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Ligninolytic enzyme production and dye decolorization by *Trametes trogii*: application of the Plackett–Burman experimental design to evaluate nutritional requirements

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Abstract

The effects of medium components on laccase, manganese peroxidase and glyoxal oxidase production by *Trametes trogii* (MYA 28-11) have been studied using Plackett–Burman (PB) experimental design. Copper had the highest positive influence on ligninolytic enzyme production. *T. trogii* extracellular fluids obtained in the medium which rendered the highest ligninolytic production (45.32 U/ml laccase, 214.5 mU/ml manganese peroxidase and 116 mU/ml glyoxal oxidase) also showed the greatest ability to decolorize the dyes Ponceau 2R (a xylidine derivative), malachite green and anthraquinone blue (at rates of 2.14, 1.35 μg and 3 mg dye/(ml h), respectively). The relationship among decolorization rates and ligninolytic enzyme activities was analyzed by multiple regression. The results fit a linear plus interactions model. The comparison of the response surfaces obtained, suggests that while laccase activity has a greater importance in xylidine degradation, manganese peroxidase activity plays the major role in malachite green decolorization.

Keywords: Plackett–Burman design; Ligninolytic enzymes; Dye decolorization; Nutritional requirements; White rot fungi; *Trametes trogii*

1. Introduction

Approximately 10,000 different dyes and pigments are produced annually worldwide and used extensively in the dye and printing industries. Synthetic dyes are chemically diverse, with the commonly used in industry divided into those of azoic, triphenylmethane or heterocyclic/polymeric structures. Product processing methods often cause a loss of large amounts of dyes to wastewaters, representing 10–15% of the dyes applied. Several of these dyes are very stable to light, temperature and microbial attack, making them recalcitrant compounds, many of them are also toxic [1].

White-rot fungi, a group of lignin-degrading basidiomycetes, have received considerable attention for their bioremediation potential. This capability to degrade lignin is due to their extracellular nonspecific and nonstereoselective enzyme system composed by laccases (EC 1.10.3.2), lignin peroxidases (LiP, EC 1.11.1.14) and manganese peroxidases (MnP, EC 1.11.1.13), which function together with H_2O_2-producing oxidases and secondary metabolites. White-rot fungi secrete one or more of the three enzymes essential for lignin degradation. The same unique nonspecific mechanisms that give these fungi the ability to degrade lignin also allow them to degrade a wide range of pollutants, among them: polycyclic aromatic hydrocarbons, chlorinated phenols, polychlorinated biphenyls, dioxins, pesticides, explosives and dyes. Purified laccases, LiPs and MnPs are able to decolorize dyes of different chemical structure [2].

A majority of the previous studies have focused on the lignin-degrading enzymes of *Phanerochaete chrysosporium* and *Trametes versicolor*. Recently however, there has been a growing interest in studying the lignin-modifying enzymes of a wider array of white-rot fungi, not only from the

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standpoint of comparative biology but also with the expectation of finding better lignin-degrading systems for use in various biotechnological applications [3]. The white-rot fungus *Trametes trogii* has received little research attention although it is an outstanding laccase producer [4] and its ability to degrade high priority pollutants such as polychlorinated biphenyls, polyaromatic dyes and polycyclic aromatic compounds has been recently demonstrated [5–7].

For a broad application, the cost of enzymes is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium is the basic research for industrial application. The use of different statistical designs for medium optimization has been recently employed for xylanase, amylase and laccase production by fungal cultures [8–12]. These statistical methods, as compared to the common ''one-factor-at-a-time'' method proved to be powerful and useful tools.

The objective of this study was to evaluate the relative importance of various nutrients for ligninolytic enzyme production by *T. trogii* in submerged fermentation. The ability of the culture fluids to decolorize different synthetic textile dyes and the role of ligninolytic enzymes in the decolorization process was also investigated. The Plackett–Burman (PB) experimental design [13] was applied to maximize enzyme production and decolorization efficiency.

2. Materials and methods

2.1. Microorganism

Strain MYA 28-11 (BAFC 463) of *T. trogii* (*Funalia trogii*) (Berk. In Trog.) Bond & Singer (Aphyllophorales, Basidiomycetes), maintained at 4 °C on malt agar slants.

