Adsorption of Congo Red dye on hazelnut shells and degradation with Phanerochaete chrysosporium

ADSORPTION OF CONGO RED DYE ON HAZELNUT SHELLS AND DEGRADATION WITH *Phanerochaete chrysosporium*

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The present work concerns the experimental evaluation of hazelnut shells as a low cost natural biosorbent. Adsorption of the direct azo dye Congo Red was performed within a concentrations range of 50-5000 mg/L. Hazelnut shells were employed as organic support for *Phanerochaete chrysosporium* cultures to study the best cultural medium composition for the MnP production. The capability of *Phanerochaete chrysosporium* to take macronutrients as carbon and nitrogen from hazelnut shells was demonstrated. Cultures of *Phanerochaete chrysosporium* were carried out with hazelnut shells coming from Congo Red adsorption tests, showing that 43% of the adsorbed dye was degraded.

Keywords: Hazelnut shells; White-rot fungi; Manganese peroxidase; Adsorption; Dyes

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INTRODUCTION

Research about dye removal from industrial effluents is receiving increasing attention as legislation is becoming more and more restrictive. More than 100,000 dyes are commercially available (Nigam et al. 2000) and more than 700,000 tons/year are produced in the world for the textile industry alone (Will et al. 2000). Most of these dyes are difficult to decolorize due to their stability, complex structure, and synthetic origin, necessary characteristics due to the fact that they have to be fast upon exposure to sweat, light, water, and oxidizing agents.

Generally dyes are not easily biodegradable, and for this reason they are not fully removed in conventional biological plants. Therefore, a tertiary refining process is required for a complete decolourization in order to obtain an effluent of high quality suitable for reuse in the same dyehouse plant (Ferrero 2007). Tertiary treatments can be divided in three categories, as summarized in Table 1.

Table 1. Tertiary Treatments

Category of treatment	Type
Physical	Adsorption, ion exchange, electrokinetic coagulation, irradiation in oxidizing medium
Chemical	Fenton's reagent, sodium hypochlorite, photochemical oxidation, ozonation, cucurbituril, electrochemical destruction
Biological	Adsorption on living/dead biomass, anaerobic treatments with single or mixed cultures of bacteria, biodegradation by white-rot fungi

Among these advanced treatments, at this moment, adsorption is considered more effective and less expensive than other technologies. Activated carbon is the most used material, owing to its high adsorption capacity, but it is expensive and needs a thermal regeneration process when the saturation is reached, increasing further its cost.

Research has been directed towards developing more effective and economical solutions, investigating the use of various low cost organic adsorbents. These materials should be low cost, easily available, and disposable without regeneration.

Employing materials derived from natural sources, plant wastes, or industrial by-products could be a good way to reduce cost problems. Examples of these kinds of materials used as adsorbents include: orange peel (Arami et al. 2005), rice hulls, maize cob, bagasse pith, wheat straw, corn cobs, and wood chips. These have been studied and found effective and cheaper when compared with activated carbon (Nassar & El-Geundi 1991; Nawar & Doma 1989; Nigam et al. 2000). Most of them are cellulose-based and can be employed without any previous thermal or chemical treatment.

In this study the evaluation of dye adsorption on hazelnut shells and its degradation are reported. The choice of this material is justified by its large availability in some countries, as a food industry waste. Nowadays, its major use is as combustible solid fuel, owing to its considerable calorific value (near 17 MJ/kg of dried product), very close to that of wood.

The use of hazelnut shells as sorbents with high surface area and without any thermal treatment was proposed in some studies for the removal of toxic metal ions as Cr(VI) (Cimino et al. 2000; Kobya 2004) and for the removal of dyes such as Methylene Blue (Ferrero 2007; Kannan and Sundaram 2001) and Acid Blue 25 (Ferrero 2007).

