units lower compared to P. *ostreatus.* These results are probably due to the difference in the intrinsic metabolism of the mentioned microorganisms, which belong to different species. The effect of a temperature increase (treatments 5-8), is verified with a significant decrease in the percentage of BR46 degradation; in the case of *P. ostreatus,* down to 1.78%. One possible explanation for this behavior is attributed to a faster drying process that the solid medium has at this temperature compared to 20 °C, which prevents the proper growth of the microorganism. At 28 °C, the inoculum dries and contracts preventing the proper colonization of the entire substrate. It is emphasized that for treatment 8, the synergy between high levels of the C:N ratio and moisture, mitigates the impact of temperature favoring the availability of nutrients, allowing appropriate development of the microorganism, and resulting in 51.5% degradation. Meanwhile, *P. pulmonarius* shows a greater sensitivity to the temperature rise, leading to a lack of degradation (treatment 5, corresponding to the conditions of lower moisture and less addition of carbon source).

Table 2.
BR46 degradation rates by *P. ostreatus*, *P. pulmonarius* and *T. versicolor* and grouping by Scheffe's test.

Microorganism								
P. ostreatus			***P. pulmonarius***			***T. versicolor***		
Treatment	**Mean**	**Scheffe**	**Treatment**	**Mean**	**Scheffe**	**Treatment**	**Mean**	**Scheffe**
1	72.875	A	1	63.375	A	8	64.100	A
3	69.250	A	2	63.350	A	3	61.550	A
2	66.025	A	4	62.300	A	1	60.975	A
4	65.200	A	3	61.100	A	6	58.725	A
8	51.500	B	6	7.025	B	7	57.850	A
6	6.500	C	8	4.400	BC	5	56.250	A
7	5.625	C	7	0.250	BC	2	55.125	A
5	1.775	C	5	0.000	C	4	54.825	A

Source: The authors

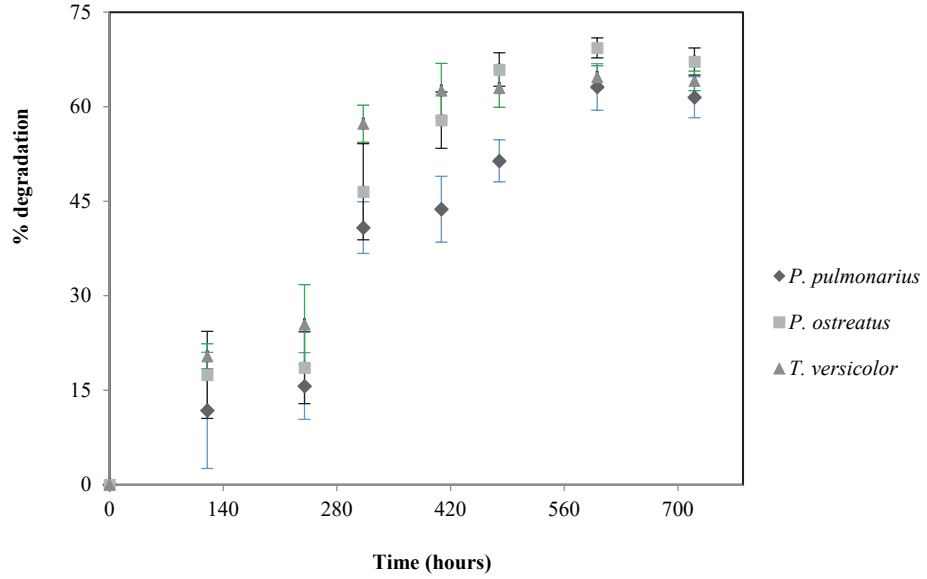

Figure 1. Progress of the BR46 dye degradation by *P. ostreatus, P. pulmonarius* y *T. versicolor*
Source: The authors

The degradation process of the BR46 dye using *T. versicolor* was not affected under the conditions of temperature, moisture or C:N ratio evaluated, since a significant statistical difference between the pairs of treatment methods was not found. These results contrast with those found with *P. pulmonarius* and *P. ostreatus*, and a possible explanation for such a trend is taxonomy. This microorganism belongs to another genus within WRF, and therefore it may adapt differently to diverse nutritional conditions, directly influencing its development [34]. In particular, for this species the additional supply of carbon source (C:N 20:1 in treatments 3, 4, 7 and 8), did not favor the degradation; it is clear that the presence of carbon can support the primary metabolism; however, ligninolytic enzymes associated with dye degradation are part of the secondary metabolism [21].