2.2. Culture media

Glucose/asparagine (GA) medium ($MgSO_4 \cdot 7H_2O$, 0.5 g; H_2KPO_4, 0.5 g; HK_2PO_4, 0.6 g; H_3BO_3, 0.07 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.02 mg; $FeCl_3$, 1 mg; $ZnCl_2$, 3.5 mg; thiamin hydrochloride, 0.1 mg; and different concentrations of glucose (10–20 g); L-asparagine monohydrate (0.5–4 g); $CuSO_4 \cdot 5H_2O$ (1.6–667 μM); $MnCl_2 \cdot 4H_2O$ (0.45–333 μM); veratryl (3,4-dimethoxybenzyl) alcohol (VA) (0–2 mM); Tween 80 (polyoxyethylene sorbitan mono-oleate) (0–1.7 mM); distilled water up to 1 l). The initial pH of the medium was adjusted to values among 4–6.5 with either NaOH 1 N or HCl 1 N after autoclaving.

2.3. Culture conditions

Incubation was carried out at 28 ± 1 °C under stationary conditions in 250 ml Erlenmeyer flasks containing 25 ml of medium, which were inoculated with two agar plugs (each of 0.25 cm^2) from a 5-day-old colony grown on Bacto-agar 2%. On day 23, fungal cultures were filtered through a filter paper using a Büchner funnel and culture supernatants were used as enzyme sources.

2.4. Experimental design and statistical analysis

The Plackett–Burman experimental design was used to evaluate the relative importance of various nutrients for ligninolytic enzyme production by *T. trogii* in submerged fermentation. This design assumes that there are no interactions between the different media constituents, x_i, in the range of variables under consideration. A linear approach is considered to be sufficient for screening.

$$Y = \beta_o + \sum \beta_i x_i \quad (i = 1, \ldots, k)$$

where Y is the estimated target function and β_i are the regression coefficients. The PB design is a fractional factorial design and the main effect (the contrast coefficient, b) of such a design may be simply calculated as the difference between the average of measurements made at the high level (+1) of the factor and the average of measurements made at the low level (−1). Contrast coefficients allow the determination of the effect of each constituent. A large contrast coefficient either positive or negative indicates that a factor has a large impact on titre; while a coefficient close to zero means that a factor has little or no effect. The P-value is the probability that the magnitude of a contrast coefficient is due to random process variability. A low P-value indicates a ''real'' or significant effect. The significance of each variable was determined by applying the Student's t-test [9,13]. Seven variables representing six nutritional components and initial pH of the medium were used. For each variable a high (+1) and a low (−1) concentration were tested (Table 1). The rows in Table 2 represent the 12 different trials and each column represents a different variable. Experiments were replicated three times. The statistical analyses were performed by use of multiple regressions and ANOVA with the softwares Minitab v 13.1 and Essential Regression v 2.2.

Table 1
Plackett–Burman design for medium optimization, positive (+1) and negative (−1) levels of independent variables used in trials

Factor setting	Experimental variables						
	Glucose (g/l)	Asparagine (g/l)	Tween 80 (mM)	VA (mM)	Cu^{2+} (μM)	Mn^{2+} (μM)	pH
High (+1)	20	4	1.7	2	667	333	6.5
Low (−1)	10	0.5	0	0	1.6	0.45	4

Table 2
Plackett–Burman design for medium optimization and measured response

Trial	Experimental variables							Volumetric enzyme activity			Decolorization ability (ΔA/(min l))[a]		
	Glucose (g/l)	Asparagine (g/l)	Tween (mM)	VA (mM)	Cu^{2+} (μM)	Mn^{2+} (μM)	pH	Laccase (U/ml)	MnP (mU/ml)	GLOX (mU/ml)	Ponceau 2R	Malachite green	Anthraquinone blue
1	10	0.5	0	2	667	333	4	18.18	47	31	1.73	6.94	528.95
2	10	0.5	1.7	2	667	0.45	6.5	2.14	10.5	35.5	0.53	3.51	450.17
3	20	4	0	2	667	0.45	6.5	45.32	214.5	116	1.88	7.64	677.50
4	10	4	0	0	1.6	333	6.5	1.05	4	5.3	0.34	2.24	94.53
5	20	4	0	2	1.6	0.45	4	2.13	62	24.5	0.65	3.44	508.69
6	10	0.5	0	0	1.6	0.45	4	0.09	2	0	0.04	0.39	9.00
7	20	4	1.7	0	667	333	4	18.38	105	40.5	0.74	4.76	544.70
8	20	0.5	1.7	0	1.6	0.45	6.5	0.28	3	0	0.07	0.48	13.50
9	20	0.5	0	0	667	333	6.5	1.38	4	6.5	0.22	2.20	38.26
10	10	4	1.7	2	1.6	333	6.5	0.82	10	6.8	0.20	0.91	24.76
11	20	0.5	1.7	2	1.6	333	4	0.56	3	2.1	0.14	0.87	29.26
12	10	4	1.7	0	667	0.45	4	2.46	21	22	0.42	2.39	182.32

