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Full Length Research Paper

Biotransformation of indigo carmine to isatin sulfonic acid by lyophilized mycelia from *Trametes versicolor*

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Indigo carmine (IC) was biotrasformed to 5-isatinsulfonic acid using intracellular and associated enzymes from *Trametes versicolor* lyophilized mycelia; even when extracellular enzymes were absent, in high concentration solutions of IC (4 000 mg L^{-1}) and non-sterile condition. *T. versicolor* was grown in wheat strew and malt extract liquid medium and harvested during the stationary growth phase, it was lyophilized and made to react with indigo carmine. Experimental series were performed at different IC concentrations (from 100 to 4000 mg L^{-1}). Color removal was 99.90, 98.75, 88.35, 79.47, 70.0 and 40.35% for 100, 500, 1000, 2000, 3000 and 4000 mg L^{-1} of IC, respectively after 120 h with exception for 100 mg L^{-1} of IC, which reached total color removal after 1 h. Reacted mixture byproducts were separated by column chromatography. IC biotransformation to 5-isatinsulfonic acid was confirmed by HPLC, UV-VIS, FT- IR, ^{1}H and ^{13}C NMR spectroscopy. Activity of laccase from lyophilized mycelia was conserved after one year at 4°C. Dehydrated biological material in colorant biodegradation is a new method which allows obtaining high discoloration efficiencies. Lyophilized mycelia could be more stable than traditionally used wet biomass or liquid culture for biodegradation of color dye.

Key words: Biodegradation, indigo carmine, *Trametes versicolor.*

INTRODUCTION

Indigo carmine (IC) discoloration by white-rot fungi has been widely documented (Campos et al., 2001; Cing et al., 2003; Lorenzo et al., 2005; Birhanli and Yesilad, 2006). Most of the reported research attributes textile dye biodegradation to the extracellular laccases and peroxidases from white-rot fungi, (examples of these class of enzymes are lignin peroxidase (LiP), manganese peroxidase (MnP) and phenoloxidases), which are present in the liquid culture (dos Santos et al., 2007). Generally, discoloration is carried out using indigo carmine as a co-substrate (Levin et al., 2004; Ramya et al., 2008). The main inconvenience for using fungi in liquid cultures is to maintain growth conditions allowing extracellular enzyme excretion. Therefore, under these conditions, it is hard to perform a pilot scaling for applying it at an industrial scale although, there are some research about using culture filtered (Pazarlıoglu et al., 2005; Birhanli and Yesilada, 2006); enzymatic extract (Singh et al., 2007); fungal biomass (Cing et al., 2003; Anastasi et al., 2009); as well as immobilized fungi (Dominguez et al., 2005; Rodríguez et al., 2006). These methods are limited by both their low enzymatic viability and sterile conditions (Birhanli and Yesilada, 2006). Until now, employment of dehydrated mycelium have not been studied, this may be an option to have better results on color removal and overcome the inconvenience of the earlier mentioned methods, since dehydration by a lyophilization process allows for conservation of intracellular and associated enzymes.

The aim of this paper is to show that the biodegradation of textile dye indigo carmine in high concentrations can

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be obtained by using intracellular and associate enzyme contained in lyophilized mycelia from *Trametes versicolor* in non sterile conditions and without using extracellular enzyme from liquid culture media.

MATERIALS AND METHODS

Chemical products

Reagents were obtained from Sigma Aldrich and Bioxon. *T. versicolor* 8273 was a gift from Dr. Rafael Vázquez-Duhalt, Instituto de Biotecnologia (UNAM, México). Commercial laccase was provided by Novozymes (Deni Lite II).

Culture

Cultures were grown in Petri dishes at 30°C using a media composed of: glucose (10 g L^{-1}), malt extract (3 g L^{-1}), soybean peptone (5 g L^{-1}), yeast extract (3 g L^{-1}), and dextrose potato agar (40 g L^{-1}). After 8 days, a 2 × 2 cm section of fungi mycelia was cut and transferred to an Erlenmeyer flasks (15) containing 150 ml of medium prepared with extract obtained from wheat straw chopped (0.5 cm) (100 g L^{-1}) and malt extract (20 g L^{-1}) boiled at 90°C for 1 h, as reported by Sainos et al. (2006). After this, the media was enriched with 0.05 g $CaCl_{2*}.2H_20$/L, 0.05 g $CuS0_{4*}.5H_20$/L and 0.05 g $MnS0_{4*}.H_20$/L. The medium was sterilized at 121°C and 15 psi for 15 min, inoculated and incubated at 30°C for 15 days under static conditions.

