

Energy consumption by *in vitro* fungal decolorization of synthetic colorants and an industrial effluent

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ABSTRACT

The present study evaluated the decolourisation capacity of four native fungal strains on synthetic dyes and assessed the biotechnological potential of the selected strains in the decolourisation of a real industrial effluent. Discolouration assays were performed on solid medium using six dyes, and the production profiles of laccase, lignin peroxidase, and manganese peroxidase were determined in liquid cultures. Laccase was the most abundantly produced enzyme in all strains. Based on decolourisation potential and enzymatic production curves, *Trametes maxima* CU1 and *Trametes hirsuta* CS5 were selected. Eighteen-day-old culture supernatants were used for the decolourisation of Reactive Blue 19 (RB19), Reactive Black 5 (RB5), Crystal Violet (CV), and the industrial effluent. *T. maxima* CU1 showed the highest discolouration percentages of synthetic dyes, reaching 97% in RB19 and 61% in RB5 at six hours, while CS5 required nine hours for 41% and 25%, respectively. For CV, CU1 reached 50% at 48 h, and CS5 only 6%. In the industrial effluent, CS5 achieved > 50% discolouration at 293 nm at six hours. CU1 and *T. versicolor* UAMH 8272 reached 21% and 17%, respectively. At 613 nm, CU1 showed the highest activity (60% at six hours). The process consumed 10,869.43 MJ, the electricity accounting for 76.13% of total energy use. These results confirm *T. maxima* CU1 high efficiency, highlighting the need to optimize energy consumption for sustainable applications.

1. Introduction

Industrial dye pollution constitutes a pervasive yet insufficiently

recognized environmental crisis, exerting a sustained and deleterious impact on one of the planet's most essential resources: water. Each year, an estimated 280,000 tons of unused synthetic dyes, representing

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10–25% of global production, and up to 50% for reactive dyes are discharged into wastewater streams, transforming once-pristine aquatic ecosystems into vibrant yet hazardous chemical landscapes. These persistent pollutants, often carcinogenic and mutagenic, not only suffocate aquatic life and contaminate agricultural lands but also pose profound, long-term risks to human health [1]. Although conventional treatment methods such as coagulation, adsorption, and advanced oxidation processes are widely deployed, they are constrained by high energy demands, heavy reliance on chemical reagents, and the generation of vast quantities of toxic sludge, frequently failing to achieve complete detoxification. This unsustainable trajectory necessitates a radical shift towards innovative, biologically driven solutions, where the precision and inherent eco-friendliness of enzymatic degradation offer a compelling and scientifically robust pathway to genuine environmental remediation [2].

Textile dyes are chemical substances used to impart colour to textile fibres, a use that can be traced back centuries. Initially, natural dyes were used in dyeing processes, however, over time, they were gradually replaced by synthetic dyes, which are noted for their vibrant colours, ease of application, and colour fastness [3]. This versatility is due to their complex chemical structure, which determines their physical and chemical characteristics [4]. However, the environmental impact and health risks associated with these compounds are of great relevance, particularly due to their adverse effects on aquatic ecosystems [5]. In addition, there are reports of DNA damage and cell death, which represent a risk to human health through ingestion of contaminated water or dermal contact [6]. For this reason, industrial effluent treatment has been the subject of extensive research, aimed at addressing the unique challenges posed by various industries, such as paint, textile and pulp and paper manufacturing, due to their complex composition and contaminant content [7]. This has led to the exploitation of different methods, including physicochemical, biological and advanced oxidation processes, such as electrochemical and hybrid techniques [8]. It is worth mentioning that these methods have advantages and disadvantages, specifically in the case of physicochemical processes which includes the generation of by-products resulting from incomplete oxidation, and the production of sludge from iron salts, while also incurring significant costs associated with the treatment of by-products and electricity consumption [9]. On the contrary, biological treatments offer benefits such as good dye removal efficiency, and some organisms can be applied on a large scale, decreasing biochemical oxygen demand and suspended solids (reference). However, they also require constant maintenance and optimal conditions for effective dye removal [10]. In this context, white-rot fungi (WRF), also known as ligninolytic basidiomycetes, stand out as an efficient alternative for degrading recalcitrant xenobiotic compounds, including synthetic dyes, owing to their extracellular lignin-degrading enzyme system [11]. The main activities of this system are laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP), which are characterised by their relative non-specificity and high oxidative power, responsible for the degradation of lignin and chemically related compounds [12,13]. Despite the advantages of these enzymes in decolourisation processes, energy consumption remains an important limitation.

To the best of our knowledge, there are no reports of quantifying energy consumption in laboratory, pilot plant or large-scale wastewater dye degradation. Therefore, in this work while studying the *in vitro* degradation of synthetic dyes and an industrial effluent, we also estimated the associated energy consumption.

2. Material and methods

2.1. Biological material

The native strains used were *Trametes maxima* CU1, *Trametes hirsuta* CS5, *Pycnoporus sanguineus* GE01 and *Funalia troglia* CS200, and *Trametes versicolor* UAMH 8272 was included as a reference strain. All strains are

maintained in the Natural Sciences Laboratory of the Faculty of Agronomy and preserved through periodic reseeded every three months on YMGA medium (4 g•L⁻¹ glucose, 4 g•L⁻¹ yeast, 10 g•L⁻¹ malt extract and 15 g•L⁻¹ agar) [14].

2.2. Decolourisation in solid medium

For decolourisation assays on solid medium, a modified Kirk medium (KM) was used. The composition per litre of the medium was: glucose, 10 g; yeast extract, 0.5 g; peptone, 5 g; KH₂PO₄, 1 g; MgSO₄, 1 g; NH₄NO₃, 1 g; KCl, 0.5 g; solution of trace elements, 10 mL; and CuSO₄, 35 mM, 10 mL. The composition of the trace element solution per litre was FeSO₄ 0.01 g, ZnSO₄ 0.028 g and CaCl₂ 0.025 g [15]. For solid media, bacteriological agar was added at a concentration of 1.5%. In the case of dyes, they were added at a final concentration of 200 ppm in the previously sterilised media at 121 °C for 15 min. Subsequently, each plate was then inoculated with a 0.5 cm agar piece taken from the periphery of a five-day-old actively growing fungal culture and placed at the center of the plate. The plates were then incubated at 28 °C.

The dyes used were Reactive Blue 19 (RB19), Crystal Violet (CV), and Reactive Black 5 (RB5), representing the anthraquinone, triaryl methane, and diazo groups, respectively. Ligninolytic potential was evaluated based on the production of lignin-modifying enzymes and LiP production, by the decolourisation of Poly-R478 and Azure B, respectively. The oxidation of syringaldazine was detected to indicate Lac production. Non-supplemented media were used as negative controls. Discolouration was measured using a 150 mm Caliper® vernier with an accuracy of 0.01 mm. The measurements were recorded daily from day 1 to day 7 and then on days 9, 12, 15, 20, 25, and 30 for the estimation of growth velocity and bleaching. The decolorization index (DI) was determined as the ratio of the bleaching diameter to the growth diameter [16], as shown in the formula (1):

$$D.I. = \frac{D}{G} \quad (1)$$

Where D is the decolourisation diameter (or reaction in the case of syringaldazine), and G is the growth diameter. All experiments were conducted in quadruplicate.

2.3. Enzyme production

For the analysis of enzyme production, KM medium (as previously described in Section 2.2) and Kellogg's All Bran Flakes medium (BF) [2% (w/v) Kellogg's® Bran Flakes in 60-mM potassium phosphate buffer pH 6.0] [14] were used. Both media were placed in 250 mL Erlenmeyer flasks with a working volume of 120 mL. The previously sterilised flasks were inoculated with mycelium transfer using the strains *T. maxima* CU1, *T. hirsuta* CS5, *F. troglia* CS200, *P. sanguineus* GE01, and *T. versicolor* UAMH 8272, with three inoculum cylinders in each. The cultures were incubated at 28 °C, with shaking at 150 rpm. Sampling was carried out over 30 days, with 2 mL aliquots collected every two days in Eppendorf® tubes for the analysis of enzyme activity. The trial lasted 30 days, with sampling every two days. The aliquots were centrifuged at 15,000 rpm for 5 min using a Mini Spin microcentrifuge (Eppendorf, Massachusetts, USA). The production curves of Lac, MnP and LiP activities were determined by an adaptation of Périé and Gold, [17] and Field et al. [18] methods. Lac activity was determined by the oxidation of 2,6-dimethoxyphenol (DMP) at 50 mM in a sodium succinate buffer solution (50 mM, pH 3.5). LiP activity was quantified in a 50 mM sodium succinate buffer, pH 4.5, 2 mM hydrogen peroxide (H₂O₂) and 20 mM DMP. MnP activity was estimated by the formation of the Mn³⁺-malonate complex, measured through the oxidation of DMP in a 50 mM sodium malonate buffer (pH 4.5) and 2 mM H₂O₂. DMP oxidation was performed at 468 nm ($\epsilon = 49\,600\text{ M}^{-1}\text{ cm}^{-1}$), using a Shimadzu® 1800 UV-VIS spectrophotometer (Kyoto, Japan), where an

enzymatic unit was defined as the amount of enzyme required for the oxidation of one μmol of substrate in one minute. This definition was consistently applied to all enzymes evaluated in this study, as enzymatic activities were determined using the same substrate (DMP) under their respective assay conditions, ensuring methodological uniformity and comparability of results.

