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Polymerization study of the aromatic amines generated by the biodegradation of azo dyes using the laccase enzyme

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ABSTRACT

Four different azo dyes were decolorized (color reduction >90%) by bacteria isolated from a textile wastewater effluent. Dye decolorizing was carried out under microaerobic conditions until completion, after which the aromatic amine concentration was determined. A laccase from *Myceliophthora thermophila* was used to catalyze coupling reactions of the aromatic amines produced from decolorizing the dyes. The reaction was carried out with stirring (100 rpm) in a weak acidic buffer solution (pH 5.0) at 45 °C for 3 days. The presence of aromatic amines in the samples after bacterial decolorizing confirmed the azo bond was reduced in the process. In addition, the UV–vis spectrum was shifted significantly after the sequential bacterial-laccase treatment also indicating a chemical transformation of the dyes. After laccase treatment the solutions formed colored soluble and precipitated products. The particles sizes making up the precipitates formed after laccase treatment varied between 105 and 483 nm as determined by Photon Correlation Spectroscopy (PCS). The laccase treatment also reduced the COD of the dye solutions by ~20%. We show that successive bacterial-laccase treatment is effective in decolorized azo dyes by reduction of the azo bonds, and promoting coupling reactions between the aromatic amines formed. Promoting coupling reactions between the aromatic amines using enzymes may prove useful for the physical removal and reuse of these amines.

1. Introduction

Azo dyes constitute the largest and most versatile class of synthetic dyes used in the textile, pharmaceutical, food and cosmetics industries. These dyes are characterized by the presence of one or more azo bonds (–N=N–). These are prepared by azo coupling between a diazonium compound and an aniline, phenol or other aromatic compound. During the dyeing process in the textile industry, approximately 10–15% of the dyes used are released into the wastewater.

Aromatic amines, with known carcinogenic potential, such as aniline and sulphanilic acid appear in liquid effluents as a consequence biological reduction of the azo bond in azo dyes [1]. The presence of these dyes and their by-products in aqueous ecosystems leads to aesthetic and health problems due to the coloring of waters, the obstruction of light penetration and inhibition of oxygen transfer [2,3].

Dye wastewaters are usually treated by flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation, ozonization and Fenton's oxidation [4]. These methods are often expensive and can generate large amounts of sludge, which increases process costs [5]. The use of biological methods in the treatment of textile wastewaters may be a cost effective alternative to the physico-chemical and photochemical methods currently used. Aerobic biological methods are largely ineffective in the treatment of textile wastewaters, resulting in little or no color removal in the case of most of the dyes, especially azo dyes. Anaerobic and microaerobic treatments are effective in removing the color, but the products from azo dye degradation are frequently carcinogenic aromatic amines and these amines can inhibit further aerobic degradation [6,7].

Conventional processes for the removal of aromatic amines from industrial wastewaters include extraction, adsorption onto activated carbon, bacterial and chemical oxidation, electrochemical

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techniques and irradiation. All of these methods suffer from drawbacks including, high costs, formation of hazardous by-products and low efficiency [8,9].

Laccases are multi-copper phenol oxidases, which reduce oxygen to water and simultaneously catalyze the oxidation of aromatic pollutants such as anilines and phenols [10,11]. However, laccases can catalyze the coupling and polymerization of products resulting from the dye decolorizing process, and also of various halogen, alkyl and alkoxy substituted anilines [12,13]. The enzymatic polymerization of aniline has been investigated for the removal of aromatic amines and aromatic compounds from effluents [14]. The polymeric structures formed can precipitate spontaneously from solution due to their low solubility, and can be removed from the effluent in a further treatment process, or industrially reused, depending on the characteristics of the polymeric product formed [15].

Recently a one-step, simple and environmentally friendly enzymatic synthesis of polymers derived from aromatic amines such as aniline, has been developed [16–18].

