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Research article

Biological decolorization of Amaranth dye with *Trametes polyzona* in an airlift reactor under three airflow regimes

R. Pérez-Cadena [a], Y. García-Esquivel [b], Y.E. Castañeda-Cisneros [b], M.G. Serna-Díaz [c], M.R. Ramírez-Vargas [b], C.R. Muro-Urista [d], A. Téllez-Jurado [b,*]

[a] *Ingeniería en Energía, Universidad Politécnica Metropolitana de Hidalgo, Boulevard Acceso a Tolcayuca No. 1009, ExHacienda de San Javier, C.P. 43860, Tolcayuca, Hidalgo, Mexico*

[b] *Laboratorio de AgroBiotecnología, Universidad Politécnica de Pachuca, Carretera Pachuca-Cd. Sahagún, km 20, ExHacienda de Santa Bárbara, C.P. 43830, Zempoala, Hidalgo, Mexico*

[c] *Departamento de Ingeniería, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca-Tulancingo, km 45, Col. Carboneras, C.P. 42184, Mineral de la Reforma, Hidalgo, Mexico*

[d] *Departamento de Posgrado e Ingeniería Química, Instituto Tecnológico de Toluca, Av. Tecnológico s/n, Col. Agrícola Bellavista, C.P. 52149, Metepec, Estado de México, Mexico*

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ABSTRACT

In the present work, a strain of the basidiomycete fungus *Trametes polyzona* was used to decolorize the Amaranth dye. The decolorization was carried out in an Airlift reactor with three flow regimes: 1, 2, and 3 vvm. The results showed that the decolorization was a function of the flow regime. The decolorization times for the regimes of 1, 2, and 3 vvm were 30, 25, and 19 days, respectively. The COD (Chemical Oxygen Demand) decreased from 1600 to 72 mg COD/L. The enzymatic activity kinetics of laccase (Lcc), lignin peroxidase (LiP), and manganese peroxidase (MnP) were determined. In all the treatments, the enzyme LiP was expressed during the first 6 days, at which point 80% decolorization was observed, whereas Lcc and MnP enzymes were produced from day 6 until the end of the decolorization process. The effluent generated showed no inhibitory effects on the growth of the algae *Nannochloropsis salina*. *T. polyzona* showed great versatility in the decolorization of synthetic effluents containing the Amaranth dye, and the fungus was able to use this dye as its only carbon source starting at the beginning of the process. LiP was the enzyme that contributed the most to the decolorization process, and on average, 95% decreases in color and the COD were observed.

1. Introduction

The textile industry is characterized by generating large volumes of effluents highly contaminated with dyes and additives, and many of the dyes are considered dangerous for human and animal health [1, 2]. Textile effluents, when not properly treated and discharged into rivers, affect aquatic flora and fauna because they block sunlight, which prevents the photosynthetic processes of the flora trap dissolved oxygen in water due to their high COD levels, which further aggravates the problem of contamination [3, 4]. The chemical treatment of textile effluents involves physicochemical processes such as filtration, adsorption, coagulation, photodegradation, and precipitation, which mainly require many resources, time, and disposal of the obtained sludge [5]. The biological treatment of textile effluents has been carried out in two ways: using anaerobic systems with bacteria consortia and using aerobic systems that generally consist of filamentous fungi. The use of bacterial consortia for the decolorization of textile effluents is widespread; however, given the reaction conditions where reducing conditions prevail, products derived from amines can be generated, which results in a greater contaminating risk [6]. The use of filamentous fungi, specifically basidiomycete fungi, presents several advantages because these organisms carry out oxidation reactions that favor the removal of dyes without generating toxic compounds. Basidiomycete fungi can simultaneously carry out textile dye degradation processes in two ways: using extracellular enzymes and bioadsorption when the mycelium is present in the effluent [7]. The high efficiency of basidiomycete fungi in degrading textile dyes is due mainly to their enzymatic ligninolytic complex composed of laccase (Lcc), lignin peroxidase (LiP), and manganese peroxidase (MnP). These enzymes have

* Corresponding author.
E-mail address: alito@upp.edu.mx (A. Téllez-Jurado).

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high redox potential, and their ligninolytic capacity is enhanced in the presence of oxygen [8]. A great diversity of fungi, such as *Bjerkandera adusta* [9], *Coriolus* sp. [7, 10], *Pleurotus ostreatus* [11], *Phanerochaete* sp. [12, 13], *Trametes versicolor* [14], *Trametes trogii* [15] and *Trametes hirsuta* [16], has been used for decolorization processes of textile dyes. *T. polyzona* is a white-rot basidiomycete fungus with the ability to secrete enzymes such as Lcc, LiP, and MnP, which are the enzymes to which the ability to degrade dyes of textile origin has been attributed [17]; thus, its study is of interest for the development of wastewater treatment processes of textile origin. The present work evaluated the ability of *T. polyzona* to decolorize azo Amaranth dye. Three levels of aeration were tested using an airlift reactor, and the Amaranth dye was included as the only carbon source. We determined the decolorization kinetics, the decrease in the COD, and the production of ligninolytic enzymes (Lcc, LiP, and MnP) over 30 days.

