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ORIGINAL PAPER

Effect of soya lecithin on the enzymatic system of the white-rot fungi *Anthracophyllum discolor*

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Abstract The present work optimized the initial pH of the medium and the incubation temperature for ligninolytic enzymes produced by the white-rot fungus *Anthracophyllum discolor*. Additionally, the effect of soya lecithin on mycelial growth and the production of ligninolytic enzymes in static batch cultures were evaluated. The critical micelle concentration of soya lecithin was also studied by conductivity. The effects of the initial pH (3, 4, and 5) and incubation temperature (20, 25, and 30°C) on different enzymatic activities revealed that the optimum conditions to maximize ligninolytic activity were 26°C and pH 5.5 for laccase and manganese peroxidase (MnP) and 30°C and pH 5.5 for manganese-independent peroxidase (MiP). Under these culture conditions, the maximum enzyme production was 10.16, 484.46, and 112.50 U L^{-1} for laccase, MnP, and manganese-independent peroxidase MiP, respectively. During the study of the effect of soya lecithin on *A. discolor*, we found that the increase in soya lecithin concentration from 0 to 10 g L^{-1} caused an increase in mycelial growth. On the other hand, in the presence of soya lecithin, *A. discolor* produced mainly MnP, which reached a maximum concentration of 30.64 ± 4.61 U L^{-1} after 25 days of incubation with 1 g L^{-1} of the surfactant. The other enzymes were produced but to a lesser extent. The enzymatic activity of *A. discolor* was decreased when Tween 80 was used as a surfactant. The critical micelle concentration of soya lecithin calculated in our study was 0.61 g L^{-1}.

Keywords Soya lecithin · White-rot fungi · *Anthracophyllum discolor* · Ligninolytic enzymes · Phytogenic surfactant

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Introduction

Ligninolytic enzyme production in white-rot fungi is affected by many factors, such as the presence of inductors, temperature, pH, type and concentration of nutrients, and others [16, 18, 35]. Some studies have demonstrated that the presence of a surfactant may increase extracellular enzyme production in various filamentous fungi, including the white-rot fungi [13, 15, 22]. Additionally, its application in bioremediation processes may allow for an increase in mass transfer and the availability of hydrophobic organic contaminants [11].

Surfactants are organic molecules with a polar or ionic hydrophilic group and a nonpolar or hydrophobic chain, known as the head and tail groups, respectively [3, 36]. At low concentrations in aqueous solutions, the surfactants are present as single molecules [33, 36]. Above the critical micelle concentration (CMC), surfactants form micelles that consist of monomers organized in a more or less spherical structure. The ability to form micelles in solution confers certain properties on surfactants such as

emulsifying, foaming, dispersing, and the ability to act as a detergent, as has been reviewed by Desai and Banat [6]. The CMC depends on surfactant structure, composition, temperature, ionic strength, and the presence and types of organic additives in the solution [10]. At the CMC of surfactant solutions, a drastic change occurs in many physicochemical properties (surface tension, conductivity, or turbidity) [14, 39]. Surfactants can be biologically produced by a wide variety of microorganisms, such as bacteria, yeast, and fungi. This group of surface-active agents includes phytogenic surfactants such as saponins and lecithins [26].

The response of certain microorganisms to a surfactant will depend on several factors, such as cellular ultrastructure, surfactant concentration and bioavailability, and environmental and culture conditions [32]. Soeder et al. [26] showed that soya lecithin (SL) had a lower bacterial toxicity than Quillaya saponin.

Several studies have reported that the addition of some surfactants increases extracellular enzyme production in various filamentous fungi [13, 15]. Nevertheless, the mechanism by which surfactants enhance enzyme production has not been established. Garon et al. [12] evaluated the effect of surfactants on different fungal strains; their results showed the inhibition of fungal growth at the CMC by an anionic surfactant, while a nonionic surfactant was well tolerated at doses far above the CMC in most tested fungi.

White-rot fungi are widely studied for their ability to degrade a wide range of persistent or toxic environmental contaminants, such as chlorophenols, polycyclic aromatic hydrocarbons, pesticides, synthetic dyes, and others [1, 21, 24, 27, 29]. The potential of these microorganisms is based on their enzymatic system, which is unique, nonspecific, and secreted into the extracellular environment [8]. White-rot fungi secrete one or more of three extracellular enzymes that are essential for lignin degradation; those enzymes include lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) [9, 21], and are often referred to as lignin-modifying enzymes. Ligninolytic systems have been widely studied in several white-rot fungi, such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pycnoporus cinnabarinus*, *Pleurotus ostreatus*, and *Bjerkandera adusta* [18, 23, 30, 35]. However, increasing attention has recently been paid to the evaluation of the lignin-modifying enzymes of new fungal strains that would not alter ecosystems. In preliminary studies, *Anthracophyllum discolor*, a native Chilean fungus, demonstrated ligninolytic activity with a high level of MnP production and pentachlorophenol degradation potential [23].