2.4. Enzyme decolourisation assay

Decolourisation of synthetic dyes was performed using culture supernatants obtained after 18 days of growth in BF medium from the fungal strains that exhibited the highest dye-degrading activity on solid medium. Prior to the decolourisation tests, the Lac, LiP and MnP units present in the supernatants were titrated and subsequently sterilised using 0.2 μm regenerated cellulose membranes in a vacuum ultrafiltration system (Merck Millipore, Massachusetts, USA). In 500 mL Erlenmeyer flasks, 200 mL of a 5 $\text{U}\cdot\text{mL}^{-1}$ supernatant solution and 200 ppm of the dye were placed. The dyes selected for this stage were RB5, RB19, and CV. Flasks were incubated at 28 $^{\circ}\text{C}$ on an orbital shaker at 150 rpm for 72 h. Heat-treated (100 $^{\circ}\text{C}$) supernatants containing the same dye concentrations served as negative controls. Aliquots were collected at 0, 0.1, 3, 6, 9, 12, 24, 48, 60 and 72 h, and spectrophotometric analysis were conducted in the range of 200–800 nm using a Shimadzu® 1800 UV–VIS spectrophotometer (Kyoto, Japan). All measurements were performed in triplicate, and the mean values were used to generate absorption spectra graphs. Once the maximum peaks in the visible region for each dye were identified, the percentages of discoloration over time were calculated [14].

2.5. Discolouration of a wastewater sample

An industrial effluent sample was kindly provided by Servicios de Agua y Drenaje de Monterrey, I.P.D., collected from wastewater discharges of various industries and the municipal network of Monterrey and its Metropolitan area. A standard physicochemical characterisation of the sample was performed according to the guidelines of the National Water Commission, including measurements of pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The fungal strains used for discoloration assay were *T. maxima* CU1, *T. hirsuta* CS5, and *T. versicolor* UAMH 8272 (reference strain). Given the nature of the water, the discoloration was evaluated in a reaction mixture consisting of 2.9 mL of the supernatant, 1 mL of Britton–Robinson universal buffer (0.04 M boric acid, 0.04 M phosphoric acid, and 0.04 M acetic acid) adjusted to pH 4.0, and 0.1 mL of the effluent. Heat-treated supernatants at 100 $^{\circ}\text{C}$ were used as controls. Samples were taken at 0, 0.1, 6, 12, 24, 48, and 72 h. The spectrophotometric reading was performed as previously written in the range of 200–800 nm. All assays were performed in triplicate, and the average values were plotted for the construction of the bleaching spectra graphs. After identifying the maximum absorbance peaks in the visible region, the percentage of decolourisation over time was calculated.

2.6. Energy consumption

To establish energy consumption, the calculation methodology described by López-Sandín et al. [19] was used. This methodology determines the energy inputs in the process, including the energy necessary to operate equipment, as well as the inputs and labour required to comply with each stage of the process, considering the times, operating conditions, quantities, and the respective energy equivalent of each carrier consumed (Table 1).

Table 1

Energy equivalents.

Concept	Unit	(MJ·unit ⁻¹)	Source
Human labour	Manual	h	1.96 [19]
	Mechanized		1.05 [19]
Electricity	kW·h ⁻¹	11.93 [19]	
Water	kg	0.005 [20]	
Yeast	kg	6.32 [21]	
Chemical	kg	8.74 [21]	
Glucose	kg	2.56 [22]	

The following equation determined the energy input (E_{IMP}) or energy consumption in MJ (2):

$$E_{IMP} = \sum E_{EC} + E_{HL} + E_{CR} + E_{OI} \quad (2)$$

Where: E_{EC} is the energy consumed (MJ) in electricity; E_{HL} is the energy consumed (MJ) by human labour; E_{CR} is the energy consumed (MJ) in chemical reagents; E_{OI} is the energy consumed (MJ) by other inputs such as water, enzymes, glucose and yeasts.

Electricity consumption is considered the electricity used by electrical devices directly involved in the biotransformation process of synthetic dyes. Consumption data were recorded by an electrical meter (STEREN® CAJ-HER-432, DF, China) every hour. Equipment used for limited periods was measured during its period of use. To calculate the energy consumed in electricity (E_{EC}) was used the following Eq. (3):

$$E_{EC} = \sum_{i=1}^{i=n} E_C * Q_E \quad (3)$$

Where: E_C is the electricity consumption (kWh) by the equipment(s) used; Q_E is the energy equivalent of electricity (MJ·kWh⁻¹) shown in Table 1.

The following Eq. (4) determined the energy consumed in human labour (E_{HL}):

$$E_{HL} = \sum_{i=1}^{i=n} L_T * Q_H \quad (4)$$

Where: L_T is the labour time (h) of the operator(s) per unit of production, Q_H is the energy equivalent of human labour (MJ·h⁻¹) shown in Table 1.

The energy consumed (E_{CR}) in chemical reagents was determined by the following Eq. (5):

$$E_{CR} = \sum_{i=1}^{i=n} C_R * Q_R \quad (5)$$

Where: C_R is the chemical reagent(s) consumed (kg), Q_R is the energy equivalent of the chemical reagent (MJ·kg⁻¹) shown in Table 1.

The energy consumed in other inputs (E_{OI}) was determined by the following Eq. (6):

$$E_{OI} = \sum_{i=1}^{i=n} I_C * Q_I \quad (6)$$

Where: I_C is the input(s) consumed (kg), Q_I is the energy equivalent of the input (MJ·kg⁻¹) shown in Table 1.

2.7. Experimental design

In the first stage of the solid media bleaching assay, a factorial design with four replications was used to record the experimental data

associated with the combined effects of time and fungal strains. The data generated from the interactions between dye, strain, and time were statistically analysed through a multivariate analysis of variance (MANOVA) to determine the presence of statistically significant differences ($p \leq 0.05$). In the second and third stages, the enzymatic profiling assays and synthetic dye bleaching treatments were performed in

triplicate, and the data obtained were analysed using analysis of variance (ANOVA) to verify the existence of statistically significant differences ($p \leq 0.05$). For mean comparisons, Tukey's test was applied at a 95% confidence level (5% error). All statistical analyses were performed using the Infostat software package, version 2020.

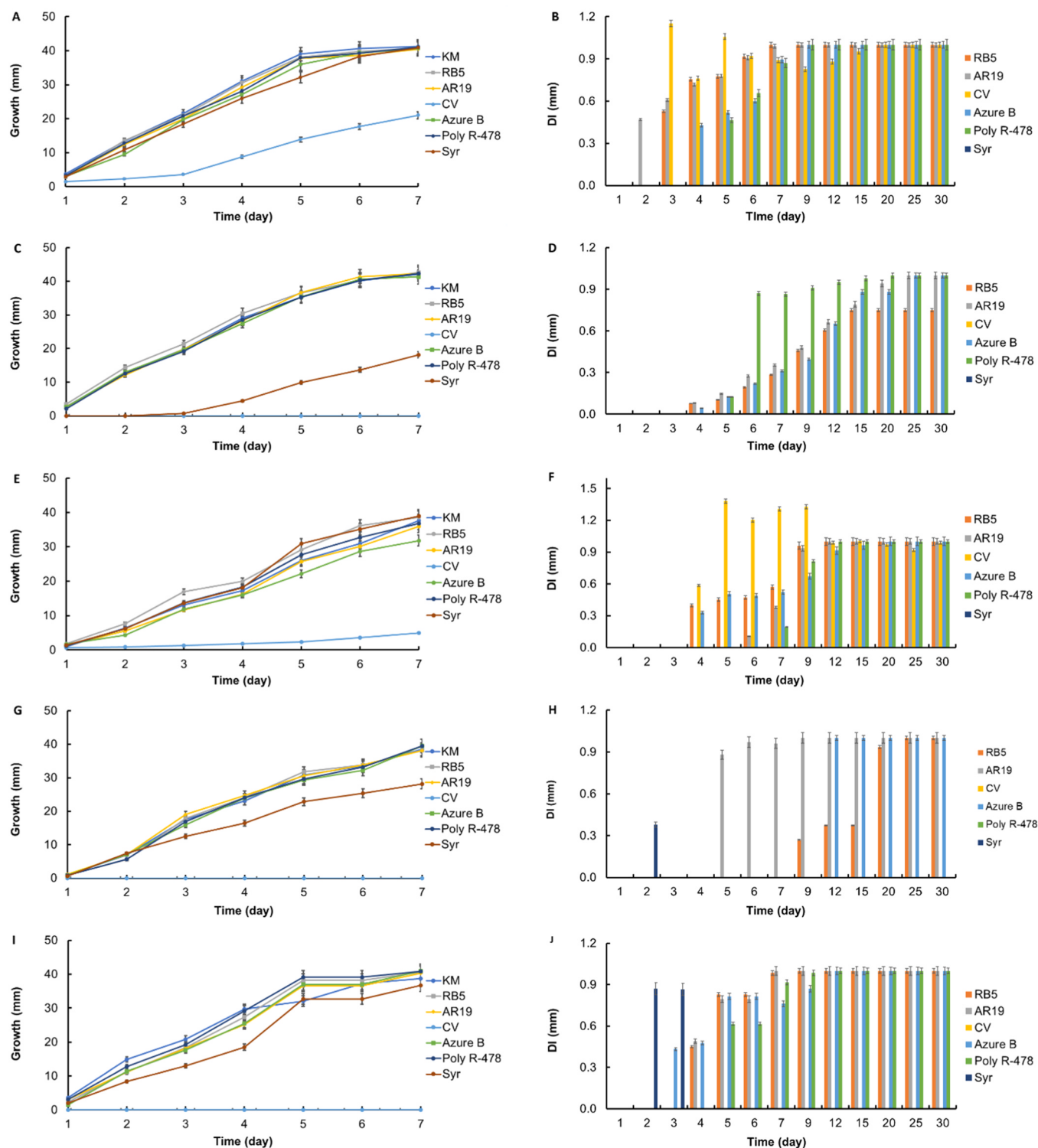


Fig. 1. Growth kinetics and decolorization indices (D.I.) in solid medium. Figures A, C, E, G and I show the growth kinetics of *T. maxima* CU1, *T. hirsuta* CS5, *F. troglia* CS200, *P. sanguineus* GE01 and *T. versicolor* UAMH 8272, respectively. On the other hand, figures B, D, F, H and J show the decolorization indices (D.I.) corresponding to each strain, in the same order.