2. Materials and methods

2.1. Strain propagation and maintenance

The basidiomycete fungus *Trametes polyzona* [18] used in this study was isolated from Huejutla, Hidalgo, Mexico (21°08′32.38″ N, 98 °26′28.39″ W). The strain was grown on Petri dishes with sterile potato dextrose agar (PDA) for 7 days at 37 °C and was kept refrigerated (4 °C) until use. The strain was replanted every month to maintain its viability.

2.2. Preparation of the preinoculum

For preparation of the preinoculum, a plate in which the fungus was grown for 7 days was cut into small agar squares (0.5 cm^2) using a sterile blade under aseptic conditions, and the squares were added to a 250-mL Erlenmeyer flask containing sterile water. The flask was kept under stirring (150 rpm) for 12 h at room temperature. The obtained supernatant, which contained the mycelium detached from the agar, was used as the inoculum for the decolorization treatments. The preinoculum was standardized to one Petri dish in which the fungus was grown for 7 days for every 100 mL of sterile water.

2.3. Inoculation of the airlift reactor

An Airlift Reactor (SEV) instrumented with dissolved oxygen, pH, and temperature sensors, as well as a rotameter, was used to measure the airflow. The reactor had a height of 6.5 cm and an internal diameter of 12.5 cm, and the inner tube had a height of 40 cm and an internal diameter of 7 cm. The reactor containing 3.6 L of the defined chemical medium described by Kirk and Farrel [19] without glucose was sterilized for 20 min under 15 lb of pressure before inoculation. Once the reactor reached room temperature, a solution of the filtered Amaranth dye was added through a 0.2-μm nylon membrane until a dye concentration of 100 mg/L was reached inside the reactor. Subsequently, the inoculum was added to a final working volume of 10%.

2.4. Decolorization tests

To carry out the decolorization tests, the sterile and inoculated Airlift reactor was incubated at 37 °C for 30 days; during this process, the concentration of the Amaranth dye (CI 16185) (100 mg/L) and the inoculum concentration were maintained constant, and the aeration conditions were varied (1, 2 and 3 vvm). Three milliliters of medium was collected daily and used to determine the decoloration, the enzymatic activities of Lcc, LiP and MnP, the COD, and the extracellular protein concentration. All the treatments were performed in triplicate.

2.5. Decolorization curve

A dye stock solution (500 mg/L) was prepared and subjected to serial dilutions, and the absorbance of each dilution was read with a spectrophotometer (BioMate) at 521 nm. Using the obtained data, a calibration curve was constructed to determine the percentage of decoloration.

2.6. Determination of enzymatic activities

2.6.1. Lcc activity determination

The method described by Bourbonnais et al. [20], which is based on determining the oxidation of ABTS by the action of the Lcc enzyme for 1 min by measuring the absorbance of 420 nm, was used.

2.6.2. LiP activity determination

The method reported by Maganhotto et al. [21], which is based on the production of veratraldehyde through the oxidation of veratryl alcohol by the enzymatic action of LiP, was used. The enzyme activity was read using a spectrophotometer (BioMate) at 310 nm.

2.6.3. MnP activity determination

To determine the activity of MnP, the method described by Kuwahara et al. [22] was used. This method is based on the oxidation of phenol red by the enzymatic action of MnP in the presence of peroxide. The enzymatic activity was read using a spectrophotometer (BioMate) at 610 nm.

2.6.4. COD determination

The COD was determined with a commercial kit (HACH) using tubes, a high COD range, and a spectrophotometer (HANNA).

2.6.5. Determination of the extracellular protein concentration

The method described by Bradford [23] was used to estimate the concentration of extracellular protein generated during the decolorization process. The protein concentration was used to estimate the specific activity unit (SAU) as the activity unit/mL·μg protein.

2.7. Dye adsorption

To estimate the amount of dye adsorbed by the biomass, the method reported by Medina-Moreno et al. [24], which estimates the adsorption isotherms between the inert biomass and the dye present in the medium, was applied.

2.8. Phytotoxicity determination

To assess the effluent toxicity generated by the decolorization processes, the survival of the algae *Nannochloropsis salina* was determined. The effluent was supplemented with NaCl (4%), and the growth conditions were those described by Sáenz et al. [25]. Every 24 h, a sample was collected, and the cell density was read at 560 nm for 5 days.