In this study, the initial pH of the medium and the incubation temperature for ligninolytic enzyme production were optimized by response surface methodology (RSM). Also, the effect of the natural surfactant soya lecithin on mycelial growth and ligninolytic enzyme production by the white-rot fungi *A. discolor* was evaluated. The CMC of soya lecithin was experimentally determined.

Materials and methods

Microorganism and growth conditions

The white-rot fungi *A. discolor* was obtained from the culture collection of the Environmental Biotechnology Laboratory of the University of La Frontera. The fungus was transferred from slant tubes to glucose malt extract agar plates (agar 15 g L^{-1}, glucose 10 g L^{-1}, malt extract 30 g L^{-1}, pH 5.2), maintained at 4°C for each plate and then incubated at 30 ± 2°C for 7 days. *A. discolor* was first grown in 1-L Erlenmeyer flasks containing 100 mL of modified Kirk medium [28] and five 6-mm diameter agar plugs of active mycelium. The culture was incubated statically at 30 ± 2°C for 7 days in darkness. After this, the fungal culture was homogenized in a sterilized blender for 1 min. The modified Kirk medium contained (per liter of distilled water) 10 g of glucose, 0.2 g of ammonium tartrate dibasic, 2 g of KH_2PO_4, 0.59 g of $MgSO_4{\cdot}7H_2O$, 0.1 g of $CaCl_2$, 3.3 g of sodium acetate, and 10 mL of mineral salts. The initial pH of the medium was adjusted to 4.5 with either 1 N NaOH or 1 N HCl.

Surfactants

Natural soya lecithin (SL) surfactant was purchased from PRINAL®, contain 1.99 Mn mg L^{-1} analysed by Mn as measured by atomic spectrophotometry in our laboratory. Tween 80 (TW 80) is a synthetic surfactant that was purchased from Merck; it has a molecular weight of 1,310 g mol^{-1} and a density of 1.07 mg L^{-1}.

Soya lecithin and TW 80 were chosen for this study due to the fact that no studies had evaluated the effect of SL on the enzyme production of white-rot fungi. TW 80 demonstrates no toxic effect on either fungal growth or the stimulation of ligninolytic enzyme production [7]; both are nonionic surfactants, which means that they are nontoxic [32, 33].

Optimization of pH and temperature on the production of ligninolytic enzymes of *A. discolor* by response surface methodology (RSM)

Effects of the initial pH of the medium (4, 5, and 6) and the temperature (20, 25, and 30°C) on the ligninolytic activity of *A. discolor* were evaluated using 50 mL of modified

Kirk medium in 100-mL Erlenmeyer flasks. The initial pH of the medium was adjusted with either 1 N NaOH or 1 N HCl. The culture medium was autoclaved at 121 ± 2°C for 15 min, and 2 mL of homogenized mycelium was used as the inoculum. Ligninolytic activities of laccase (Lac), lignin peroxidase (LiP), manganese-independent peroxidase (MiP), and manganese peroxidase (MnP) were evaluated periodically for up to 22 days. These assays were carried out in triplicate for each treatment.

Effect of SL on the mycelial growth of *A. discolor*

This test was used to determine the effect of SL (0, 0.3, 0.4, 1, and 10 g L^{-1}) on the mycelial growth of *A. discolor* on potato dextrose agar (PDA). Several SL concentrations were added to the PDA medium, which was autoclaved at 121 ± 2°C for 15 min and aseptically transferred to Petri dishes. Plates were inoculated at the plate center with one 6-mm agar plug of active *A. discolor* mycelia containing a 7-day-old culture on PDA medium. The plates were incubated in the dark at 26 ± 2°C. Mycelia growth was measured daily from the edge of the agar disk to the inner edge of the Petri dish. PDA plates without SL and inoculated with *A. discolor* were used as a control. The results are reported as the mean of four replicates for each SL concentration.

Experimental determination of the critical micelle concentration of SL by conductivity

The critical micelle concentration (CMC) was determined by the breakpoint of conductivity versus the surfactant concentration curve (0.1–1.5 g L^{-1} SL). The electrical conductivity of each surfactant solution was measured with a Hach sensION5 conductivity meter at 25 ± 2°C. All solutions were prepared in 50 mL of modified Kirk medium at the optimized pH of 5.5. Deionized water was used to prepare the SL solution and modified Kirk medium in these experiments. The results are reported as the mean of three replicates.

Effect of SL and TW 80 on the production of ligninolytic enzymes by *A. discolor*

The effects of SL and TW 80 were evaluated at concentrations of 0–1 g L^{-1} using modified Kirk medium (50 mL) in 100-mL Erlenmeyer flasks. The culture medium was autoclaved at 121 ± 2°C for 15 min, and 2 mL of homogenized mycelium was used as the inoculum. The cultures were statically incubated at 26 ± 2°C. The ligninolytic activity of Lac, LiP, MiP, and MnP were evaluated periodically for up to 33 days. The results are reported as the mean of three replicates per treatment, with the standard deviation of the mean represented by error bars.

Enzyme assays

The enzymatic activities were measured from the supernatant of a previously centrifuged sample (2 min at 3,000 rpm at 4°C) of the culture medium.