Lyophilized mycelium preparation

Each mycelia biofilm was separated from liquid culture and lyophilized in a Labconco freeze dryer for 24 h. After biofilm, lyophilized mycelia were triturated with liquid nitrogen in a mortar to obtained powder which was stored at 4°C until it was required.

Intracellular and associated enzyme extraction

A mixture of 0.030 g lyophilized mycelia and 5 mL of sterilized water were stirred at 17.8 × *g*, for 2 h; the suspension was centrifuged at 12 857 × *g* and 4°C for 20 min. The supernatant was collected (crude extract) and used to assay for laccase activity.

Laccase activity

Laccase activity was measured spectrophotometrically with a Genesys 5 spectrophotometer using 1 µmol 2,2-azino–bis (3-ethylbenzothiazoline- 6-sulfonate) (ABTS) as substrate. One unit of enzyme activity was defined as of ABTS oxidized per minute at 25°C (ε_{436}= 29 300 $M^{-1}cm^{-1}$) (Wolfenden and Wilson, 1982).

Decolourization of indigo carmine

Degradation ability of lyophilized mycelia in IC solutions was tested in two ways: (1) using 6, 9 and 12 g L^{-1} lyophilized mycelia with samples containing 100 mgL^{-1} of IC; and (2) a fix amount of mycelia (12 g L^{-1}) with 100, 500, 1 000, 2 000, 3 000 and 4 000 mg L^{-1} of IC solution. Discoloration experiments were performed at 17.8 × *g*, in a non-buffered medium, room temperature and non- sterile condition. Samples were withdrawn at regular time intervals and centrifuged (12 857 × *g*, 15 min and 4°C). Supernatant was used for UV-Vis analysis of IC. In order to clarify whether discoloration was due to adsorption or an enzymatic action, UV-VIS spectra, in the range 190 to 1100 nm, were obtained for a 100 mg L^{-1} IC solution treatment as follows: (1) a reaction solution (IC reacted with *T. versicolor* lyophilized mycelia); (2) control solution (IC reacted with sterile lyophilized mycelia at 121°C and 15 psi); (3) reference solution (IC without treatment). Bioassays were performed under the same condition as described earlier and run in duplicate.

UV-VIS analyses

Discoloration was spectrophotometrically measured with a Hewlett Packard HP 8453 diode array UV-VIS spectrophotometer, absorption spectra were recorded from 190 to 1 100 nm and color removal efficiency was calculated with readings at IC λ_{max} (610 nm).

Purification and characterization of reaction product

A mixture of 14 g L^{-1} lyophilized mycelia with 4 000 mg l^{-1} IC solution was assayed at 17.8 ×*g* in non-buffered medium, at room temperature. Reaction products were centrifuged (12 857 × *g,* 15 min and at 4°C); the supernatant was ultra filtrated with a 1 kDa disk membrane; filtered liquid was lyophilized by 24 h. The products of reaction were separated by column chromatography over silica, using CH_2Cl_2:CH_3OH 8:2 as mobile phase. Obtained products were concentrated on a vacuum rota vapour (Buchii R124 Germany). After this procedure, each concentrated product was lyophilized for 8 h and analyzed by UV-VIS, HPLC, FT- IR, ^1H and ^{13}C NMR techniques.

HPLC analysis

Samples of the reaction product were analyzed by HPLC technique using 1100 series Hewlett-Packard model equipped with a diode array detector and a C-18 reverse-phase column (250 × 4.6 mm). The flow rate was 0.3 ml min^{-1}, the mobile phase was methanol: water (30:70 v/v), sample injected volume was 5 µl, IC detection was done at 210 nm for byproducts and 610 nm for IC and the total run time was 10 min.

FT- IR analysis

FT- IR spectra, using attenuated total reflectance (ATR) technique, were recorded using Bruker vertex 70 apparatus with a resolution of 4 cm^{-1}, in ranges of 800 to 4 000 cm^{-1} and at room temperature. Samples of the reaction product and standard of 5-isatinsulfonic acid were solubilized in methanol, placed on a glass plate for solvent evaporation and introduced as a thin film in the FT- IR.

Nuclear magnetic resonance (NMR) ^1H and ^{13}C- NMR analysis

The completely dried sample was dissolved in $(CD_3)_2SO$ and transferred into NMR tubes;^1H, ^{13}C-NMR spectra, as well as gHMBC were obtained on a Varian VNMR 500 instrument (500 MHz) using $(CD_3)_2SO$ as the solvent and TMS as an internal standard.