In the present paper the degradation of four azo dyes was carried out under microarobic conditions, using a set of bacteria isolated from a textile activated sludge process. This was followed by a secondary treatment with laccase to determine if the biodegradation products from the azo dyes, such as aromatic amines, could be polymerized in coupling reactions. The enzymatic polymerization experiments were carried out for 3 days. The spectra and particle sizes of the products were analyzed by UV–vis and Photon Correlation Spectroscopy respectively, and the changes in Chemical Oxygen Demand (COD) were also determined to evaluate whether laccase could reduce the COD content.

2. Materials and methods

2.1. Chemicals and media

The azo textile dyes investigated: C.I. Reactive Yellow 107 (RY107), C.I. Reactive Black 5 (RB5), C.I. Reactive Red 198 (RR198) and C.I. Direct Blue 71 (DB71), were kindly provided by a textile company in Brazil. The structures of the dyes are shown in Fig. 1. Laccase (EC 1.10.3.2) from *Myceliophthora thermophila* (final activity 1.0 U mL^{-1}) was kindly provided by Novozymes, Denmark. The mineral salts medium (MM) at pH 7 used in all the batch experiments contained: K_2HPO_4 (1.6 g L^{-1}), KH_2PO_4 (0.2 g L^{-1}), $(NH_4)_2SO_4$ (1.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.2 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (0.01 g L^{-1}), NaCl (0.1 g L^{-1}) and $CaCl_2{\cdot}2H_2O$ (0.02 g L^{-1}). The medium was supplemented with 100 mg L^{-1} of dye, 3 g L^{-1} of glucose and 1 g L^{-1} of sodium pyruvate, and was autoclaved at 121 °C for 15 min and designated rich mineral medium (MMR). Aniline-2-sulphonic acid (95% pure) was used as a model product of azo dye reduction, was purchased from Sigma.

2.2. Strain isolation and characterization

The bacteria were isolated from the activated sludge process of a textile company. Serial dilutions (10^{-1} to 10^{-6}) of the samples collected were inoculated into Nutrient Agar Medium using the spread plate technique. Isolated strains were inoculated into MMR with a mixture of azo dyes (100 mg L^{-1}/dyes) and incubated under microaerobic conditions (O_2 limited environment) at 30 °C for 7 days. The strains that achieved the best decolorizing were selected for this study.

Identification of the isolated strains was performed using 16S rRNA gene sequence analysis. Genomic DNA was obtained according to Ausubel et al. [19]. The pure cultures were harvested at the end of the exponential growth phase by centrifugation at 18,600 × *g* for 3 min. The DNA samples obtained were re-dissolved overnight at 4 °C in 50 μL of sterile, deionized water.

The 16S rRNA gene was amplified by PCR using primers, 27f and 1401r specific for the Universal Bacteria Domain. Fifty microliter reaction mixtures were used containing 100 ng of total genomic DNA, 2 U of Taq polymerase (Invitrogen®), 0.2 mM of deoxynucleoside triphosphates and 0.4 μM of each primer. The PCR amplifications were carried out using an initial denaturation step of 2 min at 94 °C, followed by 10 cycles of 1 min at 94 °C, 30 s at 69 °C, decreasing 0.5 °C each cycle, and 3 min at 72 °C, followed by another 10 cycles of 1 min at 94 °C, 30 s at 63 °C and 3 min at 72 °C, in an Eppendorf thermal cycler (Eppendorf Mastercycler Gradient). The PCR product was purified using a GFX™ PCR DNA Kit and a Gel Band Purification kit (GE HealthCare) for automated sequencing in the MegaBace DNA Analysis System 1000. The sequencing was carried out using the 10f (5′-GAG TTT GAT CCT GGC TCA G-3′); 765f (5′-ATT AGA TAC CCT GGT AG-3′); 782r (5′-ACC AGG GTA TCT AAT CCT GT-3′) and 1100r (5′-AGG GTT GGG GTG GTT G-3′) primers and the DYEnamic ET Dye Terminator Cycle Sequencing Kit for the automated MegaBace 500 system (GE Healthcare) according to the manufacturer's instructions. Partial 16S rRNA sequences obtained from the isolates were assembled in a contig using the phred/Phrap/CONSED program [20].