The Lac activity was determined with 2,6-dimethoxyphenol (DMP) as the substrate in sodium malonate (pH 4.5). The enzyme activity unit was defined as an increase in absorbance per minute at 468 nm and 30°C [4]. The MnP activity was determined by monitoring the oxidation of 2,6-DMP spectrophotometrically at 30°C. The reaction mixture (1 mL) contained 200 μL of 250 mM sodium malonate (pH 4.5), 50 μL of 20 mM 2,6-DMP, 50 μL of 20 mM $MnSO_4 \cdot H_2O$, and 600 μL of supernatant. The reaction was initiated by adding 100 μL of 4 mM H_2O_2, monitored at 468 nm and corrected by the Lac activity. The MiP activity was determined in a reaction mixture containing 200 μL of 250 mM sodium malonate (pH 4.5), 50 μL of 20 mM 2,6-DMP, 100 μL of 20 mM EDTA, and 550 μL of supernatant. The reaction was initiated by adding 100 μL of 4 mM H_2O_2, monitored at 468 nm, and corrected by the laccase activity [4]. The extinction coefficient was 49,600 M^{-1} cm^{-1}. The LiP activity is based on the oxidation of veratryl alcohol. The reaction mixture contained 1,420 μL of disodium tartrate dihydrate (0.1 M, pH 3.0), 400 μL of veratryl alcohol (20 mM), and 100 μL of supernatant. The reaction was initiated by adding 80 μL of H_2O_2 (10 mM) [5] and was monitored at 310 nm for 2 min. The extinction coefficient was 93,000 M^{-1} cm^{-1}.

Experimental design and statistical analysis

The independent variables selected in this study were the initial pH of the medium (4, 5, and 6) and the temperature (20, 25, and 30°C). Central composite methodology was applied according to the Design Expert 6.0.6 statistical software (Stat-Ease, Minneapolis, MN, USA). Selection of the low and high levels of each variable was based on the results of a single-factor method investigation.

The experimental design consisted of 11 trials, including three replications at the central point. All experiments were performed in triplicate, and the average of each enzymatic activity was taken as the dependent variable. Statistical analysis and analyses of variance (ANOVA) were carried out using the Design Expert 6.0.6 statistical software (Stat-Ease, Minneapolis, MN, USA).

Where required, the data were discussed using the ANOVA and the Duncan test was used to compare means. The statistical significance level for all treatments was 5%.

Results

Optimization of pH and temperature on the production of ligninolytic enzymes of *A. discolor* by RSM

To examine the combined effect of different culture conditions (initial pH of the medium and the incubation temperature) on ligninolytic enzyme production, a central composite design of two variables at three levels of work each plus three center points, which led to a total of 11 trials, was performed.

The everyday values of different enzyme activities (Lac, LiP, MnP, and MiP) were used to determine the accumulated enzyme activity. The values of the variables analyzed and the responses measured are shown in Table 1.

The accumulated enzyme activity data were fitted by a polynomial quadratic equation (Table 2). The lack-of-fit value indicates variation due to model inadequacy. Under the conditions tested, the lack-of-fit test was not significant for the experimental data for LiP activities, which were, therefore, not adjusted to any type of equation.

The equations show that there were negative interactions between the independent variables for enzyme production (−0.13, −6.81, and −2.92 for Lac, MnP, and MiP, respectively). However, it also showed a positive effect for both variables in the linear term, but with a greater influence of pH. This result indicates that the production of Lac, MnP, and MiP increased with increasing values of initial pH of the medium and incubation temperature. On the other hand, the negative quadratic coefficient of pH indicates the existence of a maximum activity of Lac, MnP, and MiP as a function of pH. The response surface plot obtained as a function of the initial pH of the medium versus the incubation temperature is shown in Fig. 1.

Figure 1 shows that MnP, Lac, and MiP demonstrated no variations when the incubation temperature varied between 20 and 30°C. However, it is shown that the initial pH of the medium has a greater effect on the ligninolytic activity of *A. discolor*.

The optimal conditions to obtain maximal ligninolytic enzyme production were an initial pH of the medium of 5.5 and an incubation temperature of 26°C for MnP and Lac. For MiP, those conditions were pH 5.5 and 30°C. The modeled values predict that the maximum enzyme productions that can be obtained by using the abovementioned, optimized conditions of the variables are 484.46 U L^{-1} for MnP, 10.16 U L^{-1} for Lac, and 112.5 U L^{-1} for MiP.

Effect of SL on the mycelial growth of *A. Discolor*

Figure 2 presents the results of fungal growth in the presence of different concentrations of SL (0, 0.3, 0.4, 1, and 10 g L^{-1}). As the SL concentration increased from 0 to 10 g L^{-1}, an increase in mycelial growth was observed. After 4 days of incubation, the mycelium of *A. discolor* grew 3.3, 3.7, 3.4, 3.5, and 3.9 cm when PDA was supplemented with 0, 0.3, 0.4, 1, and 10 g L^{-1} SL, respectively. At all of the tested concentrations of SL, including the control with no natural surfactant, the mycelium of *A. discolor* covered the full Petri dish within 5 days.