Reaction of commercial laccase enzyme with IC

In order to prove that IC biotransformation can be attributed to lacasse activity, a set of experiments were carried out with a 4 000

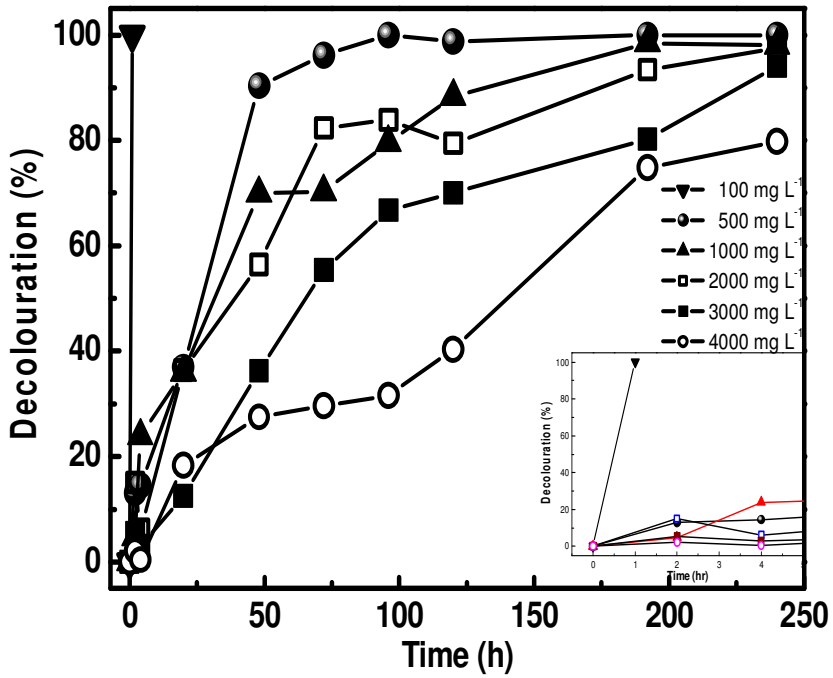

Figure 1. Effect of IC concentration reaction conditions: 6 g lyophilized mycelia /L of IC solution, room temperature, 17.8 × *g* in non-buffered media. Concentration IC: (▼), 100 mg l^{-1}, (●), 500 mg l^{-1}, (▲), 1 000 mg l^{-1}, (□), 2 000 mg l^{-1}, (■), 3 000 mg l^{-1}, (○) 4 000, mg l^{-1}

mg L^{-1} IC solution and commercial laccase enzyme (12 g L^{-1}) and tests were run under same conditions as those with *T. versicolor* lyophilized mycelia. Purification and identification of reaction products followed the protocol applied for *T. versicolor* lyophilized mycelia.

RESULTS

Decolourization of indigo carmine

The assay of three *T. versicolor* lyophilized mycelia concentrations (6, 9 and 12 g L^{-1} lyophilized mycelia) showed that the time to reach total discoloration was 60, 30 and 20 min, respectively. Therefore, it can be inferred that there was a specific amount of enzyme in the lyophilized mycelia, since increasing lyophilized mycelia amount decreased the reaction time for discoloration. The different concentration of IC solution (from 100 to 4 000 mg L^{-1}) that reacted with the lyophilized mycelia (Figure 1) showed that at low concentration of IC (100 mg L^{-1}), rate of the biodegradation was high in comparison with higher concentration (500 to 4 000 mg L^{-1}). It may be due to the saturation of active sites of enzyme by colorant molecules, which reduce interaction between IC molecules and enzyme while in low concentration there are more free active sites for interaction with IC molecules. These phenomena suggested there is a relationship between IC substrate and enzymatic activity.

IC adsorption on mycelia

It was observed that after 1 h, absorbance spectra of initial IC and control solutions maintained their initial absorbance since their spectrum profile remained unaltered, while the test solution spectra disappeared completely (Figure 2). This behavior indicated that IC dye was not absorbed on *T. versicolor* lyophilized mycelia, but rather the IC was transformed by an enzymatic action.