Identification was achieved by comparing the contiguous 16S rRNA sequences obtained with the 16S rRNA sequence data obtained from the reference and type strains available in the public databases GenBank and RDP (Ribosomal Database Project II Release 9, Michigan State University, USA) using the BLASTn and Seqmatch, respectively. The sequences were aligned using the CLUSTAL X program and analyzed with MEGA software version 4.0 [21,22]. Evolutionary distances were derived from sequence-pair dissimilarities, calculated as implemented in MEGA using Kimura's DNA substitution model [23]. The phylogenetic reconstruction was done using the neighbour-joining (NJ) algorithm, with bootstrap values calculated from 1000 replicate runs, using the routines included in the MEGA software [24]. The 16S rRNA partial sequences determined in this study were deposited in the Genbank database.

2.3. Decolorizing assays

Decolorizing assays were performed in Erlenmeyer flasks containing 350 mL of MMR (pH 7) supplemented with 100 mg L^{-1} of the dyes. Before starting the experiment, the bacteria were grown individually in 250 mL flasks containing 100 mL of MMR containing no dye. The flasks were incubated with stirring at 150 rpm for 24 h at 30 °C, and 1% of inoculum added. The samples were incubated under microaerobic conditions at 30 °C for 168 h or until no color was observed.

Decolorization was measured in a UV–vis spectrophotometer (Shimadzu 2101) and the percentage decolorized was calculated. The samples were then frozen and freeze dried (FTS System model Dura-Dry MP).

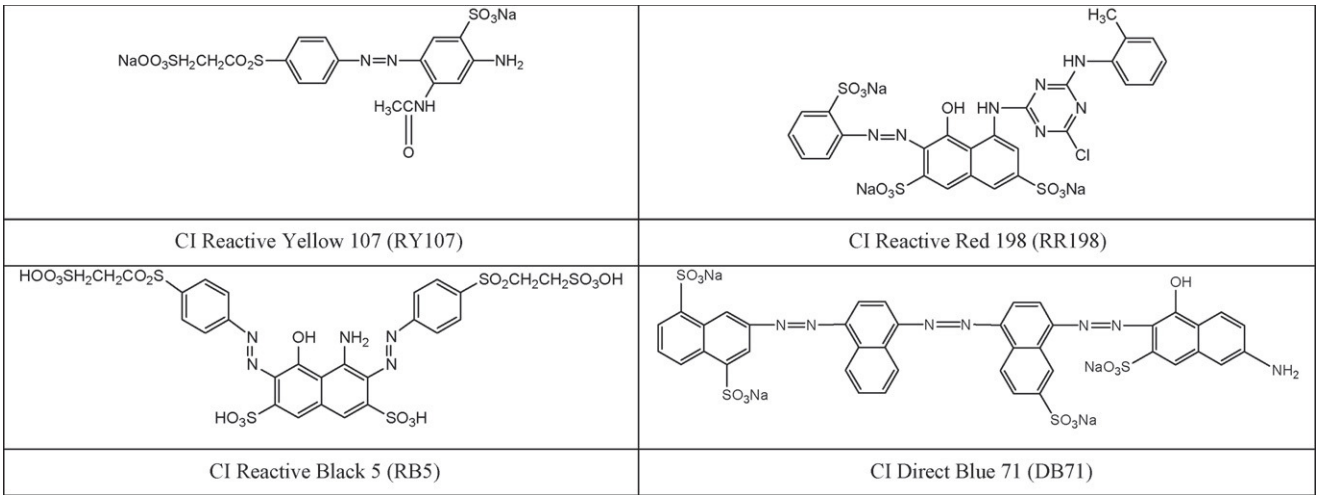

Fig. 1. Chemical dyes structures.