The dye used in this work was Congo Red, a direct azo dye employed for dyeing cellulosic fibers such as cotton. The adsorbed dye was biodegraded by the white-rot fungus *Phanerochaete chrysosporium*. This is a well known ligninolityc white-rot fungus that is able to degrade and in some cases mineralize several xenobiotic compounds as polychlorinated biphenyl (Eaton 1985; Morris & Lester 1994), DDT (Bumpus et al. 1985; Bumpus & August 1987), dioxin (Hammel et al. 1986), benzo(a)pyrene (Hammerli et al. 1986), trinitrotoluene (Fernando et al. 1990) and dyes (Wesemberg et al. 2003).

The present work has two main aims: the evaluation of Congo Red adsorption on hazelnut shells and its biodegradation by the white-rot fungus *Phanerochaete chrysosporium*, employing hazelnut shells with adsorbed dye as support-substrate for the microorganism growth.

EXPERIMENTAL

The experimental protocol followed in this study can be summarized as below:

- Evaluation of Congo Red adsorption rate on hazelnut shells.
- Evaluation of *Phanerochaete chrysosporium* growth on this material, focusing on the cultural medium composition in order to determine the best composition for the manganese dependent peroxidase (MnP) production. Three types of cultures were studied:

  - Complete medium.
  - Medium without carbon source.
  - Medium without carbon and nitrogen sources.
- Evaluation of *Phanerochaete chrysosporium* growth on hazelnut shells containing the adsorbed dye, employing the cultural medium without carbon and nitrogen sources.
- Determination of degraded dye percentage, after hazelnut shells extraction.

Strain and Culture Conditions

Phanerochaete chrysosporium ATCC 24725 (BKM-F-1767) was maintained on 2% malt extract agar plates. Culture media were inoculated with conidia (5×10^{10}/L) suspended in distilled water.

A nitrogen limited medium with glucose as the carbon source was used, prepared according to Tien and Kirk (1988) plus asolecitin from soya bean (0.75 g/L). The initial pH value was 5.5, and the initial glucose and ammonium concentrations were 10.0 and 0.04 g/L, respectively. The cultures were grown at 39°C during the trophophase and at 30°C during the idiophase, in pure oxygen atmosphere (Chimirri 2004).

Dye Adsorption Studies

Hazelnut shells were obtained from a nut processing factory situated in the geographical area of Asti, northern Italy, where the variety *Corylus avellana* is cultivated.

Before their use, hazelnut shells were chopped to sizes of approximately 0.7 cm^2, and then washed with deionized water according to the following procedure: 30g of hazelnut shells were soaked in 100 mL of deionized water in 500 mL Erlenmeyer flasks provided of baffles and agitated at 170 rpm on an horizontal orbital shaker for 24h at 30°C in a thermostatic room.

This washing was repeated three times; a sample of each washing liquid was taken. Finally hazelnut shells were dried in air oven at 105 °C for 24 h.

The samples of liquid coming from the washing pre-treatment required 24h at 30°C for the natural sedimentation of suspended particles. At the end of this time samples of the clarified supernatant were taken and analysed spectrophotometrically using a Hewlett Packard 8452 Diode Array spectrophotometer.

Stock solution of 10 g/L of Congo Red (Aldrich Chemical Company, Inc.) was used to prepare dye solutions at four different concentrations: 50, 500, 1000, and 5000 mg/L.

Adsorption experiments were carried out in 500 mL Erlenmeyer flasks; a ratio of 10 g dry substrate : 100 mL dye solution was used. Hazelnut shells were soaked in these solutions for up to 48h at 30°C in static conditions.

Dye solution samples were taken at regular intervals during the test and centrifuged at 9000 rpm for 7 min using an ultracentrifuge Centrikon T-42K. The clarified supernatants were analysed spectrophotometrically. Dye removal was calculated from the decrease of absorption at the λ_{max} of Congo Red: 490 nm.

Hazelnut shells separated from each dye solution were dried in air oven for 24 h at 105°C.