2.9. Data analysis

The statistical software SPSS (v 20.0) was used to analyze the data obtained. Single-factor ANOVA and mean comparison tests (Tukey and Duncan) with a significance level of 0.05 were used.

3. Results and discussion

3.1. Relationship between decolorization and the COD

In the first stage of the work, the effect of aeration on the COD was assessed. The results showed that the decolorization process was a function of aeration. With an aeration of 1 vvm, the decolorization time was 30 days (Figure 1A), and the fastest decolorization rate was detected until day 12, when the decolorization reached 81%. On day 30, the decolorization was 100%. The application of 2 vvm reduced the decolorization time to 25 days (Figure 1B); during the first 6 days, a decolorization percentage of 61% was reached, and this process was then

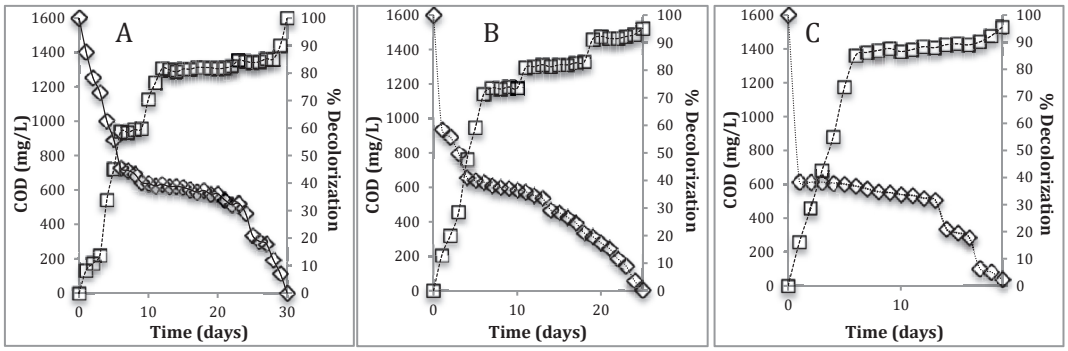

Figure 1. The decolorization process of the Amaranth dye related to the decrease of COD at the three airflow regimes tested. A = 1 vvm; B = 2 vvm; C = 3 vvm.

continued until 95% decolorization was reached on day 25. With 3 vvm, the maximum decolorization time was 19 days (Figure 1C); on day 6 of the process, a decolorization of 85% was achieved; from days 7–19, the decolorization increased by 10%, and the maximal decolorization percentage on day 19 was 95%. The decolorization was compared to the decrease in the COD. In the treatment with 1 vvm, a 52% decrease in the COD was reached on day 6 (Figure 1A), which corresponded to a COD consumption rate of 138.9 mg COD/L·day, and the final COD was 72 mg COD/L, which corresponds to a total decrease of 95.2%. When 2 vvm was applied, 50% of the COD decrease was observed over the first 4 days of the process (Figure 1B), corresponding to a COD consumption rate of 202.5 mg COD/L·day, and the COD at the end of the process was 72 mg COD/L, which represents a total decrease in the COD of 95.4%. In the treatment with 3 vvm, a 61% decrease in the COD was observed over the first 24 h (Figure 1C), which corresponded to a COD consumption rate of 985 mg COD/L·day. In this treatment, the minimum COD value of 59 mg COD/L was detected at 19 days, which corresponded to a total decrease in the COD of 96.2%.

Textile effluents are characterized by high BOD and COD levels, which are mainly due to azo compounds and other additives [26]. A decrease in the COD of textile effluents is essential because these effluents reportedly contain recalcitrant compounds that can cause serious problems to human health and the environment [27]. A wide variety of genera of basidiomycete fungi and bacteria have been used to treat textile effluents. Agrawal et al. [28] used the Enterobacteria *Providencia* sp. SRS82 for the degradation of Acid Black 210 dye and observed a 69% decrease in the COD. In contrast, Wu et al. [29] tested five basidiomycete fungi to decolorize black liquors from the paper industry and observed that *T. versicolor* decreased the COD by approximately 30% after 16 days of treatment. Navada et al. [30] observed a decrease in the COD of 60% in solutions with Remazol Brilliant Blue R using the fungus *Phomopsis* sp.. Shaheen et al. [31] used *Ganoderma lucidum* to decolorize effluents containing five Sandal textile reactive dyes and observed up to 95% decolorization with a 70% decrease in the COD. Gajera et al. [32] used *Hypocrea koningii* fungi to decolorize azo dyes from the textile industry and observed 50% decolorization after 12 days of incubation in a medium supplemented with peptone and a COD reduction of 63% under the optimal conditions studied. Anastasi et al. [9] used a strain of *B. adusta* to decolorize effluents obtained from a textile company supplemented with mineral medium, yeast extract, and glucose; these researchers used a series reactor system for 70 days and observed 85% decolorization at the end of the process and a COD reduction of 69%. The results have shown that the decolorization process is associated with a decrease in the COD and depends on the basidiomycete fungus used for the process. The results obtained in the present study using *T. polyzona* show its higher efficiency in both decolorization and reducing the COD compared with that reported by other authors, and the best treatment was obtained with this fungus and an airflow of 3 vvm, which reduced the decolorization time from 30 (1 vvm) to 19 days. Another clear advantage of using *T. polyzona* is the fact that this fungus can grow in medium containing amaranth dye as the carbon source, and it was only necessary to supplement the medium with mineral salts.