2.4. The detection of aromatic amines

The aromatic amines in the solid phase were determined using a modified method of Marik and Lam [25]. After incubation, samples were taken under microaerobic conditions, frozen and freeze dried (FTS System model Dura-Dry MP). A mixture of samples treated by bacteria for each dye (5 mg) was dissolved in 5 mL of a 0.4% solution of chloranil in dimethylformamide (DMF) and heated at 100 °C for 5 min. The absorption was measured in a Helios α Unicam UV–vis spectrophotometer at 560 nm. A calibration curve was prepared using aniline-2-sulphonic acid as a model product of azo dye reduction, and the sample amine concentration was calculated in mM. The control was the MMR medium containing no dye, treated with the bacteria under microaerobic conditions, and the absorption at 560 nm was subtracted from the biodegraded samples containing the dyes.

2.5. Polymerization reaction using laccase

Polymerization of the aromatic amines generated by bacterial decolorization of azo dyes was evaluated using laccase as the polymerization enzyme. Freeze dried samples from the different bacteria for a particular dye were mixed together ($3.0\,g\,L^{-1}$) and buffered with 0.1 M sodium acetate, pH 5.0 and incubated alone and with 500 μL of Novozyme 51003 *M. thermophila* laccase at 45 °C for 72 h, with agitation at 100 rpm, and the amine and polymerizatin products produced by the laccase treatment were monitored by UV–vis analysis and Photon Correlation Spectroscopy (PCS). The spectra over the range from 200 to 800 nm was determined in a J&M Tidas UV/visible spectrophotometer equipped with a diode-array recorder (J&M Analytische Mess und Regeltechnik GmbH, Germany) before and after treatment for 0, 24 and 72 h. The same experiment was performed with the non-biodegraded azo dyes. Aniline-2-sulphonic acid (ASA) (10 mM) was used separately as a model product of azo dye reduction. The reaction was carried out three times.

2.6. Photon Correlation Spectroscopy (PCS) and Chemical Oxygen Demand (COD)

The size distribution of polymerization products with and without laccase was determined at pH 5.0 (acetate buffer) at 45 °C via (PCS) analysis (Malvern zetasizer NS). Prior to the PCS measurements, the buffer was filtered through 0.2 μm Millipore membrane filters and the samples through 0.45 μm Millipore membrane filters directly into the cuvette.

To verify the reduction of organic compounds in the samples after the laccase treatment, the COD was monitored according to the bio-available ASTM 1252 standard guidelines after centrifugation (20,000 × *g* for 15 min) of the samples obtained after 0, 1 and 72 h of laccase treatment [26].

3. Results

3.1. Strain isolation and characterization

The bacterial strains VN-1, VN-15, VN-31 and VN-38 used in this study were isolated from raw effluents from a textile industry in Itatiba-Brazil, and selected based on their ability to decolorize four azo dyes ($100\,mg\,L^{-1}$ of each) in MMR in this study. The strains VN-1, VN-15 and VN-38 were identified based on 16S rRNA gene sequence analysis. The partial sequences determined in this study were deposited in the Genbank database under the accession numbers FJ598006 (*Microbacterium* sp. strain VN-1), FJ598007 (*Brevibacterium* sp. strain VN-15) and FJ598008 (*Leucobacter albus* strain VN-38). Strain VN-1 was identified as *Microbacterium* sp., supporting values of 97% and 98% on the boot strap, similar to strain types *M. resistens*, *M. thalassium* and *M. oxydans*. Strain VN-15 was phylogenetically positioned in the genus *Brevibacterium*. The nucleotide alignment of this strain supported bootstrap values of 98 and 99%, showing similarity to different *Brevibacterium* strains including the sequences of the strains *B. linens*, *B. permense*, *B. epidermidis* and *B. Iodinum* The 16S rRNA gene sequence of the VN-38 strain supported bootstrap values of 99%, showing similarity to type strain *L. albus*. The *Klebsiella* sp. strain VN-31 was previously identified according to Franciscon et al. [27].