Biotransformation of IC by active *T. versicolor* lyophilized mycelia

Reaction product obtained from mixing 14 g l^{-1} of lyophilized mycelia with 4 000 mg l^{-1} IC, was used to confirm biotransformation of IC; was analyzed by UV-VIS, FT-IR, HPLC, ^{1}H and ^{13}C NMR analysis. The UV-VIS spectrum of the obtained reaction product had two maximum absorbance; 209 and 242 nm (Figure 3) while the standard prepared with isatin-5-sulfonic acid sodium dehydrated salt (which in aqueous phase ionized to 5-

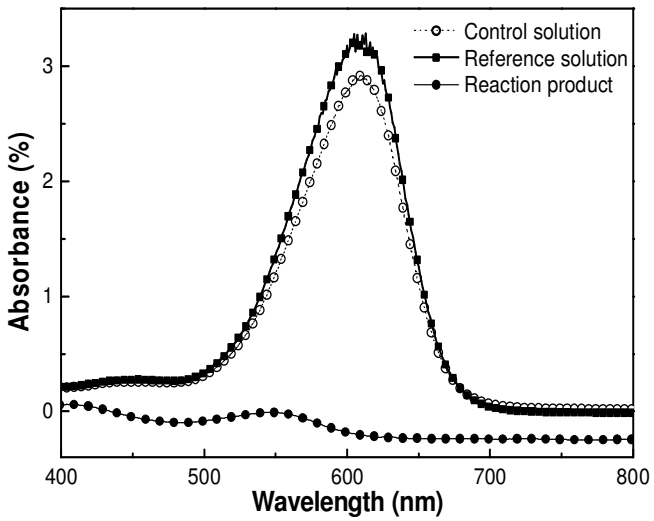

Figure 2. UV-VIS spectra of 100 mg l^{-1} IC with lyophilized mycelia of *T. versicolor* after 1 h of reaction, with 6 g mycelia/L, at room temperature and 17.8 × *g* in non-buffered medium. (■), reference solution (IC); (○), control solution; (●), reaction product.

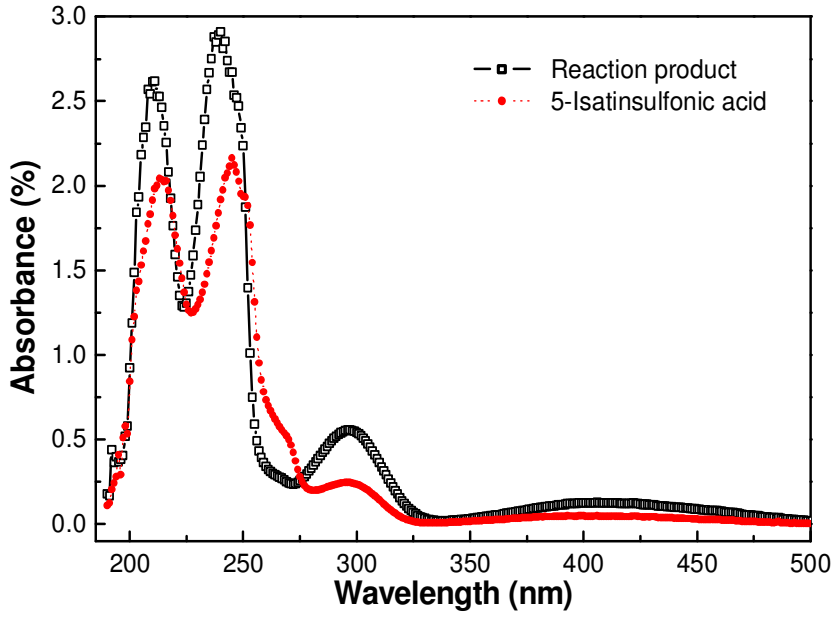

Figure 3. UV-VIS spectra of 5-isatinsulfonic acid and product reaction. Reaction condition: IC 4 000 mg L^{-1} with lyophilized mycelia, at room temperature, in a non-buffered media; concentration of mycelium/colorant 14 g L^{-1} and 17.8 × g; (●) ▬ , 5-isatinsulfonic acid; (□), reaction product.

isatinsulfonic acid), also had two characteristic peaks detected at 214 and 246. This closeness in values, suggest that the reaction product contained 5-isatin-sulfonic acid (ISAS).

