Biodegradation of the textile dye Direct Blue 69 by the White-Rot Fungus *Bjerkandera sp.* in presence of chromium salts

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Biodegradación, en presencia de sales de cromo, del colorante textil Azul Directo 69 por el hongo de pudrición blanca *Bjerkandera* sp.

Abstract. The effect of Cr^{3+} and Cr^{6+} on the growth and ligninolytic activity of *Bjerkandera* was studied using the polymeric textile dye Direct Blue 69 as the degradation substrate. The standard basal medium containing different concentrations of chromium ions and the dye was inoculated with *Bjerkandera* in static conditions under an air atmosphere at 26 °C for 13-d. The presence of chromium ions in concentrations higher than 10 ppm for CrO_4^{2-} or 150 ppm for Cr^{3+} affected the growth rate, the decolorization rate and the Mn-peroxidase enzymatic production compared with those of a control sample. The Mn-peroxidase activity of the fungus was not affected by the addition of increasing concentrations of chromium ions (up to 50 ppm of CrO_4^{2-} or 300 ppm of Cr^{3+}) to the standard basal medium, after allowing the fungus to grow for 7-d. When *Bjerkandera* was incubated for 15-d in a standard basal medium containing 10 ppm of CrO_4^{2-} or 150 ppm of Cr^{3+} it accumulated 30 % w/w of chromium ions.
Keywords: Heavy metals; White-rot fungus; Textile dye.

Resumen. Se estudio el efecto del Cr^{3+} y Cr^{6+} sobre el crecimiento y la actividad ligninolítica de *Bjerkandera* sp. utilizando como sustrato de degradación un colorante polimérico de uso textil. El medio basal estándar conteniendo diferentes concentraciones de ión cromo y el colorante Azul directo 69 fue sembrado con *Bjerkandera* sp. e incubado en condiciones estáticas (y en atmósfera de aire a 26ºC) durante 13 días. Los resultados obtenidos muestran que la presencia de ión cromo en concentraciones superiores a los 10 ppm para CrO_4^{2-} o los 150 ppm para Cr^{3+} afecta el crecimiento, la velocidad de decoloración y la producción enzimatica Mn-peroxidasa de *Bjerkandera* sp. Asimismo se observó que la actividad enzimática Mn-peroxidasa de este hongo no fue afectada cuando se agregaron concentraciones crecientes de ión cromo (hasta 50 ppm para CrO_4^{2-} o los 300 ppm para Cr^{3+}) al medio basal estándar, luego de permitir el crecimiento fúngico durante 7 días y que *Bjerkandera* sp. acumuló 30% m/m de ion cromo cuando se la incubó durante 15 días en medio basal estándar conteniendo 10ppm de CrO_4^{2-} o 150ppm de Cr^{3+}.
Palabras clave: Colorantes textiles; Hongos de la pudrición blanca; Metales pesados.

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ORIGINAL

Introduction

Synthetic dyestuffs are extensively used in textile, paper, printing and tanning industries. The effluents generated in these industries are highly colored and may cause serious hazards to public health. These wastewaters are often characterized by the presence of mixtures of dyes with high persistence and inorganic ions, e.g. Cu, Hg and Cr [12] which have toxic effects both on humans and on different micro-organisms [3, 16, 20].

Ligninolytic fungi are micro-organisms characterized by the secretion of an extra-cellular enzymatic complex, potentially capable of degrading lignin and other structurally complex pollutants such as those present in solid industrial wastes or wastewaters [5, 6]. In these fungi, metals are directly and indirectly involved in their metabolism [9]; many metals are essential for growth, e.g. Na, K, Ca and Mg, whereas others e.g. Cu, Hg, Mn and Cd have inhibitory effects either on the fungal growth or on their ligninolytic activity [2, 8, 13, 15].

Previous research has demonstrated that white rot fungi can be utilized in the biodegradation of organic pollutants present in industrial wastewater [4, 10]. Furthermore, pollution by organic xenobiotics is often accompanied by chromium salts. Therefore the aim of this research was to determine the effect of the addition of Cr(III) and Cr(VI) on the decolorization of the dye Direct Blue 69 by *Bjerkandera* sp.