Previous studies have shown that the strains of *Microbacterium* sp., *Brevibacterium* and *Leucobacter* sp. are able to degrade a range of aromatics and PAHs (Polycyclic Aromatic Hydrocarbons) [28–31]. In addition, Wong and Yuen isolated five bacteria from dye-contaminated sludge and found that two bacteria, identified as *Klebsiella* ssp. and *Klebsiella pneumonae*, showed decolorizing ability with respect to the azo dye Methyl Red [32].

3.2. Decolorizing assays

The bacterial strains were tested for their ability to decolorize four azo dyes (CI Reactive Yellow 107, CI Reactive Red 198, CI Reactive Black 5 and CI Direct Blue 71) under microaerobic conditions. Fig. 1 shows the UV spectra of the azo dyes before and after bacterial biodegradation and laccase treatment. After biodegradation of the four azo dyes by bacteria under microaerobic conditions, the absorbance peaks in the visible region disappeared, indicating their decolorization. Complete decolorization (>95%) of the azo dyes was achieved (Table 1) by all the bacteria. The decolorizing time showed a relationship with the chemical structure of the dyes. RY107 and RR198 are both monoazo dyes and showed relatively short decolorizing times. The increase in degradation time for RR198 was probably due to the triazine group, whose degradation is more recalcitrant than that of the benzene and naphthalene rings [33]. Dyes with simple structures and low molecular weights usually exhibited higher rates of color removal, whereas color removal was less effective with highly substituted, high molecular weight dyes [34–36]. This is consistent with our results with the highly substituted diazo RB5 dye and the triazo DB71 dye which showed longer decolorizing times than the monoazo dyes. Moreover, steric hindrance by substitution at the *ortho* and *ortho–para* positions of the azo bond decreased the decolorizing performance. In addition, further substitution in the proximity of the azo linkages has been shown to decrease the efficiencies of biodecolorization [37,38]. It has also been reported that the presence of sulphonates in reactive dye structures could result in low levels of color removal. However, this was not applicable to the azo dyes used in this study, which exhibited high levels of color removal, independent of the number of sulphonate groups in the dye structure.

The reductive cleavage of the –N=N– bond is the initial step in the bacterial degradation of azo dyes [39]. Decolorizing of azo dyes occurs under aerobic, anaerobic and microaerobic conditions. There are few bacteria that are able to grow on azo compounds as the sole carbon source. These bacteria reductively cleave the azo bonds and use the amines as their source of carbon and energy [40,41]. Reduction under anaerobic or microaerobic conditions is the most frequently reported form of azo dye degradation, where the dye probably acts as an electron acceptor in the electron transport chain. Recent studies have also shown that the addition of redox mediators to anaerobically incubated cultures of various taxonomically different bacterial species, could result in significantly

Table 1
Decolorization (%) ± S.D.[a] and time decolorization (h) in the samples with azo dyes treated with bacteria in microaerophilic conditions.

Strain	Decolorization (%)				Time decolorization (h)			
	RY107[b]	RB5[b]	RR198[b]	DB71[b]	RY107[b]	RB5[b]	RR198[b]	DB71[b]
VN-1	96 ± 0.2	96 ± 0.6	96.8 ± 0.2	91 ± 0.4	72 ± 4	168 ± 5	120 ± 3	168 ± 6
VN-15	95 ± 0.2	97.6 ± 0.2	95 ± 0.3	95 ± 0.7	96 ± 4	168 ± 8	120 ± 4	168 ± 5
VN-31	100 ± 0.1	94 ± 0.3	98 ± 0.3	94 ± 0.2	72 ± 3	120 ± 6	72 ± 3	144 ± 5
VN-38	100 ± 0.1	100 ± 0.3	98 ± 0.3	97 ± 0.2	48 ± 3	168 ± 6	144 ± 3	168 ± 8

[a] S.D.: standard deviation.
[b] Dyes.

Table 2
Amine concentration (mM) ± S.D. in the mix solution enclosed the biodegradated azo dyes by bacteria in microaerophilic conditions.