[a] Decolorization activity is estimated as the decrease in absorbance at the maximum visible wavelength for each dye.

2.5. Enzyme assays

Laccase activity was measured with 2,2′-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M sodium acetate buffer (pH 3.4) at 30 °C. Oxidation of ABTS was determined by the increase in A_{420} (ε_{420} = 36 mM^{-1} cm^{-1}) [14]. Manganese peroxidase activity (MnP) was measured using phenol red as the substrate in 0.1 M sodium dimethylsuccinate buffer (pH 4.5) at 30 °C (ε_{610} = 22 mM^{-1} cm^{-1}) [15]. Glyoxal oxidase activity (GLOX) was determined by using a peroxidase-coupled assay with methylglyoxal as GLOX substrate and phenol red as the peroxidase substrate (ε_{610} = 22 mM^{-1} cm^{-1}) [16]. Lignin peroxidase activity (LiP) was assayed by the Azure B method [17]. International enzymatic units (U) were used (μmol product/min). Enzyme activity was expressed as U/ml of culture filtrate.

2.6. In vitro decolorization of dyes

The reaction was carried out in test tubes at 30 °C, the reaction mixture contained sodium acetate buffer (10 mM; pH 4.5), an aliquot of crude filtrate, and either the azoic dye Ponceau 2R (8.5 mg/l), the triphenylmethane dye malachite green (2.7 mg/l), or the anthraquinonic dye anthraquinone blue (50 mg/l), in a total volume of 3 ml. Dyes were added to a concentration, which gave the same initial absorbance (1.0 absorbance unit at the maximum wavelength in the visible spectrum). Decolorization activity was estimated as the decrease in absorbance at the maximum visible wavelength for each dye (respectively, 505, 615 and 620 nm). A control in which the extracellular liquid was replaced by distilled water was conducted in parallel. Results are expressed as ΔA/(min l).

2.7. Chemicals

Anthraquinone blue was a gift from Dr. A. Vitale (Department of Organic Chemistry, Faculty of Exact and Natural Sciences, University of Buenos Aires); malachite green was from Mallinckrodt (Phillipsburg, NJ, USA). All other chemicals were from Sigma (St. Louis, Mo, USA).

3. Results and discussion

Statistical methods for medium optimization have proved to be a powerful and useful tool for biotechnology. Therefore, we attempted to improve the composition of the medium by simultaneous comparisons between two levels of several factors, applying the Plackett–Burman experimental design. The factors tested included different concentrations of the medium components: glucose; asparagine; Cu^{2+}; Mn^{2+}; veratryl alcohol (VA); Tween 80; and the initial pH value after sterilization. Those factors hold the most promise for optimizing the fermentation on the basis of previous studies in this strain [4,18]. Cultures were harvested on day 23, on that day in different media assayed previously, *T. trogii* cultures were at the beginning of idiophase. Although laccase and GLOX activities appeared before mycelial biomass peaked (i.e. they were present in the primary growth phase), they showed maximum activity at the beginning of the secondary metabolism. On the other hand, MnP activity was mainly detected in the idiophase when the glucose in the culture medium was exhausted and the mycelial dry weight was decreasing [4,18]. Samples were taken on day 23 to guarantee appreciable levels of the three enzymes tested.