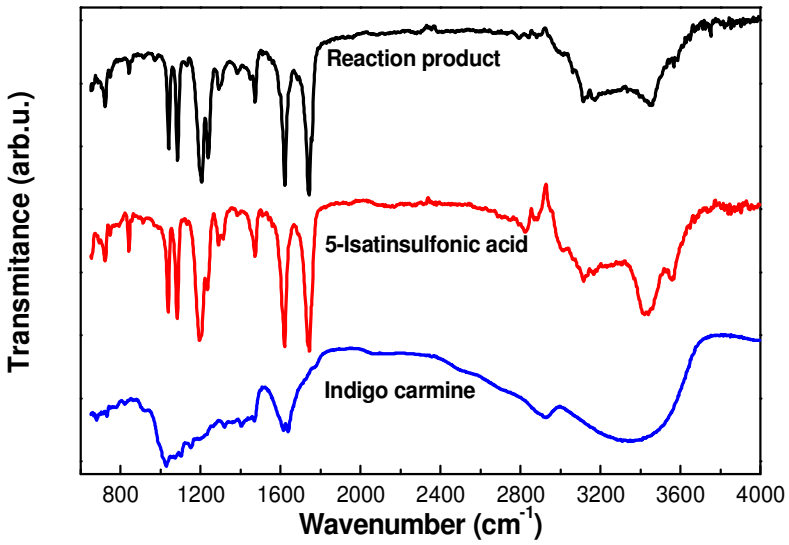

Figure 4. FT- IR spectra comparison of 5-isatinsulfonic acid and the reaction product in the frequency region of 800 to 4 000 cm^{-1}. —, 5-isatinsulfonic acid; —, reaction product; —, indigo carmine.

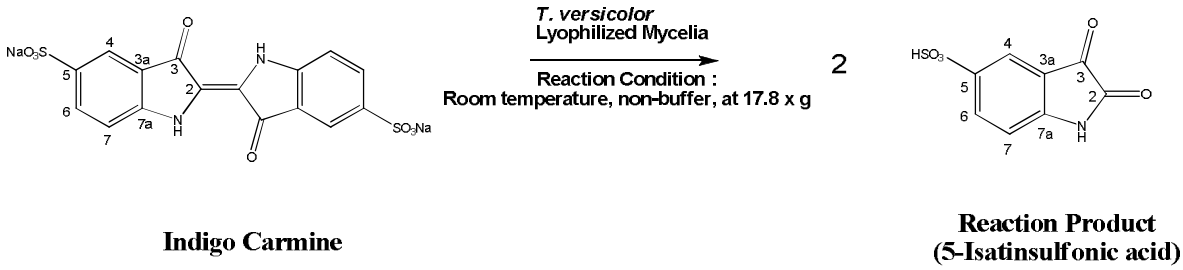

Figure 5. Chemical reaction of Indigo carmine with *T. versicolor* lyophilized mycelia. Reaction condition: room temperature and a non-buffer at 17.8 × *g*.

FT- IR spectroscopy

On the other hand, FT-IR spectrum of the reaction product (Figure 4), showed strong absorption bands at 697, 907, 1 040, 1 059, 1 202, 1 451, 1 622, 1 746, 3 121, 3 149 and 3 436 cm^{-1}. The peaks centered at 1040 and 1085 cm^{-1} were assigned to stretching vibration of sulfonic acid in IC (Vautier et al., 2001; Wade, 2008); therefore, sulfonic acid was present in the product reaction chemical structure. The absorption band located at 1 622 cm^{-1}, corresponds to the vibration of C= C aromatic bond (Wade, 2008); while the one at 1 741 cm^{-1} may be associated to a C=O bond (Pretsch et al., 1980; Wade, 2008) that implicated functional group (CO) is additional to chemical structure IC while absorption bands at 3 107 and 3 419 cm^{-1} are assigned to amine group. Moreover, spectral profile between the reaction product and ISAS standard were similar.

HPLC

The chromatogram of ISAS standard was detected at 7.40 min, while product reaction was at 7.49 min and a mixture of the standard and product reactions were registered at 7.45 min, which also corroborate that ISAS was the product of the reaction.

^{1}H and ^{13}C NMR

The ^{1}H NMR spectrum of indigo carmine had three proton in downfield at 7.81, 7.77 and 7.26 ppm, which correspond to protons aromatics and one proton at 11.25 ppm (NH). Signal at 7.81 was split into a doublet, which can be attributed to proton (4) in meta (4 and 6) positions (Figure 5) with a complement constant of 1.5 Hz. Peak at 7.77 ppm was assigned to proton (6) in meta position

Table 1. ^{1}H and ^{13}C NMR chemical shifts of indigo carmine, 5-Isatinsulfonic acid and reaction product.