Materials and methods

Bjerkandera sp. strain BOS55 was obtained from the Deparment of Chemical Engineering, University of Santiago de Compostela, Spain. $CrCl_3$ and K_2CrO_4 were obtained from Merck. All chemicals possessed analytical grade; Direct Blue 69 (CIBA) (C:I: 34210) had industrial grade.

Previous to the decolorization, Manganese Peroxidase (EC 1.11.1.13) production and biomass assays, an abiotic control was performed in a standard basal medium (SBM) containing 0.02%w/v of Direct Blue 69, to determine the maximum concentration of chromium ions which would not result in the formation of non-soluble compounds due to the interaction between the dye and the metal ion.

The SBM contained 10 mL of mineral medium [17] and Direct Blue 69 (0.02% w/v). Different concentrations of metal ions (Cr^{3+} or CrO_4^{2-}) were added. The medium (pH 4.5) was autoclaved and then a 400 mg/L thiamine solution (passed through a sterilised filter) was added (5 mL/L). Two agar plugs (5-mm diameter) were used as the inoculum in 100 mL culture flasks and incubated for 13 days under static conditions in an air atmosphere at 26ºC. Then 3 mL of solution were separated from each flask, centrifuged at 2000 rpm for 15 min and the supernatant used to determine the dye decolorization (1 mL) and MnP production (1 mL). On the remaining solution, the mycelium was separated by filtration through tared glass microfibre filters (Schleider Schuell GF50) and washed twice with 10 mL hot distilled water. Fungal growth was determined gravimetrically after drying in an oven at 80° C for 24 h, cooling to room temperature in desiccator and weighing.

For the decolorization assay of Direct Blue 69, the absorbances at 581 and 300 nm were measured in the crude filtrate with a diode array Shimadzu MultiSpec-1501 UV-VIS Spectrophotometer (SMS-1501) and expressed as a decrease in the absorbance ratio A_{581}/A_{300}.

Manganese Peroxidase (MnP) production was measured with the Paszczynski method [14]; the absorbance increase was measured with a SMS-1501 at 468 nm. One unit (UE) of peroxidase oxidizes 1mol substrate/min.

To determine if the MnP activity (rather than MnP production) was affected by the presence of chromium ions, the mycelium was grown in 100 mL culture flasks during 7 days (under static conditions in an air atmosphere at 26ºC) with 10 mL of the SBM in absence of chromium salts. Then 1 mL of solution was separated from each flask, centrifuged at 2000 rpm for 15 min and the supernatant used to determine the MnP activity. Different concentrations of the chromium ion were added prior to the determination by the Paszczynski *et al*. Method [14].

To determine the presence of chromium in the fungal strain, the mycelium was grown in 100 mL culture flasks during 7 days (under static conditions in an air atmosphere at 26ºC) with 10 mL of the SBM in presence of different concentrations of chromium salts. The mycelium was harvested and washed thoroughly. Acid digestion of the mycelium was carried out with 4 mL of nitric acid and 4 mL of sulphuric acid for 40 min at 90°C (in a sealed Teflon flask). Chromium was analyzed with a Rank Hilger Atomspek H1550 Atomic Absorption Spectrophotometer.

All experiments were performed in quadruplicate and were repeated in triplicate on separate days. Parallel controls were carried out under the same conditions without inoculum. The values reported in the figures and tables are means with standard deviations. The measurements of different assays were compared to those of the assays performed in the absence of chromium; the significance ($p < 0.05$) was validated using a GraphPad Prism Software, version 4.0.

Results and discussion

The dye Direct Blue 69 was selected as a model compound as it is often found in wastewaters proceeding from the dyeing process in the textile industry [11]. It also presents adequate characteristics essential for performing experimental assays, such as: no precipitate formation, no spectral alterations, and no redox reactions with chromium ions.

Abiotic controls were performed in SBM containing 0.02% w/v of Direct Blue 69. The results show that at concentration levels of up to 50 mg/L of Cr(VI) and 300 mg/L of Cr(III), no formation of non-soluble compounds due to the interaction between the dye and the metal ion was detected.