3.2. Effect of aeration on the ligninolytic enzymes activity

In the next stage, the action of the main ligninolytic enzymes (Lcc, LiP, and MnP) was related to the Amaranth dye decolorization process and the different levels of aeration tested (1, 2, and 3 vvm). During the treatment with 1 vvm (Figures 2A, 3A and 3B), the highest observed enzymatic activities were observed in the first days of culture: for LiP (Fig 3A), 19 SAU was observed within the first 24 h of growth, and 38 SAU of MnP (Figure 4A) was detected after 7 days of growth. Lcc activity showed a continuous increase after 30 days of culture, but low levels were detected throughout the process (Figure 2A). LiP was produced at greater quantities in the first 5 days of culture; subsequently, the activity of this enzyme remained at the basal concentrations until the end of the culture period (30 days). In contrast, an increase in the activity of MnP was observed until day 7, and the activity then gradually decreased until the end of the process. The analysis of the production of ligninolytic enzymes during the decolorization process showed that both LiP and MnP are the main enzymes responsible for this process because 80% of the decolorization occurs in the first 12 days of the process, at which time the highest activity of these two enzymes was detected. The participation of the Lcc enzyme was minimal under these growth conditions (1 vvm).

The first impact of the treatment with an airflow of 2 vvm (Figures 2B, 3B and 4B) was a decrease in the decolorization time of 5 days; specifically, the decolorization time was reduced from 30 (the time obtained with an aeration rate of 1 vvm) to 25 days. As under an aeration condition of 1 vvm, LiP was the first enzyme detected. The highest activity of LiP (17 SAU) was observed on the third day of fungal growth (Figure 3B); at later time points, the activity declined until day 25 of the process, but the enzymatic activity throughout the process was higher than that obtained with the previous treatment. For MnP (Fig 4B), a continuous increase in activity to 30 SAU was observed until day 12, and the enzymatic activity of the enzyme then declined until the end of the process. The Lcc activity (Figure 2B) does not exhibit homogeneous behavior but rather showed three maximal activities mainly after more than 70% decolorization was reached. Unlike the results obtained for the first condition studied (1 vvm), the decolorization achieved with this second treatment is mainly due to LiP and, to a lesser degree, MnP. According to the results obtained, LiP is the first enzyme detected during the process, which is the time when the decolorization rate is higher (first 6 days of the decolorization process).

In the third treatment, the aeration rate was increased to 3 vvm (Figures 2C, 3C and 4C), and a decrease of 6 days (from 25 to 19 days) in the decolorization time was achieved with this treatment compared with that obtained with 2 vvm. Consistent with the results obtained with the two other treatments, LiP was the first enzyme during the first days of culture (Figure 3C). Nevertheless, unlike the results obtained with first two conditions tested, the decolorization process with an aeration rate of

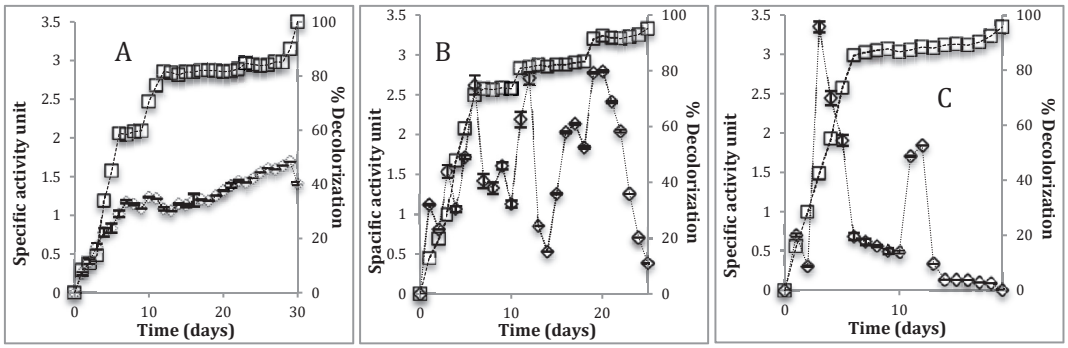

Figure 2. Production of Lcc enzyme produced during the decolorization of the Amaranth dye a three airflow. A = 1 vvm; B = 2 vvm; C = 3 vvm. ⋄ = Specific activity unit of Lcc; □ = Decolorization.