Table 1 Accumulated ligninolytic activity (U L^{-1} ± SD) of *A. discolor* in liquid medium obtained under different culture conditions after 22 days of incubation

Temperature (°C)	pH	Lac	LiP	MnP	MiP
20	4	0.42 ± 0.08	23.66 ± 2.15	8.13 ± 2.11	11.13 ± 0.68
	5	3.38 ± 0.11	73.12 ± 8.40	236.20 ± 3.23	57.60 ± 3.57
	6	4.07 ± 0.24	22.58 ± 2.84	328.27 ± 5.55	110.22 ± 2.48
25	4	0.22 ± 0.05	21.51 ± 2.00	2.40 ± 0.25	2.11 ± 0.99
	5	12.18 ± 0.48	24.73 ± 5.38	408.15 ± 4.41	98.77 ± 7.15
	6	2.14 ± 0.08	69.38 ± 6.04	584.98 ± 5.99	98.49 ± 2.68
30	4	1.02 ± 0.29	32.26 ± 2.15	2.31 ± 0.34	6.41 ± 4.44
	5	11.85 ± 0.69	10.75 ± 2.15	596.38 ± 6.73	170.54 ± 4.89
	6	2.10 ± 0.23	32.26 ± 4.30	186.18 ± 5.43	47.05 ± 4.10

The standard deviation (SD) is the mean of three replicates

Table 2 Experimental equations of Lac, MnP, and MiP produced by *A. discolor* to evaluate the combined effect of the initial pH of the medium and the incubation temperature

Enzymatic activity	Experimental equations	R^2
Lac	$-280.17 + 5.01\ T + 89.33\ pH - 0.08\ T^2 - 8.50\ pH^2 - 0.13\ T\ pH$	0.83
MnP	$-8{,}637.75 + 204.45\ T + 2{,}393.99\ pH - 3.27\ T^2 - 204.26\ pH^2 - 6.81\ T\ pH$	0.76
MiP	$-1{,}901.81 + 8.57\ T + 712.38\ pH + 0.15\ T^2 - 59.99\ pH^2 - 2.92\ T\ pH$	0.79