The effect of the addition of chromium on the growth rate of *Bjerkandera* sp. is shown in Figure 1. The results obtained show significant differences for concentrations above l50 mg/L of Cr (III) and 10 mg/L of Cr(VI). The growth

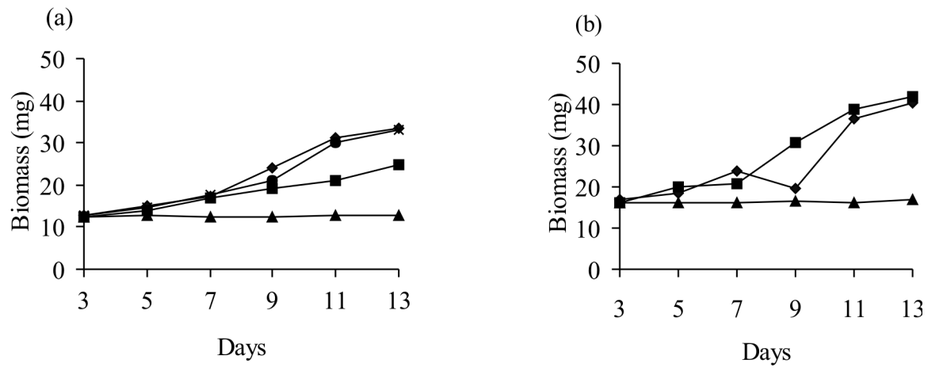

Figure 1. Evolution of the dry weight (mean values) with time (the standard deviations average is 1.9 mg). (a) different Cr^{3+} concentrations (-◆-0;-●-150;-■-200;-▲-250 mg/L); (b) different CrO_4^{2-} concentrations -◆-0;-■-10;-▲-15 mg/L).

was inhibited by 40% in a concentration of 200 mg/L of Cr(III) and the inhibitory effect was total for 250 mg/L or higher concentrations. Cr(VI) had a stronger inhibitory effect on the mycelial growth at a concentration of 15 mg/L.

These results support the hypothesis that in liquid culture media the toxic effects of Cr(III) and Cr(VI) on certain fungal strains or bacteria include inhibition of spores germination, reproduction and growth [1], [18].

The bioaccumulation of chromium in *Bjerkandera* sp. was studied in SBM with 10 mg/L of Cr(VI) or 150 mg/L of Cr(III). The results obtained indicate a bioaccumulation of Cr(III) or Cr(VI) in values of around 30% of the chromium initially added. These results suggest that this bioaccumulation as well as the lower mycelial growth are the result of the adaptation of the fungal physiology due to the polluted habitat; they are consistent with the results obtained for other fungal strains and metals. For example, Gabriel [7] reported different levels of accumulation of Pb, Cd, Al and Ca in *Daedalea quercina*, *Ganoderma applanatum, Schizophyllum commune*, and *Stereum hirsutum*; Yetis [19] also reported the sorption capacity of *Phamerochaete chrysosporium* for Pb.

The measurement of decolorization capability of *Bjerkandera* sp. showed that the presence of Cr(III) up to 150 mg/L and Cr(VI) up to 10 mg/L did not significantly alter the decolorization rate (Fig. 2). However, a reduction in the decolorization rate occurred at 150 mg/L of Cr(VI) and 15 mg/L of Cr(VI).

For the MnP production assay, the results obtained show that MnP production was significantly different for concentrations higher than 150 mg/L of Cr(III) and 10 mg/L of Cr(VI) (Fig. 3).

For the MnP activity assay, the chromium was added immediately before starting the enzymatic reaction with H_2O_2 in concentrations of up to 300 mg Cr(III)/L and 50 mg Cr(VI)/L. The results showed that neither Cr(III) nor Cr(VI) had any significant effect on the enzymatic activity (see Table 1).