3. Results and discussion

3.1. Solid media screening for dye decolorization

The MANOVA showed a highly significant statistical difference ($p < 0.0001$) in the growth and discoloration index (D.I.) or reaction to syringaldazine among the fungal strains, due to the effects of time and dye present in the medium (Supplementary Table S1). It is worth mentioning that significant interactions between the factors were also detected. Fig. 1 illustrates the effect of the dyes on the fungal growth and discoloration rates during the first seven days of incubation. Overall, all five strains displayed the exponential phase in the first days of culture and completely colonized the Petri dishes by day 7.

For *T. maxima* CU1, growth and D.I. values are presented in Fig. 1A and 1B, respectively. Growth curves on the different dyes and substrates showed similar behaviour, except for the culture supplemented with CV, which had an inhibition of 49%. Nevertheless, the D.I. values were equal to that observed in the medium with Azure B and Poly R-478. Although this strain has been previously reported as a producer of laccase with a high redox potential [14], in this study no intense reaction on syringaldazine was detected. This may indicate that the observed colour loss was due to laccase activity itself, suggesting that syringaldazine may function as a redox mediator [23]. As for the RB19 discoloration, it was observed from day two of cultivation and reached total discoloration of the medium by day seven. In the case of CV, despite the inhibitory effect, the highest activity was recorded on day 3 when the dye was transformed into a pink-coloured product. Although decolourisation of the medium began on day five and was completed at day twenty, this was the only isolate capable of degrading the dye completely. In the case of the media with Azure B and Poly R-478, a similar behaviour in the degradation index was observed over time, reaching D.I. of 1.0 at day 9 of culture.

Trametes hirsuta CS5 showed a similar growth rate in media supplemented with RB19, RB5, Azure B and Poly R-478 dyes (Fig. 1C), whereas no growth was observed in the medium with CV. While some basidiomycetes demonstrate the ability to tolerate and even decolourise CV (such as *T. maxima* CU1), suggesting potential resistance to its toxic effects [24], variability has been detected in the response to different fungal species to CV and structurally similar toxic compounds [25]. This variability likely accounts for the absence of growth in strain CS5 on CV-supplemented medium, highlighting the species-specific and complex nature of CV toxicity in basidiomycetes. Fig. 1D shows that the D.I. values observed for RB5 increased over time, reaching its maximum value (0.75) on day 15, and remained stable until day 30. A similar behaviour was recorded in the D.I. values for RB19, Azure B and Poly R-478, which increased over time, reaching a maximum value (1.0) at day 20.

In the case of the *F. trogii* CS200 strain, growth kinetics were similar among the colourants, except for the medium with Azure B and CV, which showed an inhibition of 15% and 87%, respectively (Fig. 1E). As for decolourising activity (Fig. 1F), except CV, the other substrates showed an increase in D.I. over time. Although the days of increased activity varied, with the maximum value recorded at different times: day 12 for RB5 and RB19, and days 20 and 15 for Azure B and Poly R-478, respectively. As in *T. maxima* CU1 and *T. hirsuta* CS5, no activity on syringaldazine was observed. Notably, this isolate exhibited a marked D.I. response to CV between days 5 and 7, associated with the appearance of a pink pigment similar to that produced by *T. maxima* CU1 on day 3. However, while *T. maxima* CU1 began decolorizing the pink pigment from day 5 onward, in *F. trogii* CS200 this transformation was delayed until day 30 and occurred exclusively in the growth area (supplementary Figure S1).

The growth kinetics of *P. sanguineus* GE01 (Fig. 1G) also showed linear growth up to day 4 in the presence of the dyes, except for CV and syringaldazine, which were inhibited by 100% and 27%, respectively. Regarding the behaviour of the D.I., RB5 reached its maximum value

(1.0) at day 25, while RB19 had values higher than 0.98 from day 6. In the case of the medium with Azure B, the highest discoloration was observed on day 12 with an index of 1.0. In the medium supplemented with Poly R-478, no discoloration activity was detected, which could be explained by the production of the red pigment in *P. sanguineus* cultures. Notably, it was the only isolate that at day 1 showed activity on the medium with syringaldazine (Fig. 1H), which could be due to the production of laccases with different redox potentials than those produced by the other native strains studied here, explaining the low levels of decolorization of the synthetic dyes [26]. Concerning CV, this had the same toxic effect observed in *T. hirsuta* CS5, which has been previously reported [27].

Finally, the results of the growth kinetics of *T. versicolor* UAMH 8272 showed similar behaviour in most of the treatments (Fig. 1I), except in the medium with syringaldazine, which presented an inhibitory effect of about 40% during the first 4 days of culture, while in the medium with CV no growth was observed during this time. Fig. 1J shows that the maximum D.I. values (1.0) for RB5 and RB19 dyes were reached at day 7. While for Azure B and Poly R-478 the highest decolourising activity was observed at day 12. It is worth noting that the medium with syringaldazine exhibited a reaction rate of approximately 0.9 at days 2 and 3 of culture. In general, except for *P. sanguineus* GE01, the strains showed bleaching activity on the RB5 and RB19 dyes, although the days of greatest activity varied. This finding is consistent with reports for other strains of *Pycnoporus* [28]. Regarding discoloration times, azo dyes had a slower colour removal compared to anthraquinonic dyes, as reported by Anita et al. [29]. Regarding CV, a strong inhibitory effect was observed in 40% of the strains tested, with *T. maxima* CU1 being the only one capable of clarifying the medium. In the case of the *T. hirsuta* CS5 culture, although it did not exhibit bleaching activity on this dye, from day 15, the mycelium presented a purplish colouration, associated with the phenomenon of bioadsorption. This may be due to the adhesion of the dye by the action of ionization of the mycelium surface and the formation of hydrogen bonds between them [30]. In the case of discoloration of Poly R-478 as an indicator of ligninolytic activity, only *T. maxima* CU1 and *T. versicolor* UAMH 8272 exhibited values comparable to those reported for other *Trametes* strains [31]. Finally, it is essential to note that the changes observed in the media with Azure B, might be associated primarily to lignin peroxidase discoloration [32]. However, it has also been reported that the transformation to pink can be associated with laccases, since the bleaching process often involves the transformation of the dye into compounds of different colours, which can include a pink hue. This ability is attributed to the oxidative properties of laccases, which can break down complex dyeing structures into simpler, often less coloured or differently coloured compounds [33]. Thus, the different patterns of growth and discoloration can be explained by the metabolic plasticity of these organisms.

Supplementary Figure S1 shows changes in media at 30 days of culture compared to the respective controls. *Trametes maxima* CU1 was the strain that showed the highest bleaching capacity, degrading all the substrates used and leaving a light background in the middle. In the case of *T. versicolor* UAMH8272 and *F. trogii* CS200, they showed similar results for most dyes, except for CV, which presented a pink background in the medium associated with the transformation of the dye [34]. This phenomenon was similar to that observed in the medium with RB19, which is an anthraquinone-type dye and whose discoloration was preceded by browning in the medium attributed to the breakdown of the anthraquinone structure by enzymatic action, a phenomenon that usually generates dark brown or light brown compounds [35]. In the case of the *T. hirsuta* CS5 and *P. sanguineus* GE01 strains, they did not show activity on CV. Overall, all strains showed high lignolytic activity when discoloring Poly R-478 and Azure B. However, the reaction to syringaldazine was limited. This can be explained by the action of the laccase that can act on the oxidized substrate [23], which could mean a loss of colour associated with the presence of laccases with high redox potential [26].

The variability in fungal-induced discoloration can be attributed to the combined effects of fungal species, environmental conditions, and substrate characteristics. In basidiomycetes, species-specific differences in enzymatic systems play a central role in dye degradation. For example, *Pleurotus pulmonarius* has been reported to achieve higher decolorization of Remazol Brilliant Blue R across a range of pH values and concentrations, reflecting its efficient ligninolytic enzyme machinery [36]. More broadly, white-rot basidiomycetes consistently exhibit superior decolorization performance compared to other ecological groups, primarily due to their capacity to produce oxidative enzymes such as laccases and peroxidases [37].

In addition to enzymatic degradation, discoloration is also mediated by the production of fungal pigments and secondary metabolites. In basidiomycetes, metabolic responses—particularly during fungal interactions—can induce the synthesis of compounds such as melanin, resulting in distinct color changes [38]. Importantly, these processes may occur independently of extensive structural degradation, indicating that discoloration is not always directly correlated with substrate breakdown [37].

The efficiency of dye decolorization further depends on the chemical structure of the dye and the specific fungal strain. For instance, *Coriolus versicolor* and *Pleurotus ostreatus* show markedly different decolorization efficiencies under similar conditions, highlighting the role of enzyme specificity and regulation [39]. Environmental factors, such as humidity, also influence fungal activity and the onset of discoloration,

particularly in lignocellulosic substrates [40].