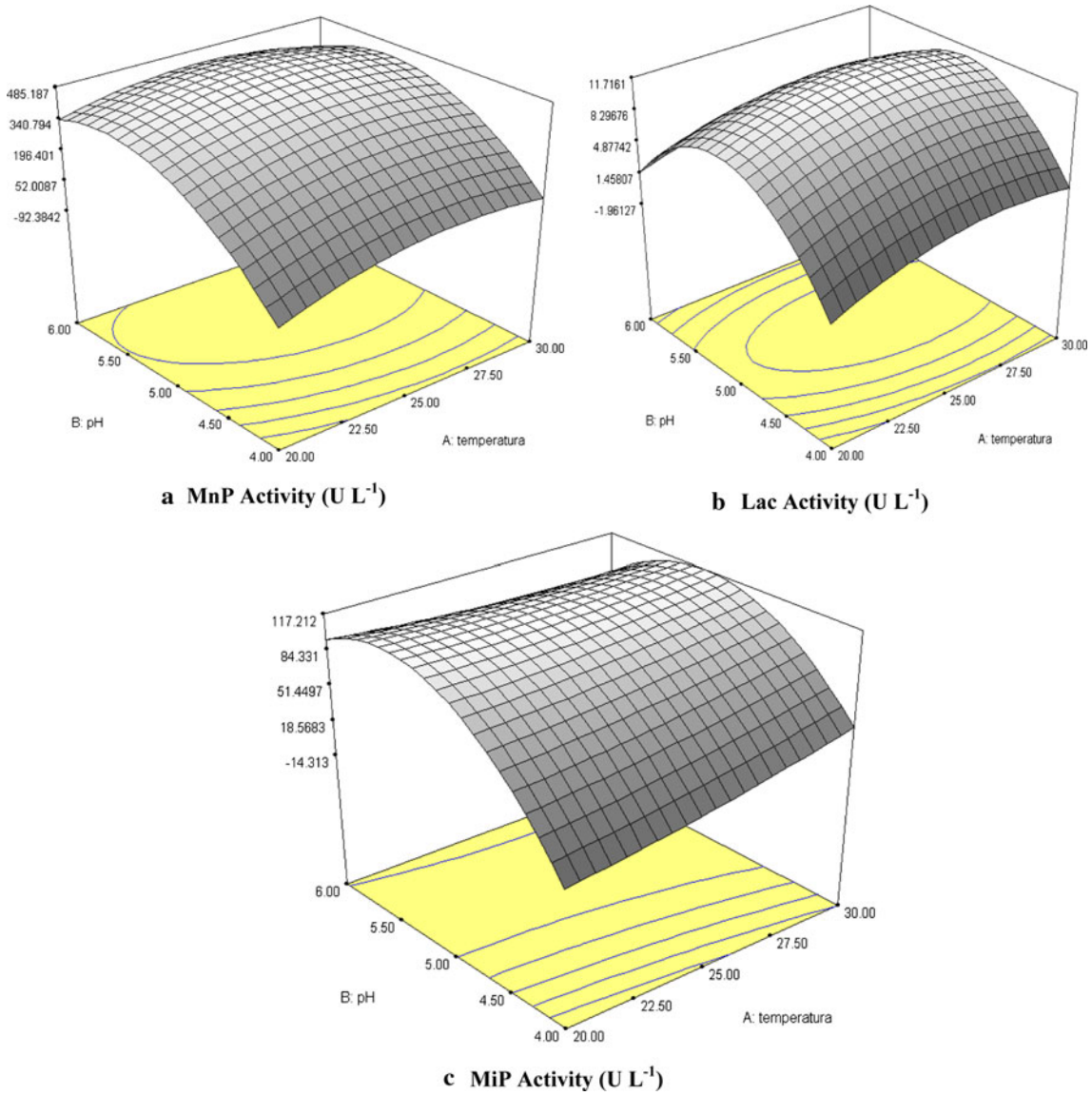

Fig. 1 Response surface plot showing the effects of the initial pH of the medium and the incubation temperature and their mutual effect on the production of MnP (**a**), Lac (**b**), and MiP (**c**) by *A. discolor*

Experimental CMC determination of SL

Figure 3 shows the variation in electrical conductivity in response to changing SL concentrations in modified Kirk medium. The specific conductivity (1/K) vs. surfactant concentration (1/SL) plot shows two straight lines with different slopes. The first zone is characterized by the equation $y = -0.0042x + 0.2622$, which corresponds to the concentration range above the CMC. At higher surfactant concentrations, micelles start to form and a slope change appears because the conductivity increases in a different manner. The equation $y = -0.0002x + 0.2556$ represents the concentration range below CMC when only monomers of surfactant exist in the solution. The intersection of these two straight lines is the CMC value of the surfactant [10, 19]. The CMC value of SL in modified Kirk medium at 25 ± 2°C obtained by conductivity measurements in this study was 0.61 g L^{-1}.

Effect of SL and TW 80 on the production of ligninolytic enzymes by *A. discolor*

SL and TW 80 concentrations of 0, 0.15, 0.3, 0.4, 0.5, and 1 g L^{-1} were evaluated. The production profiles of the ligninolytic enzymes in liquid culture of *A. discolor* containing SL and TW 80 are depicted in Figs. 4 and 5, respectively. Lac (Fig. 4a) showed low activity in all treatments and reached a maximum of 5.27 U L^{-1} after 30 days with 1 g L^{-1} of SL. The LiP (Fig. 4b) activity reached a maximum of 30.11 U L^{-1} after 30 days of

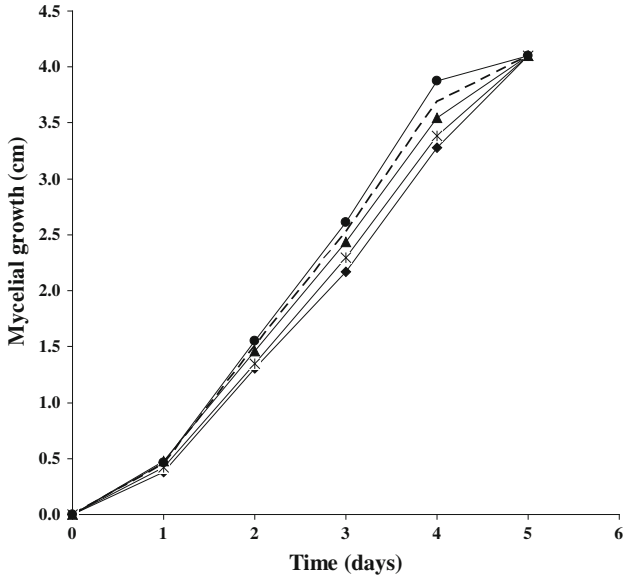

Fig. 2 Effects of SL on the mycelial growth of *A. discolor* on PDA at 26°C and pH 5.5. The evaluated SL concentrations were 0 g L^{-1} (*filled diamonds*), 0.3 g L^{-1} (*dashed line*), 0.4 g L^{-1} (*asterisks*), 1 g L^{-1} (*filled triangles*), and 10 g L^{-1} (*filled circles*). The data points represent the average values of four replicates for each SL concentration

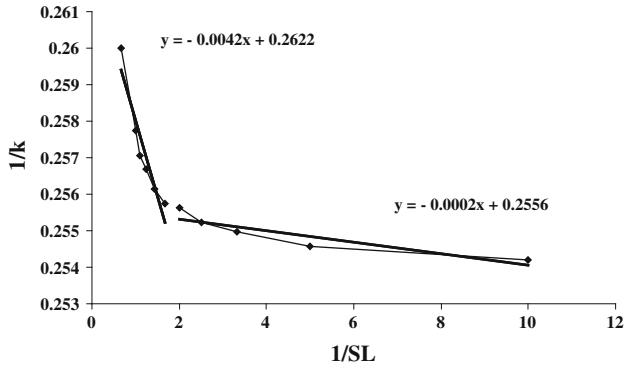

Fig. 3 Variation of electrical conductivity at several concentrations of the SL at 25 ± 2°C and pH 5.5. The points represent the average values of three replicates