A summary of the variables and their variation level is given in Table 1. The comparison of ligninolytic enzyme production in different media and the dye decolorization abilities in such media is given in Table 2, along with the design of the experiment. Medium "3" gave the maximum enzyme yield, followed by medium "7" and medium "1". The increase in yield is noteworthy. Incorporation of most suitable conditions and supplements to the medium resulted in about 100-fold increment in MnP and GLOX titers and 500-fold increase in laccase production. Attempts to detect

Table 3
Degree of positive or negative effects of various nutrients medium components and pH, on ligninolytic enzyme production by *T. trogii* according to the Plackett–Burman experimental design

Factor	Laccase activity		MnP activity		GLOX activity		Ponceau 2R decolorization		Malachite green decolorization	
	b	*P*-value	*b*	*P*-value	*b*	*P*-value	*b*	*P*-value	*b*	*P*-value
Constant	7.10[a]	0.060	37.68	0.017	24.73	0.003	0.58	0.002	3.12	<0.001
Glucose	7.22	NS[b]	49.50	0.092	14.82	NS	0.08	NS	0.50	NS
Asparagine	7.92	NS	57.84	0.058	23.34	0.078	0.26	NS	1.16.	NS
Tween	−7.26	NS	−30.16	NS	−12.74	NS	−0.46	0.098	−1.66	0.095
VA	7.58	NS	34.66	NS	23.60	0.075	0.56	0.058	1.80	0.073
Cu^{2+}	13.82[c]	0.082	53.00	0.076	35.46	0.018	0.68	0.028	3.18	0.009
Mn^{2+}	−2.01	NS	−23.34	NS	−17.64	NS	−0.02	NS	0.02	NS
pH	1.53	NS	1.00	NS	8.34	NS	−0.08	NS	−0.30	NS

Main effects (b) (laccase activity (U/ml), MnP activity (mU/ml), GLOX activity (mU/ml), Ponceau 2R and malachite green degradation (ΔA/(min l); and significance levels (*P*-values) of the experimental variables for coded values.

[a] Constants are expressed as estimated coefficients.

[b] NS: non-significant at $P < 0.1$.

[c] Indicates a significant positive effect.

LiP were unsuccessful. The negative LiP tests suggest that the fungus produces no significant levels of this enzyme or its production requires different growth conditions. LiP was detected previously, when this strain was grown in a wood-containing medium [18]. Best enzymatic activity (45.32 U/ml laccase, 214.5 mU/ml MnP and 116 mU/ml GLOX) was achieved in a medium containing glucose 20 g/l, asparagine 4 g/l, copper 667 μM, manganese 0.45 μM and veratryl alcohol 2 mM, initial pH 6.5. Culture fluids obtained in such medium also showed the greatest ability to decolorize the dyes assayed.

Table 3 shows contrast coefficients and *P*-values of the experimental variables. The *P*-values serve as a tool for checking the significance of each of the coefficients. Their analysis showed that among the factors tested only copper had a significant positive influence on laccase, MnP and GLOX production. MnP production was also enhanced by higher glucose and asparagine concentrations, while GLOX production was stimulated by the increase in asparagine and veratryl alcohol in the medium. Veratryl alcohol and copper concentrations had a significant positive influence on xylidine and malachite green decolorization as well. Other factors proved to be non statistically significant ($P < 0.1$).

Copper has been reported to be a strong laccase inducer in several species, among them *P. chrysosporium* [19] and *T. versicolor* [20]. It is known that copper induces both laccase transcription and activity [20], and the increase in activity is proportional to the amount of copper added. In the case of *T. trogii*, induction of MnP and GLOX activity has been previously observed as well [4].

Aromatic alcohols such as veratryl alcohol (a secondary metabolite produced by ligninolytic cultures of white-rot fungi, known to be a cofactor involved in the degradation of lignin and xenobiotic pollutants by LiP) have been reported to induce laccase production [21]. But in the range assayed, the addition of VA did not significantly influenced laccase and MnP production by *T. trogii*, although it induced GLOX production. On the contrary, VA has been reported to decrease extracellular GLOX activity in *P. chrysosporium* [22], and it differed in its inductive or suppressive effect on MnP production by different fungi as a function of the basal media employed [23]. VA is produced de novo by some white rot fungi and its exogenous addition may further raise the concentration to levels toxic for enzyme production [23].