Position	Indigo carmine		5-isatinsulfonic acid		Reaction product	
	δ_C	δ_H(J in Hz)	δ_C	δ_H(J in Hz)	δ_C	δ_H(J in Hz)
1		10.64bs		11.12bs		11.25bs
2	121.2		184.2		184.2	
3	187.7		159.2		159.7	
3a	117.9		117.0		116.9	
4	120.7	7.81 d (1.5)	121.6	7.62 d(2.0)	121.5	7.60 d(1.8)
5	140.5		142.8		143.0	
6	133.6	7.77 dd (8.5; 1.5)	135.6	7.82 dd (8.0; 1.5)	135.4	7.80 dd (8.5;1.8)
7	112.6	7.26 d (8.5)	111.6	6.88 d(8.0)	111.5	6.89 d(8.4)
7a	152.6		150.8		150.8	

^{1}H NMR (500 MHz, DMSO-d_6); ^{13}C NMR (125 MHz, DMSO-d_6). Chemical shifts are in ppm.

4 and 6) with J= 1.5 Hz and adjacent to ortho (6 and 7) proton with J= 8.5 Hz, giving rise to a doublet of doublets signal. That means there was a substitute group in position 5, which could be sulfonic group. Peak at 7.26 ppm may be assigned to proton (7) in ortho (6 and 7) position with J= 8.5 Hz; this signal was split into doublet. Signal at 11.25 could be assigned to proton amine. ^{13}C NMR spectrum had eight signals in downfield. Signal at 117.9, 120.7, 121.2, 140.5, 133.6, 112.6 and 152.6 ppm could be attributed to 6 aromatic carbon; signal at 187.7 ppm could be assigned to ketone proton; while signal 121.2 ppm corresponded to the carbon in position 2. As can be observed in Table 1, chemical shifts from indigo carmine with the reaction product were different. On other hand, all shifts from ^{1}H NMR and ^{13}C NMR spectrum of the reaction product and 5-isatinsulfonic acid matched (Table 1). Therefore, it was evident that majority of the reaction product was 5-isatinsulfonic acid.

T. versicolor lyophilized mycelia viability

To determine viability of the dehydrated mycelia, laccase activity was measured after four months and one year, and activities were found to be 0.56 U/g +/- 0.0033, and 0.33 +/- 0.0098, respectively. This showed that lyophilized mycelia can be conserved until one year.

DISCUSSION

The product reaction was isolated as yellow solid and the absorption band at 3 107 and 3 419 cm^{-1} in the FT- IR spectrum indicated the presence of amine group. This had UV absorption maxima at 209 and 242 nm that provided evidence of a highly conjugated molecule. The ^{1}H and ^{13}C NMR chemical shifts showed signals of aromatic trisubstituted ring. The ^{1}H NMR spectrum displayed a broad singlet for one proton at 11.25 ppm (NH), a doublet at 6.89 (H-7), a methyne at 7.60 (H-4) and double doublet at 7.80 ppm assigned to H-6. The ^{13}C NMR spectrum indicated two typical ketone carbons at 184.2 (C-2) and 159.7 (C-3) ppm; the first one showed a gHMBC correlation with the signal at 7.60 ppm (H-4), while the second one showed a gHMBC correlation with the signal at 11.25 ppm (NH). The NMR data of the other part of the molecule were in agreement with those for 5-isatin sulfonic acid standard solution. As observed in Table 1, chemical shifts from indigo carmine were different to the product reaction and absorption in UV was minor for the last one. This indicated that indigo carmine was broke into two sulfonated indole molecules (Figure 5). Although, there are reports of IC degrading to anthranilic acid (2-aminobenzoic acid) via isatin (Campos at al., 2001; Singh et al., 2007) by purified laccases either from bacterial or fungi; their reaction condition are different (pH 9 and 55°C in the case reported by Singh et al. 2007). In addition, the sulfonic group bound to the aromatic ring caused recalcitrance in the molecule due to chemical stability (Capalash and Sharma, 1992). This fact could explain why IC was not transformed into anthranilic acid by *T. versicolor* lyophilized mycelial action under the tested conditions. Therefore, biodegradation mechanism could be carried out in one step, similar to the report of Kettle et al. (2004) for a superoxide process. Biotransformation was due to enzymatic action from intracellular laccase and associated enzymes from *T. versicolor* lyophilized mycelia, since laccase activity of mycelia were conserved after dehydration. In addition, IC was reacted with commercial laccase enzyme and analysis of the obtained reaction products, showed that majority of the products were ISAS (data not shown); this also corroborates that IC is a substrate for laccase. Intracellular laccase from wet biomass of *T. versicolor* has ability to biodegrade textile dye (Young and Yu, 1997; Blanquéz et al., 2004). However, factors such as stability, preservation, control condition growth mycelia and microbial contamination of wet biomasses can be a disadvantage, which hinders industrial scale application. On other hand, *T. versicolor* lyophilized mycelia were