The data in Figure 3 and Table 1 allow us to conclude that the presence of chromium inhibits the production more than the extracellular activity of the enzymatic complex. This result is evidenced by the fact that chromium ions do not interact with the enzymatic system at the concentration levels used in this study. These results strengthen the hypothesis,

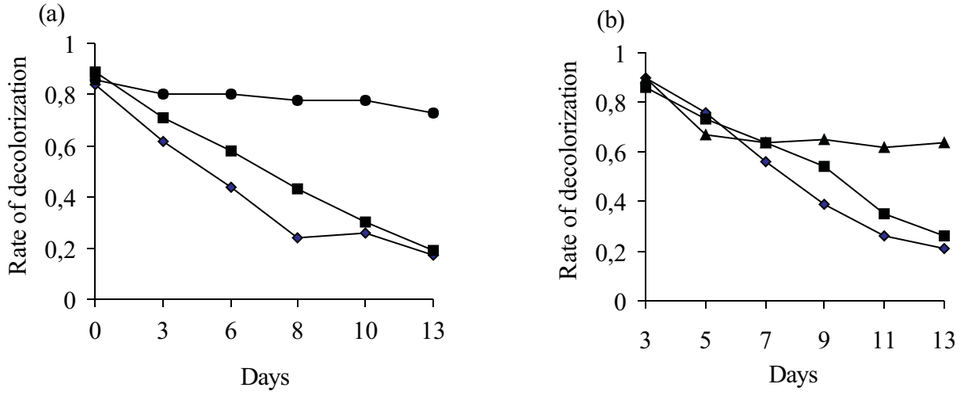

Figure 2. Rate of decolorization (mean values) of Direct Blue 69 (decrease of the ratio A_{581}/A_{300}) vs time in standard basal medium (the standard deviations average is 0.04). (a) different Cr^{3+} concentrations (-◆-0;-■-150;-●-200 mg/L); (b) different CrO_4^{2-} concentrations (-◆-0;-■-10;-▲-15 mg/L).

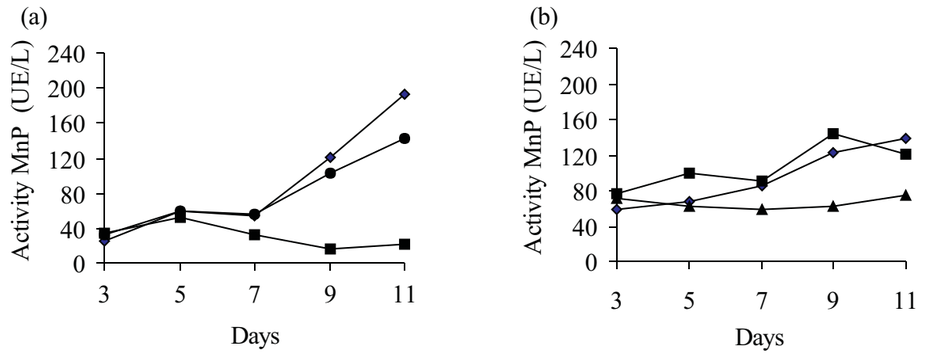

Figure 3. MnP activity (mean values) vs Time (the standard deviations average is 16.1 UE/L) (a) different Cr^{3+} concentration (-◆-0;-●-150;-■-200 mg/L); (b) different CrO_4^{2-} concentrations (-◆-0;-■-10;-▲-15 mg/L).

Table 1. MnP peroxidase activity. The enzymatic activity was determined at different Cr^{3+} and CrO_4^{2-} concentrations added before the enzymatic reaction with H_2O_2 .

Ion	*Conc Tested*	*Act. MnP (UE/L)*		*Ion*	*Conc Tested*	*Act. MnP (UE/L)*	
Cr^{3+}	0 mg/L	42.39	6.4	CrO_4^{2-}	0 mg/L	42.39	6.4
	50 mg/L	35.57	4.2		5 mg/L	43.22	4.6
	100 mg/L	42.21	9.6		10 mg/L	44.98	2.3
	150 mg/L	44.36	9.6		20 mg/L	38.07	3.4
	200 mg/L	50.90	7.2		50 mg/L	46.96	5.6
	300 mg/L	43.34	4.1				

already suggested by Gadd [9], that the toxic effects of heavy metals on ligninolytic fungi involve growth inhibition, induction of morphological changes and a variety of metabolic changes such as inhibition of enzyme production.

From the results obtained in this study it can be concluded that the *Bjerkandera* sp. BOS55 can be considered as a potential biodegrading fungus of synthetic dyestuffs in a liquid media contaminated with chromium ions in concentrations of up to 150 mg/L of Cr(III) and 10 mg/L of Cr(VI).

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