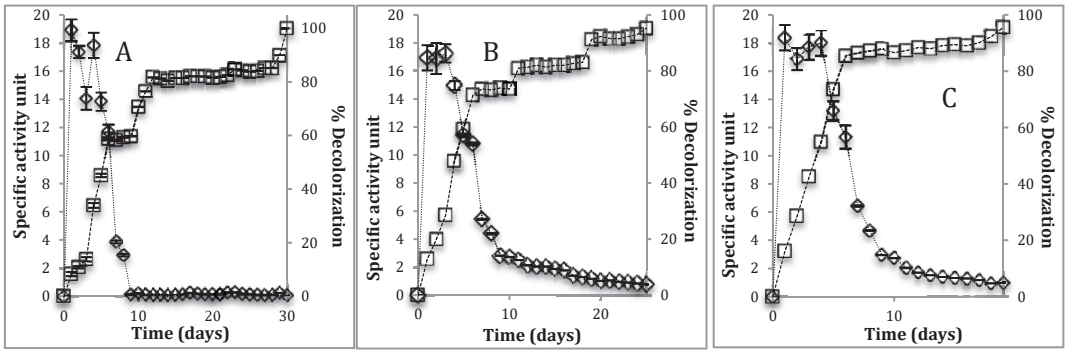

Figure 3. Production of LiP enzyme produced during the decolorization of the Amaranth dye a three airflow. A = 1 vvm; B = 2 vvm; C = 3 vvm. ⋄ = Specific activity unit of LiP; □ = Decolorization.

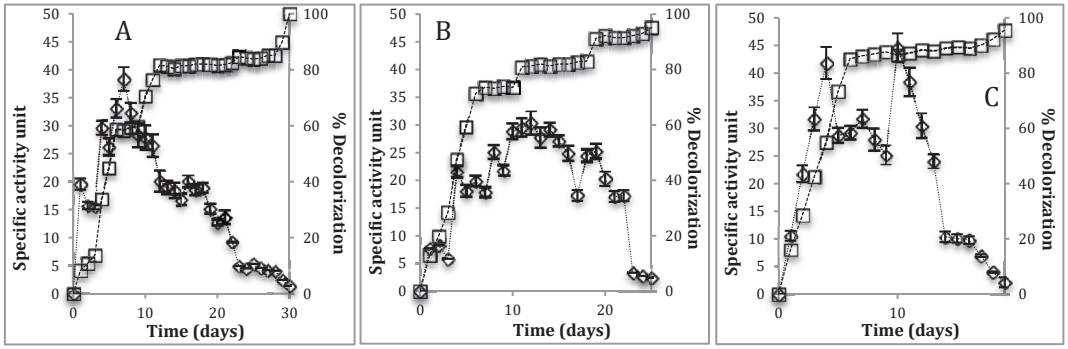

Figure 4. Production of MnP enzyme produced during the decolorization of the Amaranth dye a three airflow. A = 1 vvm; B = 2 vvm; C = 2 vvm. ⋄ = Specific activity unit of MnP; □ = Decolorization.

3 vvm was controlled by the three enzymes because all three enzymes were detected on day 6, at which point the highest decolorization speed of 85% was reached; however, the enzymatic Lcc activity remained very low but slightly higher than those observed in the two other treatments (Figure 2C). During the treatment with 3 vvm, MnP played an important role in the decolorization process, as demonstrated by its detection throughout the process; specifically, two peak MnP levels were detected (on days 4 and 10), and the activity then decreased until the end of the process (Figure 4C).