In contrast to LiP, which in general is best produced under nitrogen starvation conditions; many white rot fungi produce higher titres of laccase and MnP in N-sufficient media [23–25]. In a previous study, *T. trogii* produced the highest amounts of laccase, MnP and GLOX in the simultaneous presence of high concentrations of nitrogen and carbon in the medium in agreement with the fact that its ligninolytic system is expressed constitutively [4].

Due to the participation of peroxidases in lignin breakdown, the extracellular production of H_2O_2 is essential. GLOX activity, which is produced extracellularly and is expressed during secondary metabolism, when the ligninases are also expressed, has been suggested to be the major enzyme responsible for the production of H_2O_2 in *P. chrysosporium* [16]. High levels of GLOX activity were produced by *T. trogii* (up to 0.11 U/ml). Only a few of the 67 strains analyzed by de Jong et al. [26] tested up to 0.003 U/ml of GLOX activity. Kersten [16], using an optimized liquid medium, obtained 0.032 U/ml of GLOX activity in *P. chrysosporium*. Previous reports demonstrated that xenobiotic compound oxidation by white-rot fungi cannot be improved by overproducing peroxidases without increasing the endogenous production of H_2O_2 [25]. Thus, a crude culture filtrate with high ligninolytic and H_2O_2-producing activities such as those produced by *T. trogii* (MYA 28-11), capable of decolorizing dyes with different chemical structures, based on the magnitude of the decoloration obtained, may be a good candidate for the bioremediation of textile processing effluents.

Table 4
Analysis of variance (ANOVA) for the selected linear plus interactions model[a] for malachite green (A) and Ponceau 2R (B) decolorization by *T. trogii*

Source	Sum of squares	Degrees of freedom	Mean squares	*F*-ratio	*P*-value
(A)					
Model	63.43	6	10.57	63.23	<0.001
Linear	49.40	3	9.67	58.23	<0.001
Interaction	14.03	3	4.68	28.15	0.001
Residual error	0.83	5	0.17	–	–
Total	64.26	11	–	–	–
(B)					
Model	4.121	6	0.687	124.87	<0.001
Linear	3.361	3	0.496	90.18	<0.001
Interaction	0.760	3	0.253	46.04	<0.001
Residual error	0.028	5	0.006	–	–
Total	4.148	11	–	–	–

[a] R^2 coefficient of determination = 0.987 for malachite green and 0.993 for Ponceau 2R decolorization.

The relationship among decolorization rates obtained in the experiment and ligninolytic enzyme activities (Table 2) was analyzed by multiple regression. The results obtained for malachite green and Ponceau 2R decolorization fit the following equation: $Y_i = \beta_o + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j$ (where Y_i is the predicted response, x_i, x_j are input variables which influence the response variable *Y*, and β_o, β_i, β_{ij} represent the regression coefficients of the model). The quadratic terms were not statistically significant and therefore they were rejected. The corresponding analysis of variance (ANOVA) is presented in Table 4. The coefficient of determination (R^2) value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The models R^2 (Table 4) (0.993 for Ponceau 2R decolorization, 0.987 for malachite green and 0.968 for anthraquinone blue degradation) suggested that the fitted linear plus interactions models could explain 99.3, 98.7 and 96.8% respectively of the total variation. This implies satisfactory representations of the processes by the models. The *F*-values (63.23, 124.87 and 25.02); and $P < 0.001$ for Ponceau 2R, $P < 0.001$ for malachite green and $P = 0.001$ for anthraquinone blue decolorization, respectively, indicate that the present models are in good prediction of the experimental results. The *P*-values suggest that the coefficient for linear effects of laccase and MnP activities (with values of 0.019 and 0.049 respectively) and the interactions among MnP/laccase (0.009), MnP/GLOX (0.036) and laccase/GLOX (0.067) activities are significant for malachite green decolorization. On the other hand GLOX activity did not significantly affect the decolorization of this dye. However, it was evident from the results that although the linear effect of GLOX activity was not significant for malachite green decolorization, at least in the range assayed, its influence could not be totally overruled because of its interactive effect with laccase and MnP activities. On the other hand, whilst the interaction between laccase and MnP activities was statistically significant ($P = 0.008$) for Ponceau 2R decolorization, other factors proved to be non statistically significant at $P < 0.1$. The differences in the results obtained for both dyes suggest that this fungus implements different strategies to degrade dyes with diverse chemical structure. For anthraquinone blue decolorization all the factors analyzed were non statistically significant at $P < 0.1$. Anthraquinone blue was easily degraded by supernatants of *T. trogii*. The enzymatic activities involved in its degradation were probably not in limiting quantities, allowing significant differences to be visualized by the analysis of variance. On the other hand, anthraquinone blue decolorization might be influenced by other variables not considered in this work. The model equation fitted by regression analysis for malachite green decolorization is given by:

$$y = 0.905 + 1.448A - 0.20B - 0.012AB - 0.026AC + 0.01BC$$

The decolorization of Ponceau 2R may be best predicted by the model:

$$y = 0.085 - 0.002AB$$

where *y* is dye degradation (ΔA/(min l)); *A*, laccase (U/ml); *B*, MnP (mU/ml) and *C*, GLOX (mU/ml) activities.

The 3D response surfaces are the graphical representations of the regression equations for Ponceau 2R (Fig. 1A) and malachite green decolorization (Fig. 1B–D). They depict the effect of pair wise interaction of the parameters, when the third parameter is kept constant. The main goal of response surface is to hunt efficiently for the optimum values of the variables such that the response is maximized. Fig. 1A shows the effect of interaction of laccase and MnP activities on Ponceau 2R decolorization. Fig. 1B depicts the interaction of both activities on malachite green degradation. The comparison of the response surfaces obtained, suggests that while laccase activity has a greater importance the xylidine derivative decolorization, MnP activity plays the major role in malachite green decolorization. An increase in the decolorization of this dye was observed at higher MnP and GLOX activities (Fig. 1C), which is in good agreement with MnP being the predominant enzyme involved in malachite green decolorization, while GLOX provides the H_2O_2 necessary for its activity. On the other hand, higher titres of laccase in the supernatants decreased their malachite green decolorization efficiency, especially in the presence of high levels of GLOX (Fig. 1B and D). One possible reason may be the competition between both oxidases: GLOX and laccase, for the oxygen present in the medium.

T. trogii extracellular fluids obtained in the medium which rendered the highest ligninolytic production decolorized Ponceau 2R, malachite green and anthraquinone blue at rates of 2.14, 1.35 μg and 3 mg dye/(ml h), respectively. The different rates reflect different capacities of the crude culture