Overall, fungal discoloration should be understood as a multifactorial process driven by species-dependent enzymatic capacity, metabolic responses, and environmental conditions, which together determine the extent and nature of substrate color alteration.

3.2. Ligninolytic enzyme production

The production kinetics of laccase (Lac) in KM medium supplemented with 350 μM of CuSO_4 (KM+I) and BF are shown in Fig. 2A and 2B. The medium with the highest Lac titers was KM+I, with *T. maxima* CU1 standing out as the strain with the highest production with 16,610 U·L⁻¹. In general, production began on the 10th. In the case of *T. maxima* CU1, the peak of higher production occurred on day 12, while *P. sanguineus* GE01 presented it on day 20. *Funalia trogii* CS200 and *T. versicolor* UAMH 8272 exhibited similar behaviour by maintaining a constant production from day 15–30. It is worth mentioning that under the conditions tested in the BF medium, enzyme levels were below 4 000 U·L⁻¹. Thus, the effect of copper sulphate-supplemented medium on *T. maxima* CU1 was comparable to that reported for other strains of *T. versicolor* [41]. These differences can be explained by the metabolic characteristics of the producing organisms, as well as the composition of the medium [42]. From a metabolic perspective, different genera, species, and strains produce laccase enzymes as part of their ligninolytic

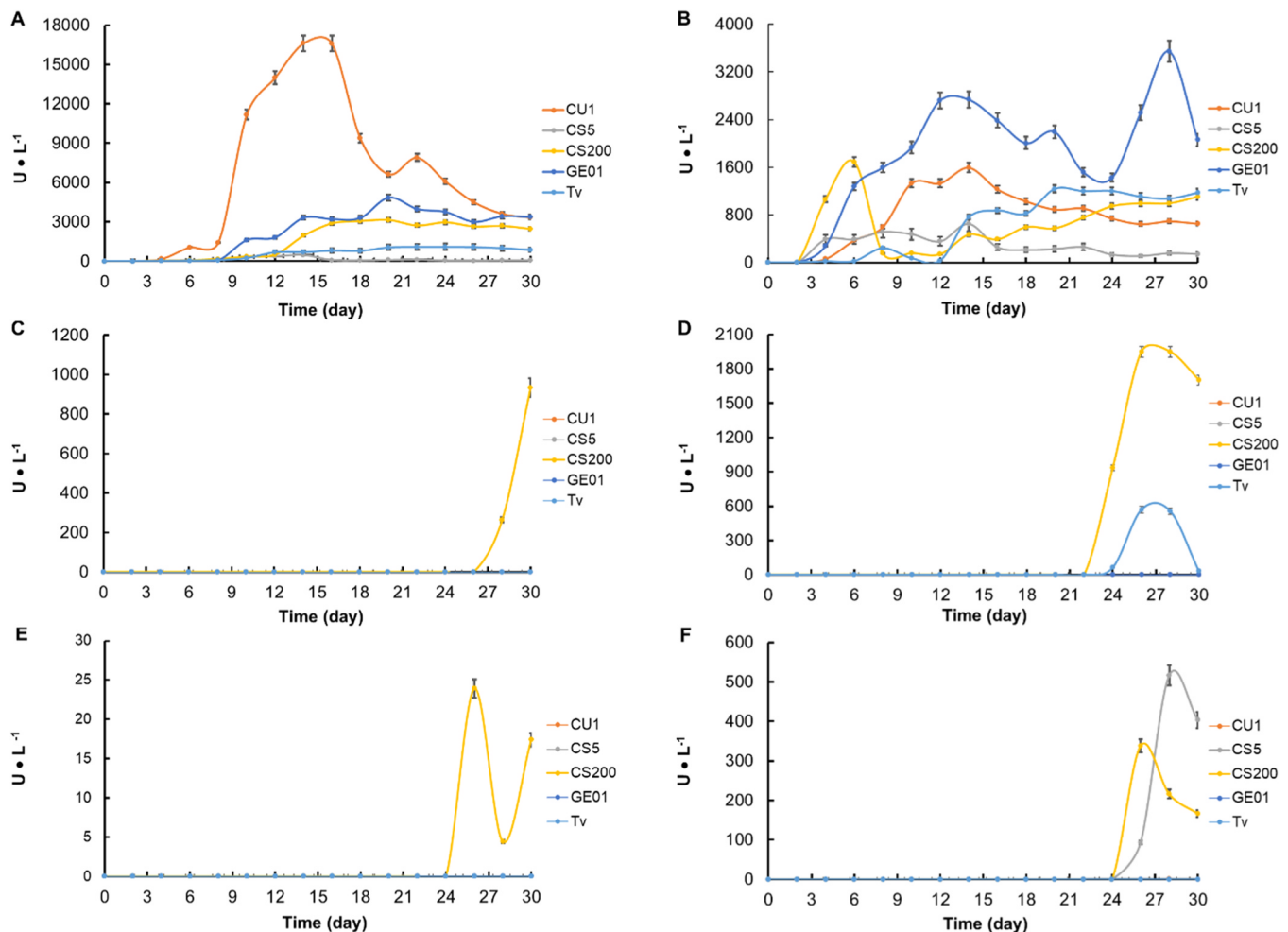


Fig. 2. Production of ligninolytic enzymes. Figs. A and B show the production of Laccase (Lac) in KM+CuSO₄ (350 mM) and BF medium, respectively. Figs. C and D correspond to the production of lignin peroxidase (LiP) in KM+CuSO₄ (350 mM) and BF medium, respectively. Finally, figures E and F represent the production of Manganese Peroxidase (MnP) in KM+CuSO₄ (350 mM) and BF medium, also respectively. CU1 corresponds to *T. maxima* CU1, CS5 to *T. hirsuta* CS5, CS200 to *F. trogii* CS200, GE01 to *P. sanguineus* GE01 and Tv to *T. versicolor* UAMH 8272. All determinations were made in triplicate, with a standard deviation equal to or less than 5%.

systems, which are crucial for the degradation of lignocellulosic materials [43]. An example of this is the presence of multiple laccase genes in *Lentinula edodes*, which exhibit varied expression under different environmental conditions, underscoring the metabolic flexibility and adaptability of basidiomycetes in laccase production [42,44]. Concerning LiP production (Figs. 2C and 2D), *F. trogii* CS 200 was the only strain producing LiP in both media. However, the highest production was measured in the BF medium ($1950 \text{ U}\cdot\text{L}^{-1}$) on the 26th day of culture. Under these conditions, *T. versicolor* UAMH 8272 did not exceed $600 \text{ U}\cdot\text{L}^{-1}$ in the same medium. These variations are consistent with what has been refuelled for other species [45], where it has been reported that LiP production varies significantly among different strains of fungi and is strongly influenced by the culture medium and environmental conditions [46]. In the case of MnP production (Fig. 2E and 2F), *F. trogii* CS200 was the only producing strain in both media. *T. hirsuta* CS5 was the most active isolate ($516 \text{ U}\cdot\text{L}^{-1}$) in the BF medium. Activity was detected for both strains from day 26 of culture. These results (like those observed in LiP production) showed that the addition of copper sulphate did not cause a significant increase in the production of both peroxidases. Similar results were reported by Vrsanska et al. [47], who observed this phenomenon in strains of *T. versicolor*, *T. suaveolens* and *T. gibbosa*, with MnP yields of less than $1 \text{ U}\cdot\text{mL}^{-1}$ (in a CuSO_4 concentration range of $0.1\text{--}1.0 \text{ mM}$). In this study, the results for *Trametes* strains also showed MnP yields below $2 \text{ U}\cdot\text{mL}^{-1}$. Thus, the low activity of this peroxidase observed in the media used reflects the complex interaction of genetic, metabolic and environmental factors that influence the biosynthesis of enzymes in fungi [48].

3.3. Dye decolourization with supernatants

The discolouration percentages of the synthetic dyes RB19, RB5, and CV over time are presented in Fig. 3, with *Trametes maxima* CU1 exhibiting the highest decolouration levels among the three dyes. In the case of RB19, it achieved 97% discolouration at 6 h, while *T. hirsuta* CS5 only discoloured 40% (Fig. 3A). In both cases, the discoloration was greater than the 10% reported for the *Volvariella volvacea* laccase in the same time interval (6 h) [49]. The percentage of discolouration of *T. maxima* CU1 was also higher than that observed with the free and immobilised laccases of *Oudemansiella canarii* EF72, which showed a discolouration of 80%. Compared to other Mexican native strains, such as *P. sanguineus* CS43 [28], the bleaching activity of the *T. maxima* CU1 strain was higher. It is worth mentioning that these variations can be attributed to metabolic differences between strains, as it has been reported that a strain of *Ganoderma lucidum* was able to discolour only 50% of the RB19 and required the use of a redox mediator to reach an efficiency of 90% [50]. In contrast, the *G. lucidum* KMK2 strain did not need a mediator to degrade 92% of this dye; however, the addition of a redox mediator was required for RB5 decolorization [51].