According to the observations, *T. polyzona* is capable of oxidizing the azo bonds present in dyes, and this activity is mainly due to the secretion of polyphenol oxidase (Lcc) and phenol oxidase (LiP and MnP) enzymes [33]. *T. polyzona* is mainly a producer of phenoloxidase enzymes such as LiP and MnP and, to a lower extent, of polyphenol oxidase enzymes such as Lcc. This oxidation process has many advantages over the reductive process used by bacterial consortia to generate more toxic products than the dye itself. Basidiomycete fungi change their metabolism depending on their carbon source, nitrogen, agitation, pH, and presence of salts, among other variables [34]. Aeration can influence the growth of biomass and increase metabolism; in response to favorable conditions, the fungus can produce large amounts of enzymes whose function is to break the intermediate molecules. The biomass increase might be related to removal of the dye. The presence of oxygen improves the oxidative process, as has been observed in decolorization processes with *T. versicolor*, where high agitation favors the decolorization of azo dyes; similar results have been reported with the fungi *Trametes villosa* and *Pleurotus sanguineus*, which improve the decolorization of the bright blue Drimaren dye in systems with agitation [35]. Other researchers have tested various conditions with agitation and without agitation during the decolorization process; for example, Jarosz-Wilkolazka et al. [36] observed that the decolorization of the Basic Blue 22 and Acid Red 183 dyes by *Bjerkandera fumosa*, *Kuehneromyces mutabilis*, and *Stropharia*

rugosoannulata was imperceptible under static cultivation conditions, whereas agitated culture conditions, which are characterized by an increased dissolved oxygen, increase the decolorization process. In contrast, the presence of the three ligninolytic enzymes during the decolorization of the dyes is sometimes essential for obtaining high decolorization percentages. Selvam et al. [37] indicated that the presence of Lcc, LiP, and MnP considerably improved the decolorization of textile dyes when these enzymes are used individually. It has also been described that complex molecules are, in principle, more difficult to degrade, and thus, all enzymatic battery fungi are needed for decolorization [38]. The results obtained show that greater aeration results in more efficient participation of ligninolytic enzymes, which improves the decolorization time by *T. polyzona*, and aeration likely improves electron transfer by accelerating the reduction of the mediator molecules responsible for the oxidation of dye molecules. Therefore, aeration favors oxidative conditions, which is reflected in increases in ligninolytic enzymes and enzymatic activity.

The basidiomycete fungus *T. polyzona* used in this work was capable of decolorizing the Amaranth dye. Other genera of basidiomycete fungi have been used to develop decolorization processes for various textile dyes. For example, Chagas and Durrant [39] used the fungi *Phanerochaete chrysosporium* and *Pleurotus sajor-caju* to decolorize the Amaranth dye and found that the use of these two fungi resulted in 98.5% and 97% decolorization at 7 and 4 days, respectively, which are similar to the results obtained in this work. Ligninolytic enzymes play a vital role in the decolorization of textile effluents. *T. polyzona* can secrete the three main ligninolytic enzymes at different stages of the decolorization process. Each ligninolytic enzyme plays a different role in the decolorization process. The activity of the enzyme Lcc, as has been described during the decolorization of the Amaranth dye by *P. sajor-caju*, actively participates in association with β-glucosidase, which generates the peroxide necessary for the action of phenoloxidase enzymes [39]. The *T. versicolor* Lcc enzyme can decolorize Orange 2 and Acid Orange 6 with 73% and 45% efficiency, respectively [14]. *T. hirsuta* Lcc has been used for the decolorization of Acid Red 97 and Acid Green 26, and an average decolorization of 90% was observed [16]. Mechichi et al. [15] reported that *T. trogii* Lcc was able to decolorize Remazol Bright Blue R solutions and reached 97% efficiency after 12 h of reaction. Das et al. [11] reported a decolorization of 36.8% Congo red solutions using *P. ostreatus* Lcc. Yavuz et al. [40] used the partially purified Lcc of *Ceriporiopsis subvermispora* to decolorize indigo carmine effluents and obtained close to 95% decolorization. Selvam et al. [37] carried out a decolorization study of the dyes Orange G, Congo red, and Black amido 10B and observed that *Thelephora* sp. Lcc was the main enzyme responsible for the decolorization.

LiP is not a substrate-specific enzyme; it has a high redox potential, and aeration conditions can favor its catalytic activity [41]. Chagas and Durrant [39] observed high enzymatic activities of LiP during the decolorization of Amaranth by *P. sajor-caju*. Oliveira et al. [42] used the LiP produced by *P. ostreatus* and *G. lucidum* to decolorize Remazol Brilliant Blue and achieved 80% decolorization. Qiu et al. [43] used the LiP produced by *P. chrysosporium* to decolorize solutions with fuchsin, rhodamine B, and pyrogallol red and found that the enzyme exhibited decolorization efficiencies of 87.2, 75.5, and 84.6% for pyrogallol red, rhodamine B and fuchsin, respectively. Selvam et al. [37] achieved 13.51% decolorization of Orange G with the LiP of *Thelephora* sp. The results obtained in the present work reveal that the *T. polyzona* LiP enzyme was the most important in the decolorization of Amaranth dye; in all the treatments, this enzyme was produced during the first days (5–6 days), when the highest decolorization percentages were observed.