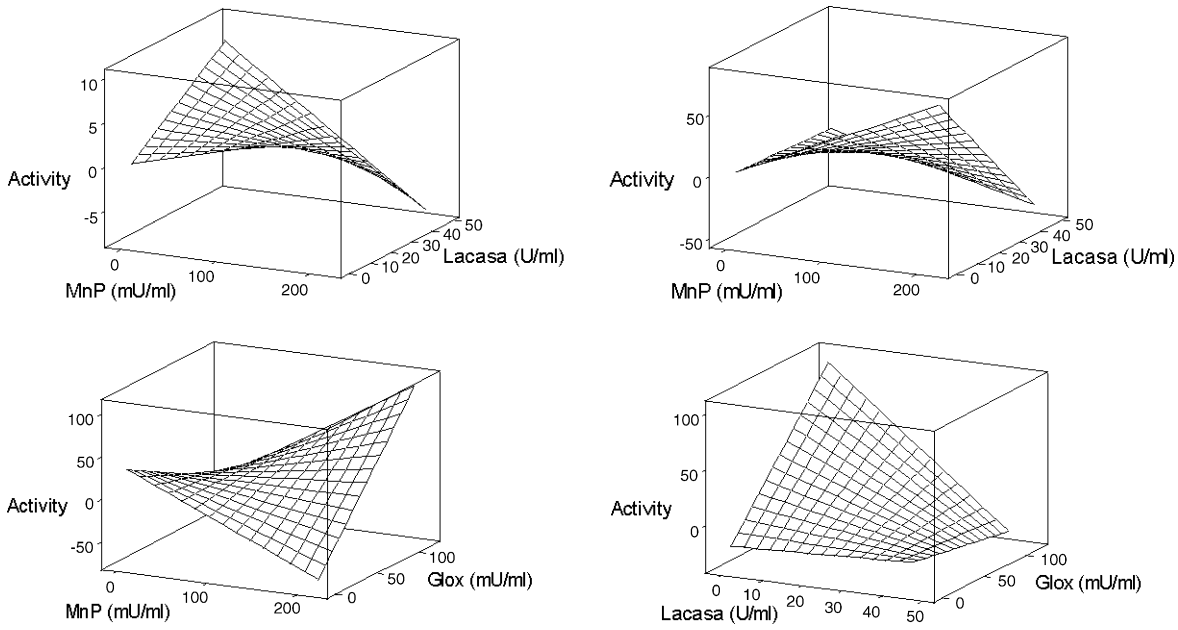

Fig. 1. Surface response for Ponceau 2R decolorization (A) by cultures of *T. trogii* as a function of laccase and MnP activities, while keeping GLOX activity at its 0 level (58 mU/ml), according to the experimental design and model in Table 2. Surface response for malachite green decolorization by cultures of *T. trogii* as a function of laccase and MnP activities, while keeping GLOX activity at its 0 level (58 mU/ml) (B), as a function of MnP and GLOX activities, while keeping laccase activity at its 0 level (22.075 U/ml) (C), as a function of laccase and GLOX activities, while keeping MnP activity at its 0 level (108.25 mU/ml) (D). Laccase activity is expressed in U/ml, MnP and GLOX activities are in mU/ml. Dye decolorization values are shown as $\Delta A/(\min \mathrm{l})$.

filtrates of *T. trogii* to remove dyes with diverse chemical structures. Jarosz-Wilkoazka et al. [27] also demonstrated that anthraquinonic dyes were decolorized easier and faster by fungi than azoic dyes. A low efficiency of decolorization of some azoic dyes, compared to other dye types, was also reported for *P. chrysosporium*, *T. versicolor* [28] and *Irpex lacteus* [29]. Microorganisms do not readily degrade azoic dyes. Sulpho and azoic groups do not occur naturally, thus sulphonated azoic dyes are recalcitrant to biodegradation. Biodegradability of azoic dyes depends on the presence of very specific changes in their molecular structure. Dyes that are anthraquinone derivatives, Remazol brilliant blue R (RBBR) and a number of polymeric dyes synthesized from RBBR, e.g. Poly B-411, serve as substrates for the lignin degradative enzymatic system of wood-destroying fungi. Their decolorization has also been used as an indicator and measure of ligninolytic activity [27]. Anthraquinone, azoic and indigoic dyes were decolorized by the laccase of *T. versicolor*, however, the mechanism of laccase-catalyzed decomposition, was different depending on dye structures. While anthraquinone was directly oxidized by the laccase, azoic and indigo dyes were not the substrates of laccase and small molecule metabolites mediated the interaction between the dyes and the enzyme. The decolorization rate of the nonsubstrate dyes was limited by the concentration of mediating compounds rather than laccase activity in the solutions [30]. Decolorization rates obtained compare favoarably with those previously reported for other white-rot fungi. Decolorization rates for the azoic dye Congo red of 2.9 and 3.9 μg/ml per day were reported by Dey et al. [31] for *P. chrysosporium* and *Polyporus ostreiformis* grown on liquid media. Respective rates for Congo red, methyl red and the group of dyes including the antraquinonic RBBR and the triphenylmethane bromophenol blue of about 10, 20 and 35 μg/ml per day, were described for cultures of *I. lacteus* [29]. *T. versicolor* decolorized the anthraquinonic dye Acid green 27 at a rate of 165 μg/(ml h) [30].

The results indicate that this strain of *T. trogii* could be used in bioprocesses to remove colour from industrial effluents. Moreover, it does not require strict secondary metabolism to produce ligninolytic enzymes [4]. This non-stringent regulation on enzyme production is an advantage over other extensive studied dye decolorizing white rot fungi such as *P. chrysosporium,* in decolorizing industrial dye effluents where N-nutrients and carbonaceous sources may still be present.

The ability of white rot fungi to degrade a wide variety of environmentally persistent pollutants indicates their potential use in anti-pollution treatments. However, only a better understanding of the mechanisms used by these fungi will allow the development of technologies to apply these organisms to the cleaning-up of aquatic and terrestrial environments [32].

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