Regarding the discolouration of the RB5 diazo dye, the maximum values observed with the CU1 isolate were recorded after 6 h, with a discolouration percentage of 62% (Fig. 3B). In the case of *T. hirsuta* CS5, the bleaching activity was lower and occurred over a longer time interval, reaching 26% in 9 h. Although the results of *T. maxima* CU1 appear to be lower than those obtained with *Pleurotus eryngii* ACCC 50757 (72.58%) [52], it is essential to note that the latter were achieved after 24 h of incubation. In contrast, *T. maxima* CU1 achieved a similar efficiency in a shorter time. Given the complexity of this colorant, it is common to use redox mediators, such as 1-hydroxybenzotriazole (HBT), to achieve approximately 90% decolorization [53], as it is a complex process that involves enzymatic mechanisms and environmental factors. Thus, the efficiency of the bleaching is affected by various factors such as the pH of the culture medium, the presence of mycelium, and the structural characteristics of the colorant. Studies have demonstrated the ability of *Phanerochaete sordida* to completely discolour RB5 within 3 days, attributing the degradation to Lac and MnP activities [54]. However, the supernatants used from *T. maxima* CU1 and *T. hirsuta* CS5 were

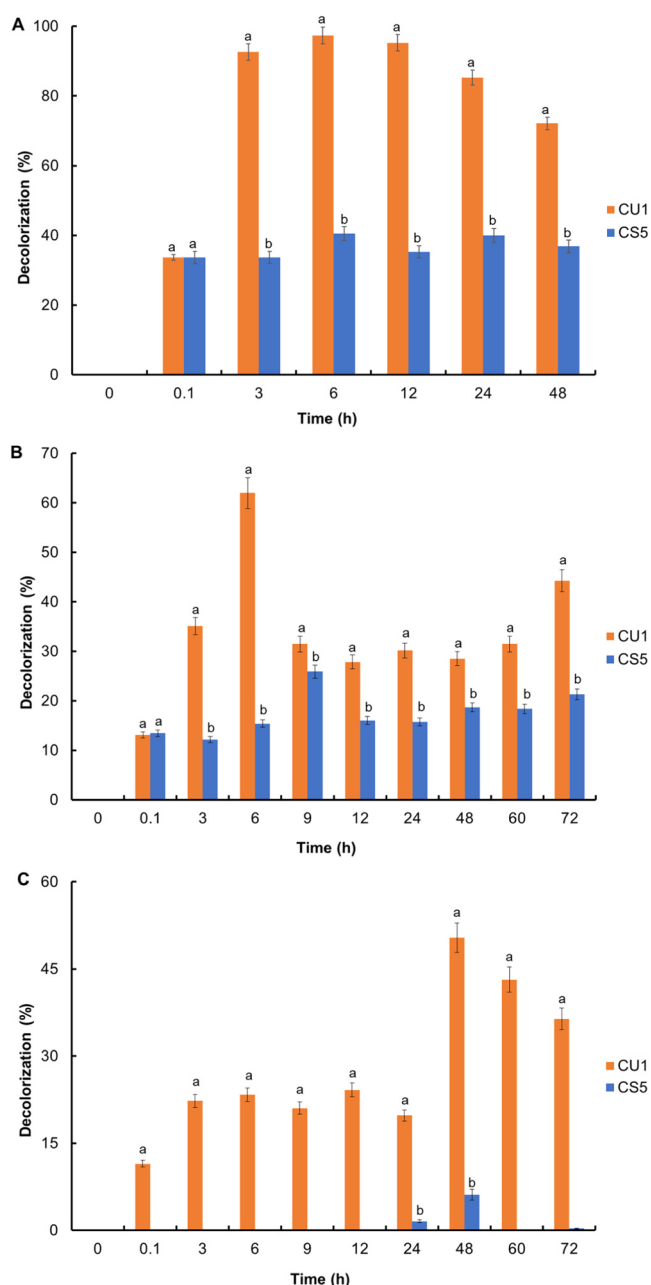


Fig. 3. Discoloration percentages. Where A corresponds to the values obtained for the dye Reactive Blue 19 (anthraquinonic), B to the Reactive Black 5 (diazo), and C to the crystal violet (triarylmethane). CU1 corresponds to *T. maxima* CU1, while CS5 corresponds to *T. hirsuta* CS5. ^{a-c} Different literals represent statistically significant differences ($p \leq 0.05$) between means at the different times evaluated.

obtained on day 18, where only the house was detected. Therefore, the discoloration results could be explained by the differences in redox potential between the different laccases and the dye [55].

CV was the dye that required the longest incubation time (48 h) to be bleached in 50%, and only *T. maxima* CU1 showed activity on it (Fig. 3C). It has been reported that the recombinant laccase of *T. hirsuta* MX2 was only able to discolour 14% of the CV, and in the presence of a redox mediator, it reached 39% [56]. In both cases, these values were lower than those observed with *T. maxima* CU1. Although lower than what was reported for the *Marasmius scorodonius* laccase NO. 42740, which under optimal operating conditions bleached 69% of this dye [57]. On the other hand, considering the complex and toxic structure of

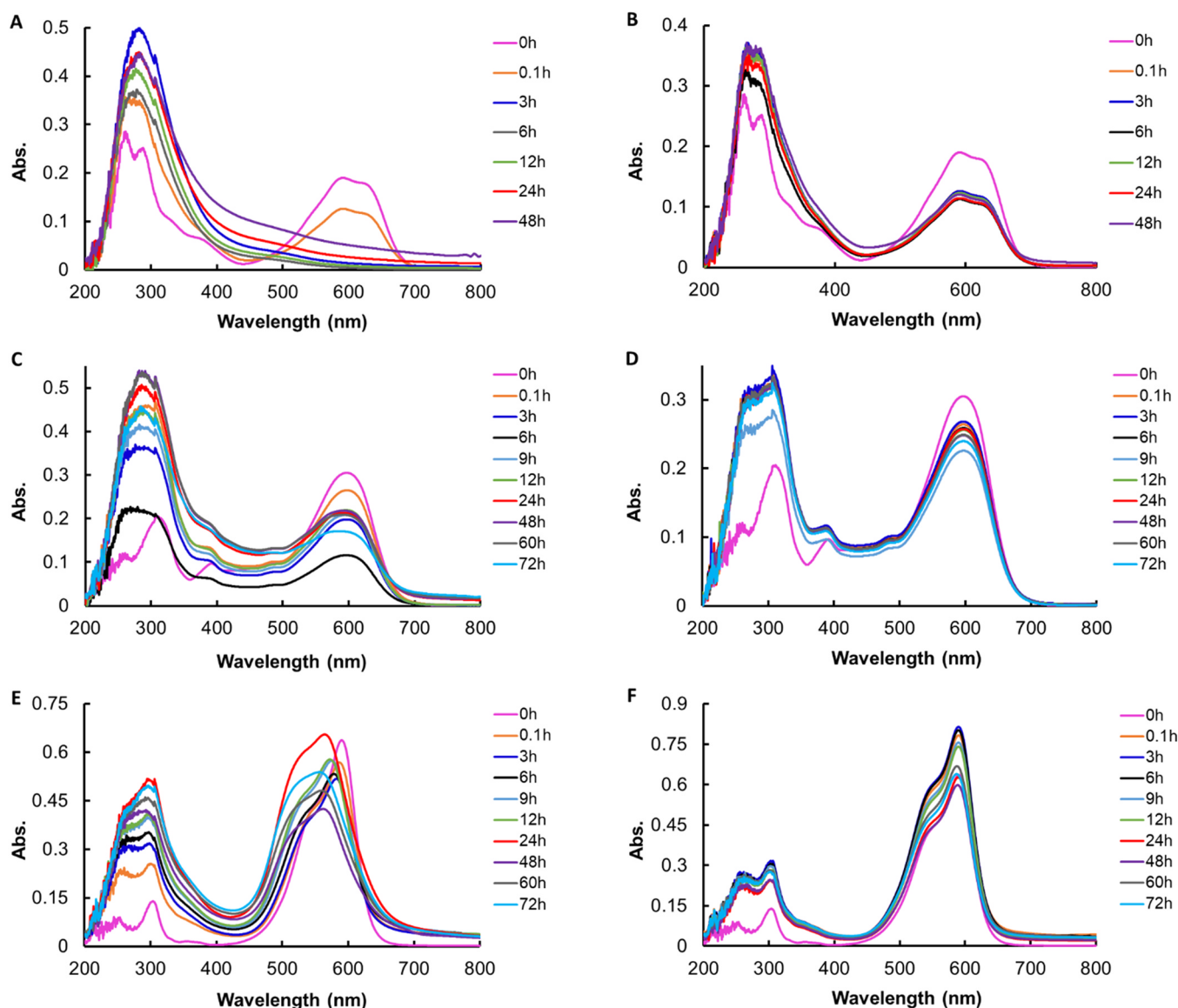


Fig. 4. Absorbance spectra during decolorization of Reactive Blue 19 (RB19), Reactive Black 5 (RB5), and Crystal Violet (CV) dyes treated with fungal supernatants. A, C and E correspond to the results obtained with *T. maxima* CU1 for the dyes RB19 (A), RB5 (C) and CV (E), respectively. B, D and F show the results obtained with *T. hirsuta* CS5 for the same dyes (RB19 in B, RB5 in D and CV in F). The curves represent the spectral evolution as a function of treatment time (0–72 h), indicating changes in absorbance (Abs) in the range of 200–800 nm.

CV, as well as its environmental impact (particularly on marine ecosystems), the bleaching potential of marine fungi has been explored [58]. In this context, the fungus *Alternaria* sp. D21 was able to discolour 58%, a value comparable to that observed in *T. maxima* CU1.

The analysis of the bleaching spectra (Fig. 4) confirmed the activity of the supernatants in structurally altering the dyes over time. Specifically, the presence of peaks in the UV/Visible region, characteristic of the aromatic rings present in these compounds, is often used as an indicator of structural changes [59].