Dyes	Aromatic amines (mM) after microaerophilic conditions
RY107	0.48 ± 0.1
RB5	0.26 ± 0.03
RR198	0.38 ± 0.1
DB71	n.d.

n.d.: not detected.

increased reduction rates of azo dyes [42]. Pure bacterial strains, including *Pseudomonas luteola*, *Sphingomonas* sp., *Staphylococcus arlettae* and *Klebsiella* sp. have been described as being capable of reducing azo dyes under anaerobic and microaerobic conditions [28,38,43,44].

3.3. Detection of aromatic amines

The production of aromatic amines from azo dyes is indicative of the reduction of the azo bond. The characteristic absorption peak of the hydrogenated azo bond structure (Ar···NH···NH···Ar) at 245 nm also decreased for all the dyes indicating the disruption of the azo bonds [48]. The decrease in absorbance of the peak at 320 nm, which is related to the naphthalene rings, from the UV spectra for RB5, RR198 and DB71, and the formation of a new peak at 280 nm for all dyes, suggested an increase in the concentration of single ring aromatic amines [47,49]. All the decolorized dye media showed the presence of aromatic amines after the bacterial treatment, with the exception of DB71, for which the measurement could not be made due to interference by the chemical structure of this dye with the methodology used (Table 2). The concentrations of aromatic amines determined correlated with the dye structure and the decolorizing time. RY107 and RR198 are both monoazo dyes and showed relatively short decolorizing times and higher amine concentrations (0.48 and 0.38 mM), respectively. The highly substituted diazo dye RB5 showed a longer decolorizing time and the lowest amine concentration (0.26 mM). Recent reports showed that the monoazo dye (Acid Orange 7) was rapidly decolorized by anaerobic cultures, and the presence of aniline (metabolite from the reductive degradation of azo dyes) was observed [45]. Also, in a pathway proposed for the biodegradation of Reactive Red 2 by *Pseudomonas* sp. SUK1, the aromatic amine aniline was one of the intermediate products originating from the biodegradation reaction [46].

3.4. Polymerization reaction using laccase

The feasibility of oxidative coupling between aromatic amines mediated by oxidoreductive enzymes for use in remediation of environmental pollution has been described [47]. Moreover recently, the one-step, simple and environmentally friendly enzymatic synthesis of polymers derived from aromatic amines has been developed [19].

As shown in Fig. 2, after 72 h of laccase treatment the UV–vis spectrum showed considerable changes. A general increase in absorbance throughout the entire visible spectra was also observed due to the formation of colored and precipitated products. In particular peaks at 330 and 360 nm became prominent. The formation of oligomeric or polymeric products as a result of coupling reactions between intermediates of the dye degradation caused by the laccase oxidative process, have been described earlier [50,51]. In addition the absorption band at 360 nm has been extensively described and ascribed to electron transitions in the aromatic π–π^* rings, on the basis of earlier studies on enzymatic polymerization of aromatic amines such as aniline [52]. The peak at 330 nm shown in the UV–vis spectra can be attributed to a reduction by O_2 caused