Finally a desorption test was carried out with the medium employed in *Phanerochaete chrysosporium* cultures, according to the following procedure: 20g of hazelnut shells coming from the adsorption tests were soaked in 250 ml of medium and incubated for 48h at 30°C. Liquid samples were collected and analyzed spectrophotometrically.

Biomass Growth Studies

The experiments were carried out in shallow stationary cultures with biomass immobilized on hazelnut shells, employing 500 mL Erlenmeyer and 2.8 L Fernback flasks. The amount of hazelnut shells was 20 g in Erlenmeyer and 90 g in Fernback flasks, with 70 and 300 mL medium volumes respectively, in order to maintain the same solid-liquid ratio of 1:3,5 in both types of flasks. These amounts of hazelnut shells were also chosen in order to cover the flasks bottom with a single layer of material. All the cultures were flushed daily with pure oxygen.

The initial temperature, corresponding to the trophophase, was 39°C, turned down to 30°C at the beginning of the idiophase.

Glucose and ammonium concentrations in extracellular fluid were determined spectrophotometrically, using enzymatic kits (Boehringer and Mannheim, numbers 10 716 251 035 and 11 112 732 035, respectively).

MnP activity was determined spectrophotometrically on the undiluted extracellular fluid, by the procedure of Paszinsky et al. (1988) at 568 nm and 22°C, with 2,6-dimethoxy-phenol as substrate.

Dye degradation studies were carried out employing hazelnut shells treated with 500 mg/L Congo Red solution. This concentration was chosen among the other three in order to reproduce the typical dye concentration of a textile industry effluent (Laing 1996; Shelley 1994; Pierce 1994). Two biotic cultures and an abiotic one were carried out in order to evaluate the adsorbed dye degradation.

These cultures were carried out in Erlenmeyer flasks, with 20 g of hazelnut shells and 70 mL of medium without carbon and nitrogen sources. Tests were carried out until MnP enzymatic activity reached to zero in biotic cultures.

Chemical extraction with 70% (v/v) methanol aqueous solution was used to recovery undegraded dye from hazelnut shells, according to the following procedure: 20 g of shells, taken from cultures were transferred in Erlenmeyer flasks and soaked in 100 mL of methanol solution. The flasks were agitated at 170 rpm on a horizontal orbital shaker for 24h, at 30°C. The extraction was repeated three times.

The liquid collected from each extraction was analyzed spectrophotometrically at λ=490 nm. The difference between the amount of adsorbed dye and the amount of extracted dye, corresponds to the amount of degraded dye in biotic cultures.

RESULTS AND DISCUSSION

Hazelnut Shells Pre-treatment

A washing treatment was necessary not only to remove the impurities and the internal peel, but principally to study the release of coloured substances from hazelnut

shells that could interfere with the successive spectrophotometric measurements at the characteristic wavelength of Congo Red.

The washing was repeated eight times, and samples taken from each one were analyzed spectrophotometrically. The results showed that after the third washing the absorbance values were negligible at 490 nm, so a pre-treatment of three washing cycles was considered adequate.

Adsorption Studies

This test was carried out with four increasing dye concentrations: 50, 500, 1000, and 5000 mg/L in order to evaluate the adsorption rate on hazelnut shells. Results are plotted in Fig. 1 and Fig. 2.

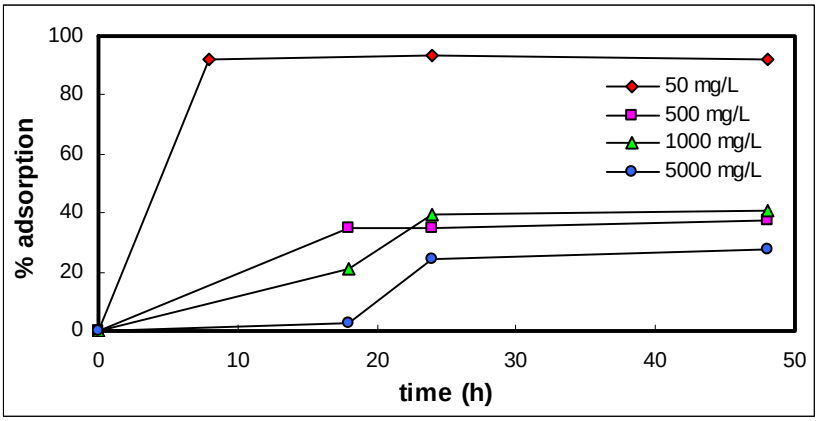

Fig. 1. Adsorption curves on hazelnut shells at different concentrations of Congo Red.