In the case of RB19, the main changes observed included a decrease in the characteristic bell in the region from 480 to 680 nm, the disappearance of the shoulder around 380 nm, and a displacement of the peaks from 290 to 300 nm and from 250 to 260 nm (Fig. 4A). The flattening of the curve in the visible spectrum region suggests an indication of biodegradation, as indicated [60]. Unlike the gradual decrease in peaks that is usually observed in absorption processes, biodegradation is characterised by the disappearance or generation of new peaks in the UV region. On the other hand, in the spectrum of the dye treated with *T. hirsuta* CS5, the main changes consisted of a slight shift of the main

peak from 590 to 580 nm, the flattening of the peak at 380 nm and the change of the peaks from 290 and 250 nm to 267 and 280 nm, respectively. The differences between the two strains could be explained by variations in the catalytic mechanisms of the laccase isoforms [61]. It is worth mentioning that the disappearance of a peak in the visible region, without the appearance of a new peak in the same area, could be associated with the rupture of the chromophore group [62]. In this context, the *T. maxima* CU1 laccase would act directly on the RB19 chromophore within a short time interval, and the resulting products could explain the signal increase observed in the UV region. However, these results are consistent with the previously described role for laccases in the degradation of synthetic dyes, through different mechanisms that involve the oxidation of these compounds into less toxic forms [63]. In the case of anthraquinone dyes, the degradation mechanism consists of the oxidation of the structure, which can occur directly or indirectly through mediating compounds [64,65]. In direct oxidation, the enzyme catalyses the transfer of an electron from the substrate, leading to the formation of radical species. These species, in turn, participate in non-enzymatic reactions that result in the decomposition of the dye [66], which would

explain the changes observed in the spectra of the dyes in the presence of the enzymes evaluated.

On the other hand, the spectrophotometric analysis of the RB5 treated with *T. maxima* CU1 revealed significant transformations in the visible region (500–680 nm). In particular, a decrease in absorption intensity was observed in this zone, accompanied by a shift and flattening of the peak at 390 nm (Fig. 4B). Additionally, there was an increase and expansion of the peak to 300 nm. On the other hand, the treatment with *T. hirsuta* CS5 presented spectral changes different from those observed with *T. maxima* CU1. Specifically, a decrease in absorbance was evidenced at 590 nm, while the peak at 390 nm and the trough at 350 nm experienced an increase in intensity. Likewise, the peak at 270 nm showed a rise, forming a plateau with the peak at 300 nm. These differences could be associated with the kinetic properties of the house isoforms present, as mentioned above. In this context, it is pertinent to consider that, in the case of azo dyes, the mechanism of action of the laccase consists of the breaking of azo bonds ($-N=N-$), which results in the formation of aromatic amines [67,68]. The spectrophotometric analysis of RB5 treated with *T. maxima* CU1 and *T. hirsuta* CS5 reveals distinct transformations in the visible region, which can be attributed to the specific isoforms of the house produced by these fungi. In this sense, laccases, as oxidoreductases, are known for their ability to oxidise a wide range of substrates, including azo dyes, breaking azo bonds to form aromatic amines [69]. The observed spectral changes, including decreased absorption intensity and peak shifts, are indicative of laccase-mediated transformation of the dye structure. The differences in spectral changes between treatments with *T. maxima* CU1 and *T. hirsuta* CS5 could be due to variations in the kinetic properties and isoform composition of the laccases produced by these fungi [45]. This is consistent with the findings for the three isoforms of *Pleurotus ostreatus* HAUCC 162 laccases, which exhibited different degradation capacities for the azo dye Methyl Orange [70].

In the case of CV treated with *T. hirsuta* CS5, no significant changes were observed in the spectral structure of the dye. The original pattern remained largely intact, with only an overall increase in absorbance, particularly at the main peak at 595 nm. The most significant decrease in the intensity of this peak occurred at 48 h, indicating low efficiency in the transformation of the dye by this strain. In contrast, treatment with *T. maxima* CU1 resulted in modifications in spectral traces over time, including a shift in the main peak from 595 nm, as well as alterations in the peaks at 300 and 250 nm. The latter were associated with an increase in wavelength and the formation of a plateau in that region, with a peak of less than 300 nm. These changes can be explained by the mechanism of action of laccase in the degradation of triarylmethane dyes, which involves monoelectronic oxidation coupled with the reduction of oxygen to water, resulting in the formation of phenoxy radicals. These radicals subsequently undergo coupling reactions that degrade triarylmethane dyes into simpler, less harmful molecules [71]. The treatment of synthetic dyes with *T. hirsuta* has shown varying degrees of efficacy, as evidenced by the contrasting results observed with different strains [56]. While *T. hirsuta* CS5 showed minimal spectral changes in CV degradation, with only an overall increase in absorbance at 595 nm and a significant decrease in intensity after 48 h, indicating a low transformation efficiency [72], *T. maxima* CU1 demonstrated remarkable modifications in spectral traces, including a shift of the main peak and alterations at lower wavelengths, suggesting a more efficient degradation process [73]. The laccase's mechanism of action, which involves monoelectronic oxidation and the formation of phenoxy radicals, plays a crucial role in the degradation of triarylmethane dyes, leading to the production of less harmful compounds [74]. In addition, studies have shown that *T. hirsuta* Bm-2 laccases can effectively decolorize various dyes, with optimal conditions that improve their catalytic efficiency [75]. This highlights the potential of utilising different strains and optimizing conditions to improve dye degradation in wastewater treatment applications. Consequently, it will be necessary to assess the toxicity of the degradation products generated in future research.

Overall, the results demonstrate that *Trametes maxima* CU1 exhibits rapid and efficient decolorization of structurally diverse dyes, particularly anthraquinone and azo compounds, within significantly shorter timeframes compared to previously reported systems. This performance, achieved without the addition of redox mediators, highlights the intrinsic catalytic efficiency of its enzymatic system. Nevertheless, further studies are required to elucidate the degradation pathways and assess the toxicity of the resulting metabolites. These findings position *T. maxima* CU1 as a promising candidate for the development of enzymatic processes for the treatment of dye-contaminated effluents.

At the molecular level, laccase-mediated dye degradation involves single-electron oxidation reactions that generate reactive radical species. These radicals can subsequently undergo a series of non-enzymatic transformations, including cleavage of chromophoric groups such as azo bonds ($-N=N-$) and structural modifications in anthraquinone and triphenylmethane dyes, leading to loss of color. In addition, laccases may act directly on phenolic-moieties or indirectly via redox mediators, which extend their substrate range and enhance the oxidation of more recalcitrant compounds. These mechanisms are consistent with the spectral changes observed in the present study [67–69].

On the other hand, the toxicity of dyes biodegraded by basidiomycete laccase is a well-researched area, with several studies indicating that the degradation products of these dyes are generally less toxic than the original compounds. Laccases, particularly those from basidiomycetes, have shown significant potential in the biodegradation of various synthetic dyes, including azo, triphenylmethane, and anthraquinone dyes, which are known for their environmental persistence and toxicity [76]. For instance, the laccase from *Lentinus* sp. was effective in degrading Congo Red, a known mutagen and carcinogen, resulting in low ecotoxicity of the byproducts [77]. Furthermore, the study involving *Pleurotus ostreatus* laccase demonstrated effective decolorization of various dyes in textile effluents, highlighting its eco-friendly and economical potential for reducing dye toxicity [78]. Additionally, the degradation of Alizarin Cyanine Green by *Stropharia* sp. laccase resulted in less toxic byproducts, as confirmed by phytotoxicity studies [79]. These findings collectively underscore the efficacy of basidiomycete laccases in not only decolorizing but also detoxifying synthetic dyes, making them valuable tools for environmental remediation efforts.

3.4. Waste water decolorization

The percentages of wastewater discoloration are presented in Fig. 5 and were calculated at wavelengths of 293 and 613 nm, corresponding to the peaks of maximum absorbance in the ultraviolet and visible regions, respectively. These wavelengths were selected because the sample, provided by Servicios de Agua y Drenaje de Monterrey, I.P.D., corresponds to an effluent of unknown composition with intense coloration, which makes it challenging to determine specific physicochemical parameters (see Supplementary Material, Figure S2). The physicochemical analysis is shown in Supplementary Material, Table S2, where key indicators of water quality, such as pH, are reported, despite the analytical limitations derived from this coloration.

In the 293 nm range, *T. hirsuta* CS5 showed the highest activity at 6 h with more than 50% discoloration. While *T. maxima* CU1 and *T. versicolor* UAMH 8272 bleached 21 and 17%, respectively. However, after 24 h, they presented similar percentages of discoloration (< 20%). Although in the visible region (613 nm), *T. maxima* CU1 showed the highest bleaching activity at 6, 12, and 24 h of incubation, with discoloration percentages of 60.0%, 46.9%, and 47.8%, respectively. In contrast, *T. hirsuta* CS5 displayed similar results after 6 h (59.7%). *T. versicolor* UAMH 8272 did not exceed 10% discoloration.