effective in degrading the 100 mg L^{-1} IC concentration. Applying *T. versicolor* lyophilized mycelia to various IC concentrations showed that the required time to reach total discoloration was shorter (1 h) than reported by some authors using either fungal or bacterial wet biomass. For example, Ramya et al. (2008) used a liquid culture from *Paenibacillus larvae* with IC solutions (100 mg L^{-1}) achieving a 100% removal after 8 h; Podgornik et al. (2001) reported biodegradation of 3 to 30 mg L^{-1} IC solutions in 2 h, by using isoenzymes of LiP and MnP obtained from *Paenibacillus chrysosporium*; Colao et al. (2006) obtained 95% of decoloration after 6 h of reaction with recombinant laccase using IC solution with 50 mg L^{-1} and with mediator and Levin et al. (2010) discolored 94% of 23.4 mg L^{-1} of IC solution in 0.5 h using enzymatic extract from *Trametes trogi* with188.3 U ml^{-1}. Special attention must be placed on IC concentration effects, since *T. versicolor* lyophilized mycelia has the ability to remove color in high concentration from 500 to 4 000 mg L^{-1} of IC providing up to 79.47% discoloration for 2 000 mg L^{-1} in 120 h; this range of IC concentration is higher than reported values (Zhang et al., 2006; Levin et al., 2010). Moreover, discoloration with lyophilized mycelia is equivalent to or greater than that attained with physico-chemical methods (Gemeay et al., 2003; Othman et al., 2007; Subramani et al., 2007). The earlier mentioned results confirm that the use of lyophilized mycelia powder for indigo carmine discoloration represent an important advantage, since it can be applied to higher IC concentrations, it does not require extracellular enzyme presence or a mediator addition, and neither sterile condition, in order to get higher efficiency.

Conclusion

In conclusion, this study shows that lyophilized mycelium powder of *T. versicolor*, has the ability to degrade IC to 5-isatinsulfonic acid by laccase intracellular and associated enzymes. The efficiency of dehydrated mycelia was greater than that obtained with other biological and physicochemical systems. The lyophilization process produced stable powders of mycelia from *T. versicolor* with an ability to preserve enzymatic activity even after one year.

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REFERENCES

Anastasi A, Prigione V, Cas L, Casieri L, Varese GC (2009). Decolourisation of model and industrial dyes by mitosporic fungi in different culture conditions. World J. Microbiol. Biotechnol. 25: 1363-1374.

Birhanli E, Yesilada O (2006). Increased production of laccase by pellets of *Funalia trogii* ATCC 200800 and *Trametes versicolor* ATCC 200801 in repeated-batch mode. Enzyme Microb. Technol. 39: 1286-1293.

Blánquez P, Casas N, Font X, Gabarrell X, Sarrà M (2004). Mechanism of textile metal dye biotransformation by *Trametes versicolor*. Water Res. 38: 2166-2172.

Campos R., Kandelbauer A, Robra KH, Cacaco AP, Gubitz GM (2001). Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*. J. Biotechnol. 89: 131-139.

Capalash N, Sharma P (1992). Biodegradation of textile azo-dyes by *Phanerochaete chrysosporium*. World J. Microbiol. Biotechnol. 8: 309-312.

Cing S, Hamamci DA, Apohan E,Yeşilada O (2003). Decolorization of Textile Dyeing Wastewater by *Phanerochaete chrysosporium*. Folia. Microbiol. 47: 639-642.

Claus H, Faber G, Koring H (2002). Redox-mediated decolorization of synthetic dyes by fungal laccases. Appl. Microbiol. Biotechnol. 59: 672-8.

Colao MC, Lupino S, Garzillo AM, Buonocore V and Ruzzi M (2006). Heterologous expression of lcc1 gene from *Trametes trogii* in *Pichia pastoris* and characterization of the recombinant enzyme. Microb. Cell. Fact. 5: 1475-285.

Domínguez A, Rodríguez CS, Sanromán MA (2005). Dye decolourization by *Trametes hirsuta* immobilised into alginate beads. World J. Microbiol. Biotechnol. 21: 405-409.

dos Santos AB, Cervantes FJ, vanLier JB (2007). Review paper on current technologies for decolourisation of textile wastewaters perspectives for anaerobic biotechnology. Bioresour. Technol. 98: 2369-2385.