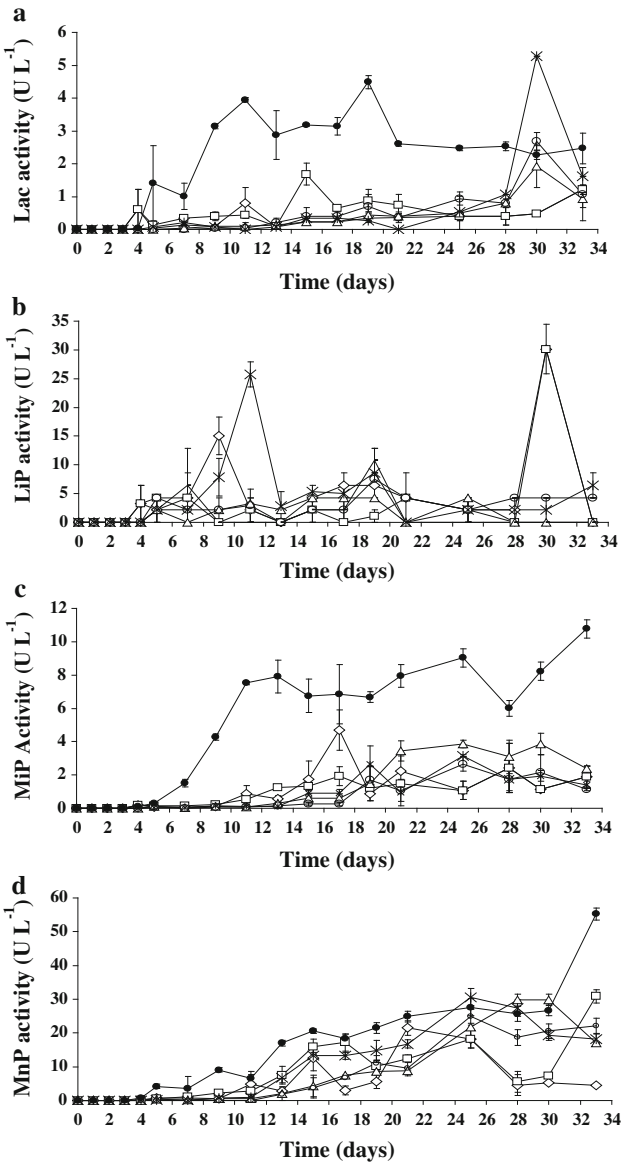

Fig. 4 Effect of different SL concentrations on the ligninolytic activity of *A. discolor* in liquid medium at 26°C and pH 5.5. The points represent the average values of three replicates. The evaluated SL concentrations were 0 g L^{-1} (*filled circles*), 0.15 g L^{-1} (*open diamonds*), 0.3 g L^{-1} (*open squares*), 0.4 g L^{-1} (*open triangles*), 0.5 g L^{-1} (*open circles*), 1 g L^{-1} (*asterisks*)

culture when the medium was supplemented with 0.15 and 0.3 g L^{-1} of SL. MiP (Fig. 4c) production increased steadily, although it showed low activity in all treatments, and did not exceed 5 U L^{-1} with 0.15 g L^{-1} of SL after 17 days of incubation. Finally, MnP (Fig. 4d) demonstrated low activity during the first 13 days of incubation, which did not exceed 10 U L^{-1} for all evaluated conditions. The MnP production then increased steadily at different speeds with increasing SL concentration, and reached a maximum of 30.64 ± 4.61 U L^{-1} on day 25 when the medium was supplemented with 1 g L^{-1} of SL.

Low Lac production (Fig. 5a) under all of the tested concentrations of TW 80 was observed; production did not exceed 4 U L^{-1}. The LiP production (Fig. 5b) demonstrated a maximum of 30.11 U L^{-1} after 30 days of culture when the medium was supplemented with 0.15 and 0.3 g L^{-1} of SL. The MiP activity (Fig. 5c) was low but increased steadily, reaching a maximum of 7.19 U L^{-1} with 0.3 g L^{-1} of TW 80 after 33 days of incubation. Finally, the MnP activity was low during the first 13 days of incubation and did not exceed 9.11 U L^{-1} for all evaluated conditions. After this period of time, the MnP production increased slowly in all treatments. The maximum MnP production was 42.14 ± 3.09 U L^{-1} with 0.3 g L^{-1} of TW 80 on day 33.