Spectrophotometric analysis (Fig. 6) of the industrial effluent revealed a complex signal with a characteristic plateau in the visible region (580–630 nm), marked by absorbance maxima at 590 and 612 nm, and sharp peaks in the UV region at 290 and 306 nm. These patterns are typical of mixtures of conjugated aromatic dyes and

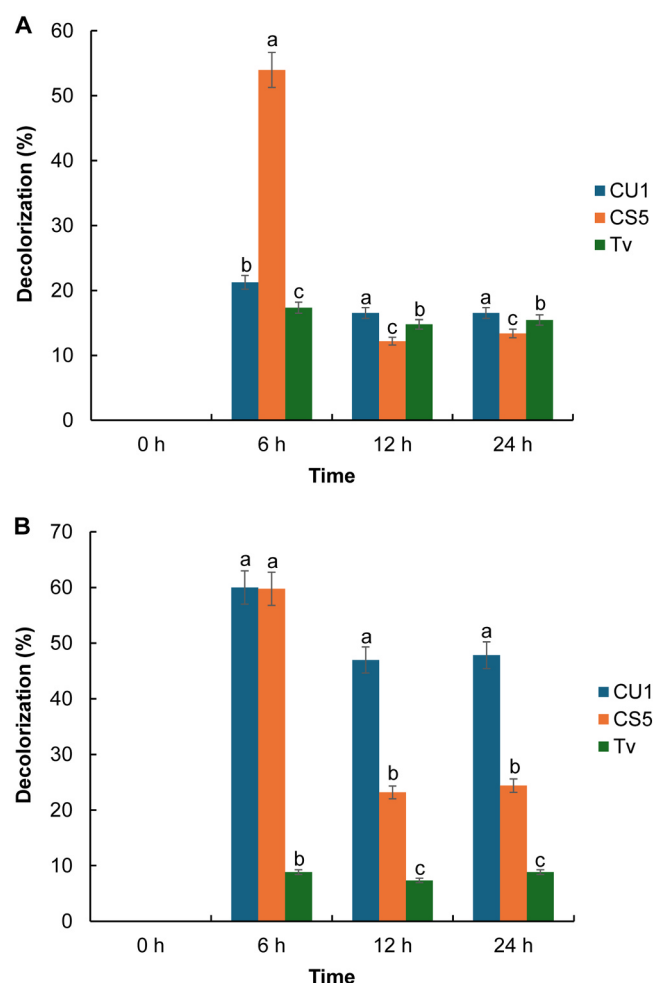


Fig. 5. Industrial effluent decoloration. A corresponds to the percentage of decoloration at 293 nm and B to that observed at 613 nm. CU1: *T. maxima* CU1, CS5: *T. hirsuta* CS5, and T.V.: *T. versicolor* UAMH 8272 as a reference strain. ^{a-c} Different literals represent statistically significant differences ($p \leq 0.05$) between means at the different times evaluated.

phenolic compounds, which are common in textile and chemical discharges [75]. During treatment with fungal supernatants, notable variations in the kinetics and spectral selectivity of the decoloration were observed. In particular, treatment with *T. maxima* CU1 resulted in a rapid decrease in the absorbance of visible peaks, with reductions of 60% and 63% at 590 and 612 nm, respectively, within the first 6 h (Fig. 6A). In contrast, the peaks in the UV region exhibited slower decoloration, with reductions of 25% and 28% in the peaks at 290 and 306 nm, respectively. In comparison, *T. hirsuta* CS5 and *T. versicolor* UAMH 8272 exhibited similar spectral behaviours, although with lower efficacy (Fig. 6B and C, respectively). *Trametes hirsuta* CS5 reached 59.76% visible decoloration after 6 h, but this efficiency dropped significantly to 24.39% after 24 h. In the UV region, CS5 exhibited decreasing kinetics (from 53.9% to 13.4%), suggesting an initial but transient mechanism of action, possibly related to a loss of enzyme activity, substrate depletion, or the generation of new products [80]. *Trametes versicolor* UAMH 8272 showed values of less than 9% in the visible region and less than 16% in the UV, suggesting a low overall bleaching efficacy. However, its kinetic profile was more stable, and its spectral selectivity suggests preferential activity on monomeric phenolic compounds, following the literature for *T. versicolor* laccases [81]. Thus, treatment with *T. maxima* CU1 was found to be the most effective for decolorising industrial dyes, especially in the visible region. At the same time, *T. hirsuta* CS5 showed initial efficacy followed by rapid loss of

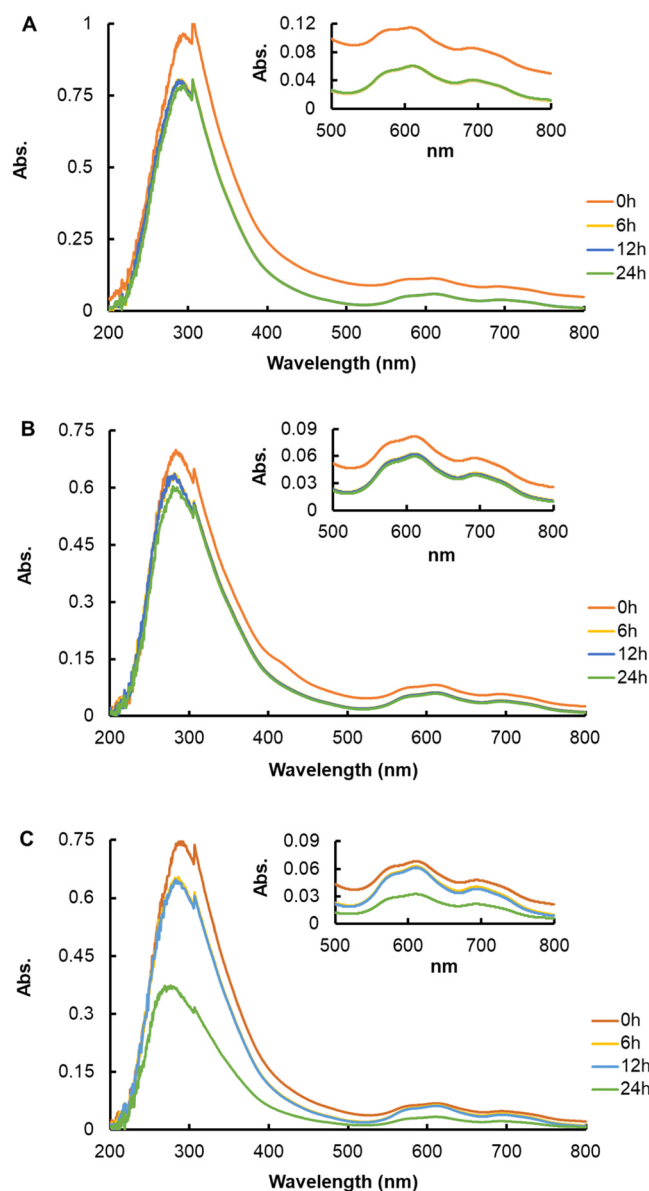


Fig. 6. Absorbance spectra during decoloration of wastewater treated with fungal supernatants. A) *T. maxima* CU1, B) *T. hirsuta* CS5 and C) *T. versicolor* UAMH 8272. The curves show the evolution of absorbance (Abs) in the range of 200–800 nm at different treatment times (0, 6, 12 and 24 h). The insets show a magnification of the visible region (500–800 nm) to detail the changes in peaks associated with conjugated dyes.

activity. *Trametes versicolor* UAMH 8272, although less effective, maintained a more stable kinetics and showed a preference for simpler phenolic compounds. These results indicate significant differences in enzyme stability and spectral selectivity between species, with important implications for biotechnological applications in the treatment of industrial effluents. These differences highlight the importance of enzymatic stability and spectral selectivity in the application of white-rot fungi for industrial effluent treatment. The implications for biotechnology applications are significant, as these fungi offer an eco-friendly and cost-effective alternative to conventional dye removal methods, which are often expensive and require specialized expertise [82,83]. The stable performance of *T. maxima* CU1, in particular, suggests its potential for long-term applications in continuous flow systems, where constant enzyme activity is essential [84]. Overall, the differential enzyme stability and substrate specificity among these *Trametes* species highlight the need for tailored approaches to industrial effluent

bioremediation, taking advantage of the unique capabilities of each fungal strain [82]. These results reinforce the need to optimize physicochemical parameters of the treatment such as pH, enzyme concentration and contact time to maximize efficiency. Previous studies have shown that *T. hirsute* strains achieved decolorization efficiencies greater than 95% under controlled conditions [85], and that enzymatic immobilization can significantly improve the stability and reuse of laccases in industrial media [70].

From an environmental and regulatory perspective, fungal-based and enzymatic treatments represent promising alternatives to conventional physicochemical processes. These systems typically operate under mild conditions and reduce the need for hazardous chemicals, which may contribute to lower sludge generation and decreased environmental impact. In addition, the use of enzymatic extracts rather than live microorganisms may facilitate regulatory acceptance by minimizing concerns associated with the release of non-native species into the environment. Furthermore, the implementation of such biotechnological approaches has the potential to reduce the ecological footprint of wastewater treatment processes, particularly in the context of dye-contaminated effluents. However, further studies are required to evaluate process scalability, long-term environmental impact, and compliance with regulatory frameworks under real operating conditions [86, 87]. However, further studies are required to evaluate process scalability, long-term environmental impact, and compliance with regulatory frameworks under real operating conditions.

3.5. Energy consumption

The determination of the E_{IMP} in the process, showed that electricity was the carrier with the highest energy consumption (Table 2), representing 76.13% of the total energy consumed (Fig. 7). This result was associated with the continuous use of electricity, since it is the primary source of energy for the operation of the equipment and technology used in the laboratory. Likewise, the was decisive in the E_{HL} representing 23.83% of the total energy consumed, subtracting only 0.04% for the consumption of other forms of energy (E_{CR} and E_{OI}).

The energy breakdown of the process showed that the team of greater ($409.44 \text{ kW}\cdot\text{h}^{-1}$) and thus energy (4884.62 MJ) was the autoclave, used for a period of 6 h to sterilize the culture media. This electrical equipment required 59.03% of the total electricity consumption, in addition, demanded $44.94\% \approx 45\%$ of the total energy consumed in the process. Although advanced heat transfer elements within autoclaves facilitate efficient temperature management and ensure uniform heat distribution, improving the quality and consistency of processed materials [88], strategies are necessary to make working with these devices more efficient. Therefore, understanding of the process involves examining a range of aspects, from measurement techniques to consumption patterns and the impact of energy sources [89].