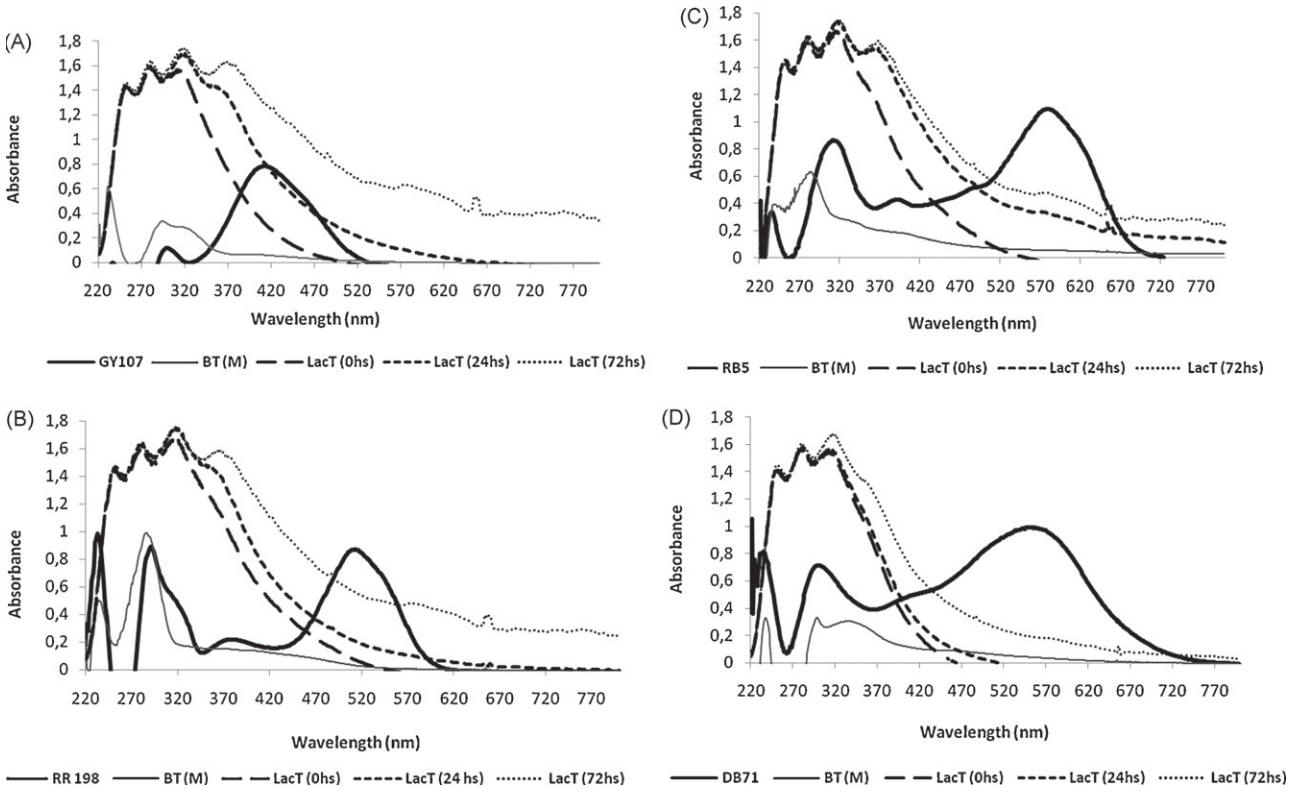

Fig. 2. UV–vis spectra of the azo dyes before (bold straight line) and after bacterial (straight line) and laccase (dashed and dotted line) treatments. (A) RY107; (B) RB5; (C) RR198; (D) DB71. BT (M): bacterial treatment in microaerobic conditions; LacT: laccase treatment in 0, 24 and 72 h.

Table 3
Photon Correlation Spectroscopy (nm) ± S.D. and Chemical Oxygen Demand (%) ± S.D. in the solution enclosed the biodegradated azo dyes by bacteria in microaerobic conditions.

Biodegradated dyes	Photon Correlation Spectroscopy (nm)		Chemical Oxygen Demand (%)
	Before laccase	After laccase	After laccase
RY107 (M)	76.91 ± 1.5	483.9 ± 5.6	12.3 ± 2
RB5 (M)	86.33 ± 1.3	165.2 ± 4.8	24.8 ± 4
RR198 (M)	79.64 ± 1.5	356.3 ± 6.3	15.6 ± 2
DB71 (M)	87.03 ± 1.9	105.2 ± 5.9	25.0 ± 3

by the laccase, and probably involves changes in the coordination geometry of at least one of the copper sites in the enzyme molecule [53]. An earlier study reported that when O_2 reacted with partially reduced laccase, extra absorbance at 330 nm was observed [54]. Confirming the present findings, a recent study about biotransformation of the azo dye Sudan Orange during enzymatic treatment with laccase, showed that the peak originally giving maximum absorption for the dye decreased, and concomitantly an increase in the absorption intensities at 325 and 530 was observed and associated with the generation of biotransformation products such as an extensive array of oligomeric products [55].