Gemeay AH, Mansour IA, El-Sharkawy RG, Zaki AB (2003). Kinetics and mechanism of the heterogeneous catalyzed oxidative degradation of indigo carmine. J. Mol. Catal. A. Chem. 193: 109-120.

Kettle AJ, Bruce MC, Clark BM, Winterbourn CC (2004). Superoxide Converts Indigo Carmine to 5-Isatinsulfonic acid Sulfonic Acid Implications for the Hypothesis that Neutrophils Produce Ozone. J. Biol. Chem. 279: 18521-18525.

Koroleva OV, Stepanova EV, Gavrilova P, Yakovleva NS , Yaropolov A (2002). Laccase and Mn-peroxidase production by *Coriolus hirsutus* strain 075 in a jar fermentor. J. Biosci. Bioeng. 93: 449-455.

Levin L, Papinutti L, Forchiassin F (2004). Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. Bioresour. Technol. 94: 169-176.

Levin L, Melignani E, Ram AM (2010). Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresour. Technol. 101: 4554-4563.

Lorenzo M, Moldes D, Sanromán MA (2006). Effect of heavy metals on the production of several laccase isoenzymes by *Trametes versicolor* and on their ability to decolourise dyes. Chemosph. 63: 912-917.

Othman I, Mohamed RM, Ibrahem F (2007). Study of photocatalytic oxidation of indigo carmine dye on Mn-supported TiO2. J. Photochem. Photobiol. A. Chem. 189: 80-85.

Pazarlioglu NK, Urek RO, Ergun F (2005). Biodecolourization of Direct Blue 15 by immobilized *Phanerochaete chrysosporium*. Process. Biochem. 40: 1923-1929.

Podgornik H, Poljansek I, Perdih A (2001). Transformation of Indigo carmine by *Phanerochaete chrysosporium* ligninolytic enzymes. Enzyme Microb. Technol. 29: 16-172.

Pretsch E, Clerc T, Seibl J, Simon W (1980). Tables for Structural Elucidation of Organic Compounds by Spectroscopic Methods Ed Alhambra. Madrid, pp. 80-90.

Ramya M, Anusha B, Kalavathy S (2008). Decolorization and biodegradation of Indigo carmine by a textile soil isolate *Paenibacillus larvae*. Biodegrad. 19: 283-291.

Rodríguez CS, Rosales E, Snromán MÁ (2006). Decolourization of synthetic dyes by *Trametes hirsuta* in expanded-bed reactors. Chemosph. 62: 1558-63.

Sainos E, Díaz G, Montiel-González AM Loera O (2006). Growth of

Pleurotus ostreatus on wheat straw and wheat grain-based media: biochemical aspects and preparation of mushroom inoculum. Appl. Microbiol. Biotechnol. 72: 812-815.
Singh PG, Capalash N, Goe R (2007). A pH-stable laccase from alkali-tolerant Proteobacterium JB: Purification, characterization and indigo carmine degradation. Enzyme Microb. Technol. 41: 794-799.
Subramani AK, Byrappa K, Ananda S, Lokanatha R, Lokanatha Rai KM, Ranganathaiah CY, Yoshimura M. (2007). Photocatalytic degradation of indigo carmine dye using TiO_2 impregnated activated carbon. Bull. Mater. Sci. 30: 37-41.
Vautier M, Guillard C and Herrmann J (2001). Photocatalytic Degradation of Dyes in Water: Case Study of Indigo and of Indigo Carmine. J. Catal. 201: 46-59.
Young L, Yu J (1997). Ligninase-catalysed decolorization of synthetic dyes. Water Res. 31: 1187-1193.
Wade Jr. LG (2008). Organic Chemistry (5th edition). Prentie Hall, pp 1208.
Wang Y, Yu J (1998). Adsorption and degradation of synthetic dyes on the mycelium of *Trametes versicolor*. Water Sci. Technol. 38: 233-238.
Wolfenden BS, Wilson RL (1982). Radical cations as reference chromogens in studies of one-electron transfer reactions; pulse radio analysis studies of ABTS. J. Chem. Soc. Perkin. Trans. 11: 805-812.
Zhang M, Wu F, Wei Z, Xiao Y and Gong W (2006). Characterization and decolorization ability of a laccase from *Panus rudis*. Enzyme Microb. Technol. 39: 92-97.