In addition to Lcc and LiP, MnP is the other ligninolytic enzyme involved in decolorization processes. MnP has high redox potential, and high aeration conditions favor its catalytic activity [44]. The MnP of *Phanerochaete* sp. can decolorize Congo red solutions with 95% efficiency. Chagas and Durrant [39] noted that high proportions of MnP and other enzymes belonging to the ligninolytic enzyme complex of β-glucosidase are responsible for the rapid decolorization of the Amaranth dye. It has also been reported that the presence of consortia can produce MnP, as reported by Yang et al. [45]. Since this enzyme was produced at higher amounts in cocultures of *Trametes* and *Chaetomium*, this enzyme decolorized Cresol Red with 93% efficiency after 6 days of incubation. Selvam et al. [37] used the MnP of *Thelephora* sp. for Orange G decolorization and observed 10.8% efficiency. The results obtained with the three conditions used in this study indicated that decolorization is related to aeration; specifically, a greater aeration is associated with a shorter decolorization time. The treatments with 2 and 3 vvm achieved 95% decolorization, whereas an aeration of 1 vvm resulted in 100% decolorization but after a longer decolorization time of 30 days. The results indicate that the LiP enzyme was the main enzyme responsible for the decolorization of the Amaranth dye because it was detected at the beginning of the process independently of the aeration condition. Lcc and MnP complement this process. Importantly, *T. polyzona* secretes the three ligninolytic enzymes without the inclusion of an additional carbon source to the decolorization medium and can efficiently use the dye as its only carbon source without any growth inhibition being observed.

3.3. Physicochemical analysis and toxicity of the effluents

The dissolved oxygen, pH, and extracellular protein were measured during the process. The dissolved oxygen concentration showed no variation among the treatments tested and was always maintained at 100%. With respect to the pH, the initial pH in all the treatments was 6.3; at the end of the first treatment (1 vvm, 30 days), the pH was 8.6, and similar behavior was observed with the other treatments (2 and 3 vvm). The results obtained from the measurements of the extracellular protein concentration are presented in Table 1. The highest final protein concentration was obtained with 1 vvm; specifically, 21 μg/mL protein was detected after 30 days. The treatment with 2 vvm resulted in a final protein concentration of 17 μg/mL, and the treatment with 3 vvm yielded a protein concentration of 14.5 μg/mL on day 19. The measurement of a lower extracellular protein at the end of the treatment with the highest aeration condition might suggest that the high oxidation conditions favor the enzymatic action of ligninolytic enzymes, as observed in this work.

Adsorption tests indicated that the *T. polyzona* mycelium could adsorb 11.9% of the dye in solution, which implies that decolorization is carried out by extracellular enzymes and by enzymes associated with the mycelium (Figure 5). On the other hand, the results obtained from the growth of the algae *Nannochloropsis salina* using the effluents resulting from the decolorization process supplemented with NaCl and Bayfolan indicated a null effect of inhibition because no significant differences in the biomass generated were observed among the treatments (1.1 g/L).

The dye adsorption tests carried out with *T. polyzona* and the enzymes attached to the mycelium simultaneously lead to decolorization at the extracellular level. Bioadsorption is a common process carried out by basidiomycete fungi, and it has been observed that the presence of biomass during decolorization makes this process more efficient in addition to generating fewer toxic compounds in the effluent [46]. It has been reported that the first mechanism that used by basidiomycetes fungi in liquid medium for decolorization is the bioadsorption of the dye. Jarosz-Wilkolazka et al. [37] detected an immediate decrease in the absorbance of the medium during Basic Blue 22 and Acid Red 183 decolorization by *B. fumosa*, *K. mutabilis*, and *T. rugosoannulata*, and this decrease was mainly due to bioadsorption processes. Anastasi et al. [9] detected a 16–34% decrease in the color during the degradation of effluents by *B. adusta* from the textile industry, and this decrease has been attributed to bioadsorption processes. On the other hand, it has been observed that *P. chrysosporium* was only fungus capable of bioadsorbing the dye in agitated cultures [47]. This phenomenon might have been obtained because agitation increases the concentration of dissolved oxygen, which results in increasing the oxidative metabolism of the fungus and accelerating its growth; this fact makes it necessary for the fungus to increase its carbon source consumption rate by increasing the expression

Table 1. Physicochemical values obtained from the effluents resulting from treatments at 1, 2, and 3 vvm with 100 mg/L of the Amaranth dye.