After laccase treatment, the spectrum of the model compound aniline-2-sulphonic acid exhibited the peaks characteristic of the enzyme and also a new peak at 360 nm (see discussion above). In the visible region the spectrum showed an increase in the regions from 400 to 460 nm. Moreover after laccase treatment, a green coloration was observed, different from the color of the solutions of the biodegraded dyes treated with laccase, which showed a light brown coloration.

The laccase enzyme in buffer, and the control runs in the absence of laccase, gave no evidence of the formation of colored or precipitated products in the solutions.

To evaluate if the laccase from *M. thermophila* used in the present study was able to decolorizing the azo dyes, the control dye solutions were treated with laccase in MMR. The UV–vis spectroscopy was not significantly modified, and showed that the azo dyes were not decolorized by the laccase treatment.

3.5. Photon Correlation Spectroscopy (PCS) and Chemical Oxygen Demand (COD)

The size of the products generated after polymerization by the enzyme laccase, were analyzed by the PCS technique.

The size profiles of the particles showed an increased after laccase treatment. The analysis showed that the particle size of the products formed in the solutions varied from 105 to 483 nm (Table 3). The size of the particles from the monoazo dyes GY107 and RR 198 (483.9 and 356.3 nm) were much greater than those from the larger and more complex diazo dyes RB5 and triazo DB71 dyes (165.2 and 105.2 nm). These results are in agreement with the amount of aromatic amines formed, since the dyes GY107 and RR 198 produced a greater quantity of aromatic amines than RB5, indicating a relationship between the quantity of aromatic amines produced and the particle size.

Table 3 shows the reduction in COD of the solution after laccase treatment. The tests were carried out using the supernatants of the solutions immediately after the addition of laccase (0) and after 1 and 72 h of laccase treatment. When the solutions were treated with laccase, the COD was reduced relative to the untreated solution by approximately 12–15% for RY 107 and RR 198 and 25% for RB5 and DB 71 after 72 h as compared with the first hour of treatment.

These results are associated with the formation of highly insoluble products. At the start of the laccase treatment (1 h) these polymeric products had not been generated significantly, large particle sized products were obtained only after treatment for 72 h, which could be removed from the samples in the form of a precipitate by centrifugation [56].

4. Conclusion

In conclusion the bacterial strains VN-1, VN-15, VN-31 and VN-38, isolated from the textile effluent and identified by 16S, were able to completely decolorized the azo dyes under microaerobic conditions. The formation of amines during the bacterial treatment was confirmed by direct measurement and by the UV–vis analysis. Over 72 h of laccase catalyzed polymerization of the aromatic amines present in the solutions polymerization products precipitated spontaneously from the solution and acquired some color, as shown by the UV–vis analysis. The particle size was also significantly higher after laccase treatment. In addition the laccase treatment reduced the COD of the solutions.

The reaction mechanism of laccases, especially the role of the metalic center, remains unknown. However, according to one model [57], laccase oxidizes the phenolic group of azo dyes or phenolics amines derived from them, with the participation of one electron generating a phenoxy radical followed by oxidation to a carbonium ion which can go on to form quinones after nucleophilic attack by water on the phenolic ring carbon bearing the azo linkage. Quinones can form stable structures by addition reactions with the radicals in the reaction with the formation of the coupling products or further polymerization.

The most effective treatments with respect to color removal for the textile effluents are anaerobic and microaerobic processes. Unfortunately they produce carcinogenic aromatic amines and are thus inadequate for the removal of organic matter. The present study subsequently showed the possibility of removing the aromatic amines using enzymatic polymerization with laccase. In addition, the possibility of obtaining polymers from the aromatic amines produced during the treatment of the effluent, in a simple and environmentally friendly way opens the possibility of developing and economic ways for reusing the effluent.

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