A maximum degradation of 64.1% was achieved with treatment 8 using T. versicolor, which corresponds to a temperature of 28 ° C, an ATRO moisture of 142% and a C:N ratio of 20:1. Since no significant difference occurs with treatment 1 and 8 for *T. versicolor* and the highest degradation with *P. ostreatus* and *P. pulmonarius*, were reached under treatment 1 conditions, these parameters were taken to evaluate the degradation kinetics curves for the three strains.

3.3. Degradation kinetics in 50 mL erlenmeyer flasks

BR46 dye degradation by *P. ostreatus*, *P. pulmonarius* and *T. versicolor*, under the best growing conditions, determined from the previous experimental designs, is shown in Fig. 1. These conditions were as follows: temperature of 20 °C, C:N ratio of 12:1, and 90% moisture. The amount of solid substrate used in each assay was 140 mg.

It is noted that in a period of 408 hours (approximately 17 days) *T. versicolor* reached the maximum degradation percentage corresponding to 63.0±3.1%. In turn, *P. ostreatus* and *P. pulmonarius* reached their maximum values of degradation in 600 hours (approximately 25 days) with percentages of 69.3±1.6% and 63.1±3.7%, respectively. Likewise, it is emphasized that the error bars indicate quite high deviations at the beginning of the process, which decrease as time advances (Fig. 1). This occurs because in the early days of the fermentation, the microorganisms have not colonized the entire substrate and the growth on the residue is not homogeneous. As time progresses, the fungus grows throughout the substrate, acting on the BP-BR46 residue-dye complex. Furthermore, while the experimental units were treated with adequate homogeneity, the inoculum of each fermentation could have come from a different Petri dish, which may contribute to the increased in difference between the assays due to the fact that the microorganisms may have exhibited different adaptation times to the solid medium. Similar degradation results were reported by Robinson *et al.* [16], who evaluated a mixture of five reactive dyes adsorbed on barley husk. In their study, the WRF *Bjerkandera adusta* was used and a maximum degradation percentage of 53.1% after 21 fermentation days was reached. This process was performed using 5.0 g of the residue-dye mixture as substrate, with culture conditions similar to those used in the present study, an incubation temperature of 25 ° C, a moisture of 85% and a medium supplemented with glucose and micronutrients. It is noteworthy that the C:N ratio is not detailed.

The results of the kinetics allow to establish that *T. versicolor* offered the best performance in BR46 degradation.

It reached a satisfactory degradation of 63.0%, in 17 days, whereas *P. pulmonarius* and *P. ostreatus* only achieved similar percentages in 25 days.

3.4. Evaluation of the degradation process in 100 mL Erlenmeyer flasks.

T. versicolor was selected to evaluate the process on a larger scale. The selection was based on its performance in kinetic studies. The fermentation was carried out at the same temperature, moisture, and C:N ratio conditions of previous experiments. However, the amount of solid substrate was increased to 2,500 mg. The degradation percentage at 15 and 30 days of fermentation are presented in Table 3.

When results of scale-up experiments are compared with the previous tests, a considerable decrease in the maximum degradation is observed. After 15 days of fermentation, the degradation percentage was 37 percentage points below the expected value (63%). However, after 30 days of fermentation, the degradation percentage corresponds to 72% of the maximum expected value. It may be noted that standard deviation is high even though each value corresponds to the weighted average of ten experimental units with three measurements for each sample. This uncertainty can be due to the heterogeneous fungal growth on the substrate: it was inoculated in the periphery of the Erlenmeyer flask, and then the substrate located in the center will be colonized at later stages. Besides, random samples were taken from each experimental unit and they could correspond to variable degradation regions.

The preliminary results suggest that the scaling-up of this process is feasible since the mass of the BP-BR46 complex increased over 18 times, but a satisfactory degradation percentage was achieved in a period that did not double the time reported for the fermentation process at a lower scale. Results also indicate that the amount of solid support to be treated has a direct impact on the rate of degradation and the time required to carry out the fermentation process. Therefore, modifications need to be implemented to promote further fungal growth and their enzyme activity. Some alternatives include the use of different inoculation techniques that enable a more homogeneous growth and a higher rate of colonization of substrate. It has also been reported that various metal inductors and co-substrates act as enhancing agents for enzyme activity and they lead to a substantial increase in the degradation of recalcitrant compounds. In particular, Baldrian and Gabriel, indicate that copper and cadmium addition led to eight and twelve fold increase of laccase activity compared to a medium without these metallic inductors [35].