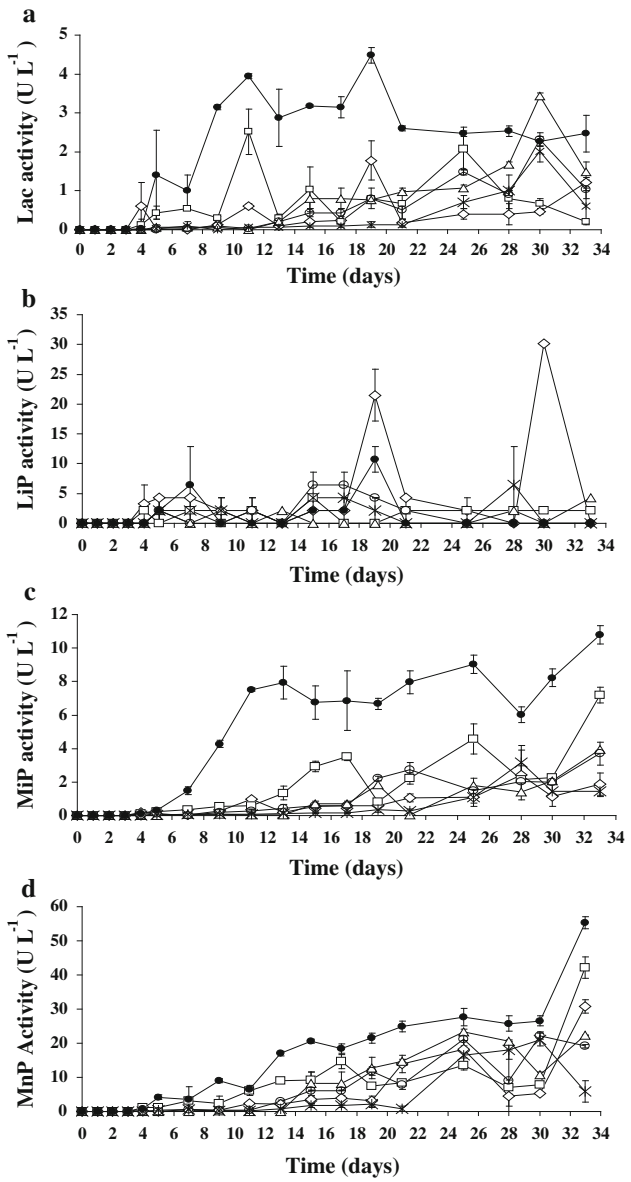

Fig. 5 Effect of different TW 80 concentrations on the ligninolytic activity of *A. discolor* in liquid medium at 26°C and pH 5.5. The points represent the average values of three replicates. The evaluated TW 80 concentrations were 0 g L^{-1} (*filled circles*), 0.15 g L^{-1} (*open diamonds*), 0.3 g L^{-1} (*open squares*), 0.4 g L^{-1} (*open triangles*), 0.5 g L^{-1} (*open circles*), 1 g L^{-1} (*asterisks*)

Figures 4 and 5 demonstrate that the ligninolytic activity of *A. discolor* gradually increased after 4 days of incubation when the culture medium was not supplemented with SL or TW 80. Under these conditions, the Lac, LiP, and MnP activities did not exceed 12 U L^{-1}. *A. discolor* produced mainly MnP, which reached a maximum of 55.26 U L^{-1} after 33 days of incubation.

Table 3 indicates that MnP activity accumulates after 33 days of incubation. At equal concentrations of SL and TW 80, the accumulated MnP activity was significantly different. The maximum accumulated activities of MnP in *A. discolor* were 162.53 ± 2.13 and 133.50 ± 2.96 U L^{-1} when the culture medium was supplemented with 1 g L^{-1} of SL and 0.30 g L^{-1} of TW 80, respectively. However, the accumulated activity of MnP in culture medium without surfactant was significantly different with respect to those activities obtained with medium supplemented with SL or TW 80.

Discussion

The ligninolytic activity of white-rot fungi depends on many factors, and each strain responds in a particular way to each of these factors. The combined study of two or more factors is important not only to evaluate the ligninolytic enzyme but also to explore possible interactions between variables. In order to enhance the production and stability of ligninolytic enzymes of *A. discolor*, several culture conditions were evaluated. First, the initial pH of the medium and the incubation temperature were studied, followed by the effect of soya lecithin.

Response surface methodology could be used for the optimization of culture conditions to obtain the maximum production of ligninolytic enzymes by *A. discolor*, which lowers the cost of biotechnological processes. The experimental design employed showed that there was no change in the enzymatic activities of *A. discolor* when the incubation temperature varied between 20 and 30°C. However, the initial pH of the medium had a greater effect on the ligninolytic activity of these white-rot fungi. When evaluating the combined effect of the initial pH of the medium and the incubation temperature, *A. discolor* was found to mainly produce MnP, with low production of Lac (Table 1). In the study by Vukojević et al. [34], there was no ligninolytic enzyme production by *Ganoderma lucidum* when the pH of the medium varied between 2.0 and 7.0 at room temperature using a rotary shaker. On the other hand, *Stereum hirsutum* demonstrated high Lac activity at pH 5, while MnP production was high at an initial pH of 6 [20].