Airflow	Dissolved oxygen (%)		pH		Protein concentration (μg/mL)	
	Initial	Final	Initial	Final	Initial	Final
1 vvm	100	6.3	8.5	4.98	14.4	20.1
2 vvm	100	100	6.3	8.6	4.09	16.9
3 vvm	100	100				

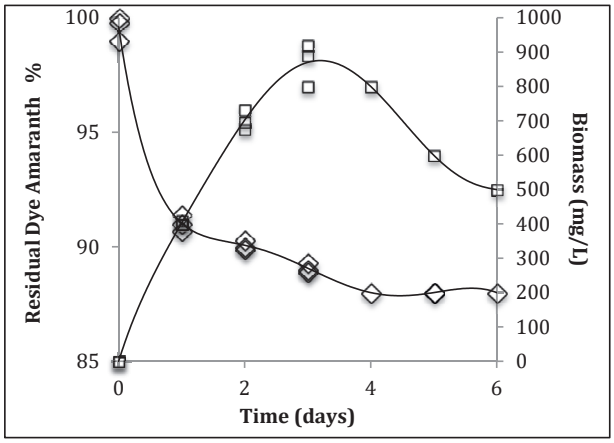

Figure 5. Adsorption isotherm of amaranth dye with inert biomass of *T. polyzona*.

of enzymes and the hydrolysis processes of the molecules present in the growth medium.

In the present work, a slight alkalinization of the decolorization medium was observed, and the pH increased from 6.3 to 8.6. Basidiomycete fungi have the characteristic of regulating their medium pH without the need to add buffer solutions. The change in pH could also have been obtained because the breaking of the azo bond generates amines, which results in a slight increase in the pH. However, no common behavior regarding the variation in pH was been observed during basidiomycete fungal growth since the pH varies depending on the substrate. Jarosz-Wilkolazka et al. [36] studied the decolorization of aniline blue using the fungus *Phomopsis* sp. at pH 5. Asgher et al. [48] reported that the LiP enzyme of *T. versicolor* exhibited its highest activity at pH 2 to 5; however, Oliveira et al. [42] detected the highest *G. lucidum* LiP activity at neutral and slightly alkaline pH, and the LiP from *P. ostreatus* presented maximal activity at a pH value of approximately 5. Knapp and Newby [49] studied the decolorization of chemical effluents with *P. chrysosporium* and *Coriolus versicolor* and found that the optimal pH for decolorization with *P. chrysosporium* and *C. versicolor* was 5.5 and 2.2, respectively. Rigas and Dritsa [50] studied the decolorization of polymeric dyes with *Polyporus* sp. and *P. ostreatus* and found that the final pH was between 5.5 and 6.5. In contrast, another strain of *P. chrysosporium* was used to decolorize the Black Amido 10B dye, and the optimal pH was 7 [13]. A study of the degradation of textile effluents using *Chaetomium globosum* observed that decolorization occurred at a pH of 5.5–9.05 [51]. These results demonstrate that there is no optimal pH for decolorization because this variable depends on the strain used for the process. With respect to effluent toxicity, no decreases in the algae growth rate were observed during the process; thus, the effluent can be considered not phytotoxic to the algae. Evaluating the toxicity of the generated effluents is of utmost importance because certain treatment conditions (such as anaerobic processes) generate highly polluting compounds. The toxicity of the effluents generated by *T. versicolor* resulting from mono-azo dye treatment has been reported, and the results showed a decrease in toxicity from 41.2 to 64.3% [14]. The analysis of the decolorization and detoxification of effluents from the kraft process using *C. subvermispora* showed that complete detoxification was achieved because the effluent was used to grow zebrafish without any toxic effects being observed [52].

4. Conclusions

The basidiomycete fungus *T. polyzona* decolorized the Amaranth dye and used it as its sole carbon source. The rate of decolorization was a function of the aeration regime, and a higher aeration rate was associated with a faster decolorization rate. The three ligninolytic enzymes that contribute to the decolorization process were detected. The results showed that the LiP enzyme was likely the enzyme with the greatest impact on the decolorization process because this enzyme was expressed during the first 6 days of all three treatments, and 80% decolorization was observed at this time point. The decolorization process was associated with a decrease in the COD. The results showed that the treatments with 2 and 3 vvm exhibited an average decolorization efficiency and a 95% decrease in the COD. In contrast, 100% decolorization was observed with the treatment with 1 vvm, and this treatment resulted in a 95% decrease in the COD. The generated effluent did not present toxicity to the algae *Nannochloropsis salina*.

Declarations

Author contribution statement

Pérez-Cadena, R., Serna-Díaz, M.G.: Conceived and designed the experiments; Analyzed and interpreted the data.

García-Esquivel, Y.: Performed the experiments.

Castañeda-Cisneros Y.E.: Performed the experiments; Analyzed and interpreted the data.

Ramírez-Vargas, M.R.: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Muro-Urista, C.R., Téllez-Jurado, A.: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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