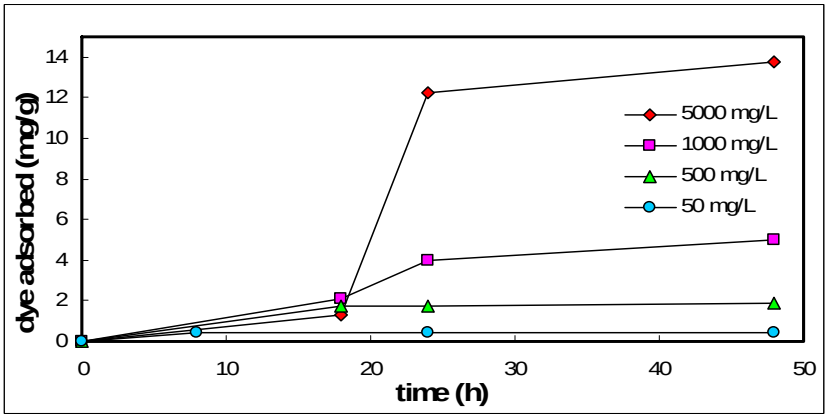

Fig. 2. Amount of dye adsorbed on hazelnut shells at different concentrations of Congo Red.

The results show that the maximum amount of adsorbed Congo Red was 13.75 mg/g_{shells} at 48 h, corresponding to 27.5% in the case of the 5000 mg/L concentration solution. In the case of the 500 mg/L Congo Red solution, the maximum dye adsorbed amount was 1.87 mg/g_{shells} at 48 h, corresponding to 37.5%.

Desorption tests done on each sample coming from adsorption tests confirmed that desorption phenomena did not take place, because no peak of absorbance was detected at the characteristic wavelength of Congo Red.

Biomass Growth and Enzymatic Production Studies

Experimental tests on three different medium compositions were carried out in 500 ml Erlenmeyer flasks, for the evaluation of *Phanerochaete chrysosporium* growth on hazelnut shells and MnP production.

It is known from the literature that the secretion of MnP is promoted by limited nutrients availability (Wesemberg et al. 2003); for this reason and in order to verify if *Phanerochaete chrysosporium* was able to take nutrients from the organic support, a complete composition medium was first considered, then the carbon source was subtracted, and lastly also the nitrogen one.

Cultural medium composition influences the trophophase time length (which occurs when nitrogen concentration reaches to zero) and as consequence on the outset of MnP activity. This effect is shown in Fig. 3. This lag time is explained by the fact that the microorganism needs more time to extract the nutrients necessary for its growth from the organic support, because they are not available in the cultural medium.

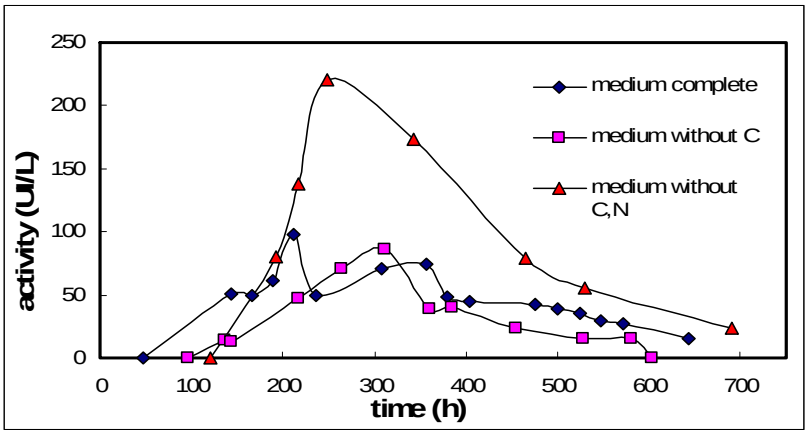

Fig. 3. Time course of MnP activity in Erlenmeyer flasks: Effect of cultural medium composition.