Table 3.
Degradation percentage and enzyme activity of *T. versicolor* in process evaluated at 100 mL Erlenmeyer flasks

Days	*%Degradation*	Enzyme activity [U/gss]		
		Lac	LiP	MnP
15	26.4±7.4	15.6±2.5	1.8±1.2	6.4±2.8
30	45.7±8.4	5.4±2.2	3.1± 1.8	1.0 ±0.4

Source: The authors

3.5. Determination of enzyme activity

The ligninolytic activity of *T. versicolor* was quantified after 15 and 30 days of the fermentative process (Table 3).

After 15 days of fermentation, enzyme activity for Lac, MnP and LiP were 15.62 ± 2.53 U/gss, 6.42 ± 2.89 U/gss and 1.83 ± 1.23 U/gss, respectively. The value obtained for laccase activity in this investigation is lower when compared to the results reported by Iandolo *et al*. [36]. They obtained an activity of 35 U/gss with *T. versicolor* at 16 days of fermentation using a mixture of tomato waste and sorghum stalks. However, it should be noted that the production of enzymes under SSF conditions without further catalytic applications was evaluated in this study. In a period of 30 days, an increase in LiP (3.1 ± 1.8 U/s) was recorded while Lac and MnP activity decreased significantly, registering values of 5.4 ± 2.2 U/gss and 1.0 ± 0.4 U/gss respectively. It is noteworthy that a greater degradation efficiency is achieved at 30 days compared to the results for 15 days, but the degradation percentage is a cumulative variable, while the enzyme activity can change from time to time.

The production of ligninolytic enzymes by different WRF depends mainly on the fungal species, the substrate composition and culture conditions [36,37]. The results obtained in this study indicate that enzyme activity varies over time, a trend also evidenced by Winquist *et al*., with enzyme production of *T. versicolor* on oat hulls [38]; Reddy *et al*. with two *Pleurotus* strains using banana waste [39]; Robinson *et al*. with grim *B. adusta* on barley husk [16]; Sun *et al*. with *Trametes* sp. AH28-2 using four agricultural byproducts [40]; and Tychanowics *et al*., with *P. pulmonarius* on corn residues [33], among others.

It is also noteworthy that Robinson *et al*. [16] evaluated the enzyme activity during the degradation of adsorbed dyes and they found that it changes over time. In the early days of fermentation, low rates of degradation were observed, which coincided with the absence or minimum enzyme activity. As the fermentation process progressed, a higher content of ligninolytic enzymes was detected, even before the degradation of the dye could be perceived. This behavior allows to suggest that the enzymes are associated initially to the lignocellulose cleavage and subsequently to the dye degradation. Enzyme content increased to a maximum value, which occurs at a different time for each enzyme, after such a maximum value is reached, the activity starts to decrease. These authors reached maximum activities of LiP, MnP and laccase of 17.0 U/gss, 15.8 U/gss and 2.0 U/gss at nine, fifteen and twelve days, respectively, obtaining a maximum degradation percentage of 53.0% after 21 days.

The results of this study point out that BR46 degradation, impregnated on an agricultural waste, with WRF, represents a highly efficient and satisfactory alternative process for its bioremediation. In particular, further studies should continue to explore appropriate conditions to achieve an efficient degradation of this azo dye with the fungal species *T. versicolor* at a larger scale.

4. Conclusions

Degradation percentages higher than 60% were achieved at 20 °C, a moisture content of 90% and a C:N ratio of 12:1

using *T. versicolor,* which proved to be the most efficient fungus between the tested species for the degradation of the azo dye BR46. Seventeen days were needed to reach the maximum level of degradation with *T versicolor*, while *P. ostreatus* and *P. pulmonarius* required 25 days.

T. versicolor is an outstanding candidate for large-scale fermentation due to its versatility against changes in the evaluated variables, which allow it to achieve efficient BR46 degradation. It is therefore a suitable species to develop an efficient and low-cost strategy with a positive environmental impact for the bioremediation of xenobiotic compounds such as synthetic dyes.

The enzymatic potential of WRF depends on their different ligninolytic enzymes, primarily laccases. Their unspecific action and great oxidative ability allow them to degrade a wide variety of organic compounds, including polymers such as lignin, chlorinated phenols, dioxins, chloroanilines and dyes. Thus, their application in several biotechnology fields and in the bioremediation of pollutants will undoubtedly be important in the future.

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