The higher E_{HL} was due to the use of the spectrophotometer that

Table 2

Energy consumption in the selection of the house with application in water treatment.

Energy Form	Stage 1	Stage 2	Stage 3	$E_{IMP}(\text{MJ})$
E_{EC}	1628.22	3171.01	3475.69	8274.92
E_{HL}	58.83	2495.15	35.58	2589.56
E_{CR}	2.06	1.13	0.87	4.05
E_{OI}				
Yeast	0.111	0.1	0.11	0.32
Glucose	0.20	0.11	0.23	0.54
Water	0.04	0.00	0.01	0.04
Total consumption (MJ)	1689.46	5667.49	3512.48	10869.43

E_{IMP} = energy consumption or energy input; E_{EC} = energy consumed in electricity; E_{HL} = energy consumed in human labour; E_{CR} = energy consumed in chemical reagents; E_{OI} = energy consumed in other inputs.

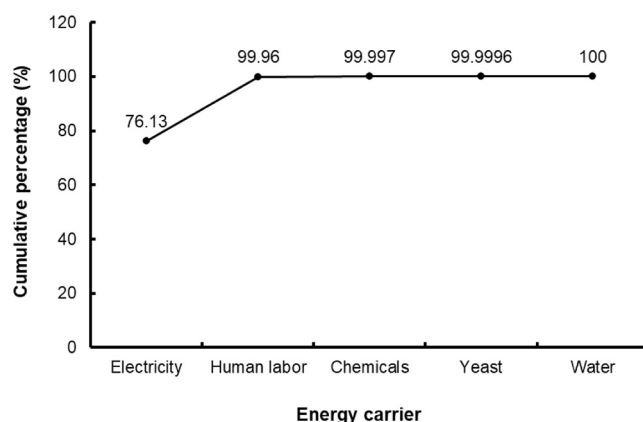


Fig. 7. Cumulative percentage of the energy consumption of the different carriers used in the process.

worked a total of 1261 h during the process, with an energy consumption of 1328.25 MJ, equivalent to 12.22% of the total energy consumed in the process. The energy associated with human labour is often neglected in traditional energy analyses. For example, in manufacturing, it is estimated that each worker-hour consumes about 30 MJ of primary energy in the United States [90]. Therefore, determining the energy demand of human labour is crucial for accurate energy assessments and for promoting sustainable practices in various sectors [91].

The consumption index established by equipment, stage and process in general (Table 2), is the beginning of the study of management, control and rational use of energy. Therefore, the control and management of electricity consumption together with labour are crucial to optimize energy use and the implementation of strategies and actions is required. In this regard, Tantidham et al. [92], propose a method that uses historical consumption data to predict future electricity needs and develop control strategies for different groups of electrical equipment. This approach addresses the complexity of managing various electrical devices and aims to improve control over electricity consumption. In terms of querying and monitoring electricity usage, methods have been developed that allow users to flexibly query the electricity consumption of specific devices over a specified period. This system updates records in real-time, providing users with accurate and adequate information about their electricity consumption [93]. Likewise, devices and methods have been used to measure electricity consumption, which involves detecting consumption values and adjusting them according to voltage variations. This method ensures accurate measurement and can be crucial for both consumers and utility providers [94]. The prediction of electricity consumption is another critical factor, as highlighted by Chen et al. [95], who use a function method to identify effective impact factors that influence electricity consumption. By incorporating these factors into a predictive model, the method improves the accuracy and speed of prediction, making it a valuable tool for energy management. Additionally, the E_{HL} can be reduced through effective time planning, concentrating workloads, and making efficient use of workspaces [96]. Likewise, technological advances can reduce labour and lead to more efficient production methods [97]. In general, energy consumption in bioprocesses is influenced by the specific methods and technologies employed, the scale of operations, and the need for precise control of environmental conditions. Therefore, the optimisation and strategic management of energy are essential to improve the efficiency and sustainability of processes. The research underscores the importance of selecting the right technologies and employing advanced optimisation techniques to minimise energy use while maintaining process efficiency. The results of this research represent the basis for a subsequent energy study and an optimization process with a sustainable approach that allows seeking and implementing strategies that contribute to reducing energy costs and making a rational use of resources.

From an energy perspective, conventional physicochemical methods for dye removal, such as advanced oxidation processes, adsorption, and chemical treatments, are often associated with relatively high energy demands due to aeration, chemical production, or pressurized operating conditions. In contrast, fungal-based and enzymatic treatments generally operate under milder conditions, such as ambient temperature and pressure, which may contribute to reduced energy requirements. In addition, strategies such as the use of crude enzymatic extracts, process optimization to reduce reaction times, and the integration of low-energy treatment configurations may further enhance the sustainability and energy efficiency of these systems [98,99].

From an economic perspective, conventional physicochemical methods for dye removal are often associated with high operational costs due to chemical consumption, energy requirements, and sludge generation and disposal. In contrast, fungal-based and enzymatic treatments may offer cost advantages by operating under mild conditions, reducing energy demand, and enabling the use of low-cost substrates or crude enzymatic extracts that avoid expensive purification steps. Nevertheless, a comprehensive techno-economic assessment is required to fully evaluate the feasibility and scalability of these systems [98,99].

4. Conclusions

Trametes maxima CU1 was the fungal strain with the highest bleaching potential over the synthetic dyes and the real effluent evaluated in the present research. After an exhaustive analysis of four basidiomycete strains and one collection strain, *T. maxima* CU1 exhibited the highest bleaching capacity in solid medium, associated with the production of laccases with high redox potential. The strain demonstrated remarkable efficiency in colour removal in Reactive Blue 19, achieving an almost complete disappearance of the dye from the first hour of treatment. However, the dyes of the triarylmethane and azo groups presented discoloration percentages of less than 60%. In the real wastewater system, *T. maxima* CU1 showed increased activity in the transformation of the sample, evidenced by changes in the spectrum, mainly in the region of 580–630 nm. The study made it possible to determine the energy expended and to establish an index of energy consumption by carrier, stage and process. Electricity (8274.92 MJ) was the energy carrier with the highest consumption, followed by labour consumption (2589.56 MJ). Thus, bioremediation with this fungal strain represents a sustainable and effective alternative for the management of this type of effluent. Although more research is required to identify the degradation products of the industrial effluent, its toxicity and the optimization of the operational factors for the scaling up of the treatment of colour-impacted wastewater using the *T. maxima* CU1 laccase.

Abbreviations

The following abbreviations are used in this manuscript:

Abs	Absorbance
BF	Kellogg's All Bran Flakes medium
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
C_R	Chemical reagent(s) consumed (kg)
CS200	<i>Funalia trogii</i> CS200
CS5	<i>Trametes hirsuta</i> CS
CU1	<i>Trametes maxima</i> CU1
CV	Crystal Violet
D.I.	Decolorization Index or reaction in the case of syringaldazine
DMP	2,6-dimethoxyphenol
E_C	Electricity consumption (kWh) by the equipment(s) used
E_{EC}	Energy consumed (MJ) in electricity
E_{HL}	Energy consumed (MJ) by human labour
E_{IMP}	Energy input
E_{OI}	Energy consumed (MJ) by other inputs such as water, enzymes, glucose and yeasts

(continued on next column)

(continued)

GE01	<i>Pycnoporus sanguineus</i> GE01
I_C	Input(s) consumed (kg)
KM	Modified Kirk medium
KM+I	Modified Kirk medium supplemented with CuSO_4 350 μM
Lac	Laccase
LiP	Lignin peroxidase
L_T	Labour time (h) of the operator(s) per unit of production
MnP	Manganese peroxidase
Q_E	Energy equivalent of electricity ($\text{MJ}\cdot\text{kWh}^{-1}$)
Q_H	Energy equivalent of human labour ($\text{MJ}\cdot\text{h}^{-1}$)
Q_I	Energy equivalent of the input ($\text{MJ}\cdot\text{kg}^{-1}$)
QR	Energy equivalent of the chemical reagent ($\text{MJ}\cdot\text{kg}^{-1}$)
RB19	Reactive Blue 19
Syr	Syringaldazine
T.V.	<i>Trametes versicolor</i> UAMH 8272
TDS	Total dissolved solids
TSS	Total suspended solids

CRediT authorship contribution statement

Abelardo Chávez-Montes: Writing – review & editing, Visualization. **Celestino García-Gómez:** Visualization, Validation, Resources. **Juan Francisco Villareal-Chiu:** Writing – review & editing, Visualization, Validation, Resources. **Laura Levin:** Writing – review & editing, Visualization, Validation, Resources. **Iqbal Hafiz M. N.:** Writing – review & editing, Visualization, Resources. **Guadalupe Gutiérrez-Soto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Parra Saldivar Roberto:** Writing – review & editing, Visualization, Supervision, Resources. **Angeles González-Urbina:** Investigation, Data curation. **Thakur Aman:** Writing – review & editing, Writing – original draft. **Iosvany López-Sandin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Gustavo Hernández-Vidal:** Writing – review & editing, Visualization, Conceptualization. **Uziel Castillo-Velázquez:** Writing – review & editing. **Joel Horacio Elizondo-Luevano:** Writing – review & editing.

Informed consent statement

Not applicable.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.enzmictec.2026.110888](https://doi.org/10.1016/j.enzmictec.2026.110888).

Data availability

Data supporting this study are openly available from <https://dspace.lib.cranfield.ac.uk/handle/1826/25197> at <https://doi.org/10.57996/cran.ceres-2850>

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