Induction of the ligninolytic system is affected by many factors, such as the type of carbon and nitrogen source [2, 18], the level of aeration and agitation during incubation, and the availability of low molecular weight mediator compounds and metals [20, 25, 35]. Ligninolytic activity is also affected by some oils and surfactants [13].

Some studies have demonstrated that the use of surfactants can stimulate fungal growth and enhance enzyme production. The results in Fig. 1 indicate that SL does not appear to have a negative effect on the fungal growth of *A. discolor*. Nonionic surfactants such as SL are often considered to be nontoxic [32, 33], and, therefore, do not affect the fungal growth of *A. discolor*. Several studies of chemical surfactants have shown that charge has an impact

Table 3 Accumulated MnP activity (U L^{-1} ± SD) of *A. discolor* in liquid culture with several concentrations of SL or TW 80 at pH 5.5 and 26°C after 33 days of incubation

Surfactant	Surfactant concentration (g L^{-1})					
	0	0.15	0.3	0.4	0.5	1
SL	216.49 ± 2.06[a]	111.39 ± 1.15[g]	128.89 ± 1.12[d]	127.96 ± 0.28[d]	116.63 ± 3.81[f]	162.53 ± 2.13[b]
TW 80		57.63 ± 1.87[i]	133.50 ± 2.96[c]	121.10 ± 1.29[e]	107.76 ± 3.00[g]	69.86 ± 0.87[h]

The standard deviation (SD) is the mean of three replicates

Different letters indicate significant differences (Duncan test, $P \leq 0.05$)

on toxicity; cationic surfactants are the most toxic and have been used as antimicrobials. Ding et al. [7] found no negative effect of TW 80 on *P. chrysosporium* growth. Garon et al. [12] evaluated the toxicity of SDS, Triton X-100, and TW 80 on fungal strains. The results showed growth inhibition by SDS (anionic surfactant), whereas Triton X-100 and TW 80 (nonionic surfactants) were well tolerated at the doses evaluated in most of the tested fungi.

The CMC value is one of the most important surfactant properties. The CMC value of SL in modified Kirk medium at 25°C obtained by conductivity was 0.61 g L^{-1}, which is greater than that obtained by Soeder et al. [26], who reported a value of 0.383 g L^{-1}. Other CMC values of soya lecithin have been reported. Wu and Wang [37] determined that the CMC value was 13.6 mg mL^{-1}, Urum and Pekdemir [31] obtained a value of 0.4 (mass%), and Wu and Wang [38] reported CMC values between 1.18 and 4.13 mg mL^{-1}. This difference can be explained because the CMC value depends on many factors, such as the determination method, phospholipid composition, electrolyte addition, buffer pH, solution temperature, ionic strength of the aqueous solutions, and the presence of organic additives, which makes this value different from that determined in pure water [10]. The importance of determining the CMC of SL lies in the fact that surfactant presence, in particular at concentrations above the CMC value, often had a toxic effect on microorganism degrading, as reported by Laha and Luthy [17]. This negative effect can be explained by disruption of the cell membranes through interactions with structural lipid components [17]. In this context, the CMC value can help to fix a limit of work when studying the surfactant effect on the enzymatic activity of a microorganism.

In the present work, SL or TW 80 was added to the culture medium in order to explore the possibility of enhancing the enzyme production by *A. discolor*. Several investigators [7, 15, 22] have shown improvements in enzyme production in the presence of some surfactants in semi-solid, immobilized, submerged, agitated, and shallow stationary liquid cultures of white-rot fungi such as *P. chrysosporium*. We chose SL because it shows lower toxicity in bacteria than Quillaya saponin [12] and because no studies have evaluated the effect of SL on enzyme production in white-rot fungi. The evaluated concentrations were 0.15, 0.3, 0.4, 0.5, and 1 g L^{-1}. At these concentrations, the detected levels of LiP and MnP were higher than those of Lac and MiP, which might be explained by the SL composition. This phytogenic surfactant contains 1.99 mg L^{-1} of Mn, a known inducer of these enzymes. Studies by Wang et al. [35] demonstrated that the presence of Mn^{2+} in the culture medium enhanced MnP and LiP production. Moreover, Ürek and Pazarlioğlu [30] determined that MnP production depended on the Mn^{2+} concentration. The presence of TW 80 stimulated LiP and MnP production with respect to Lac and MiP. Several authors have shown an improvement in enzyme excretion in the presence of certain surfactants such as TW 80 in immobilized and submerged cultures of *P. chrysosporium* [7, 15]. The mechanism by which surfactants enhance extracellular enzyme production in filamentous fungi has not been established. Several authors have suggested that surfactants promote both the uptake and exit of compounds from the cell through modification of the plasma membrane permeability [7].

The results obtained in our study show that optimal conditions for the production of high levels of Lac and MnP in *A. discolor* are the same, which is advantageous with respect to their possible synergism in bioremediation processes. SL demonstrated no negative effects on the fungal growth of *A. discolor* and increased the enzyme production of ligninolytic enzymes more than the use of TW 80.

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