Each curve shows a maximum: the highest MnP activity (more than 220 UI/L at 129 h) was reached by the culture without carbon and nitrogen sources; lower activities were reached by the other two cultures: 86 UI/L at 216 h and 98 UI/L at 164 h respectively for cultures lacking of the carbon source and complete medium. MnP activity was detectable for more than 500 hours (20 days) in the case of culture without carbon source and 570 and 596 hours in the case of culture without carbon and nitrogen sources and cultures in complete medium, respectively.

Another test was carried out employing a 2.8 L Fernback flask, in order to study the maximum time length reachable by the culture lacking of the carbon and the nitrogen sources and to simulate a scale-up of Erlenmeyer culture conditions; the results are plotted in Fig. 4.

Trophophase time length was near 120 hours, as in the case of Erlenmeyer tests with the same type of cultural medium composition. The presence of some relative maxima is due to the availability of carbon source; the second relative maximum at 362 h after the first at 180 h, is probably the consequence of the extraction of carbon from the organic support by the microorganism. In order to demonstrate this fact when the enzymatic activity fell down at 872 h, glucose was added. Triangular dots represent the time when glucose as the carbon source was added in the cultural medium, at 872, 992, and 1186 hours. In all cases the MnP activity increased, reaching 70 UI/L, and more than 80 UI/L after the second addition, and 120 UI/L after the third one. The maximum time length reached without glucose addition was near 850 hours, at more than one month.

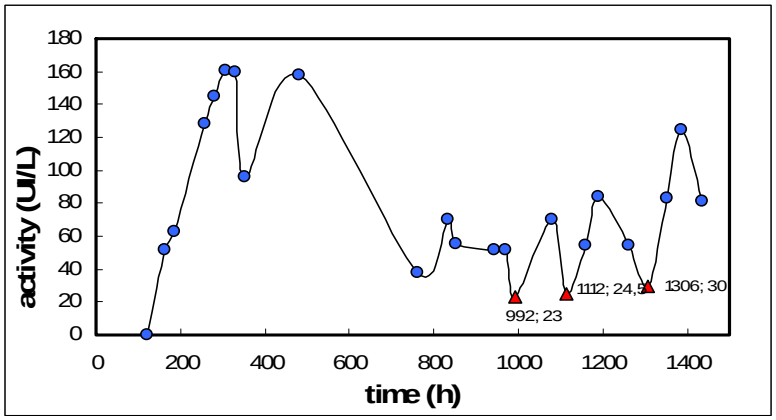

Fig. 4. Time course of MnP activity in Fernback flask (triangular dots represents carbon source additions).

Considering these results, the cultural medium composition lacking of carbon and nitrogen sources was chosen to produce *Phanerochaete chrysosporium* for dye degradation studies.

Dye Adsorbed Degradation Studies

Tests were carried out with three different cultures: one with hazelnut shells without adsorbed Congo Red and two treated with dye solution at different concentrations, in order to evaluate not only dye degradation, but also dye effect on growth and MnP production of *Phanerochaete chrysosporium*. The results are plotted in Fig. 5.

MnP activity started, in the case of the culture employing hazelnut shells treated with 500 mg/L dye solution, at 96 h, as in the case of absence of adsorbed dye, earlier than the 209 h of the culture employing hazelnut shells treated with 50 mg/L dye solution. The maximum MnP activity was quite different between the three cultures: 115 UI/L at 257 h of the no dye line, while the culture 500 mg/L dye reached 91 UI/L at 257 h, and the culture 50 mg/L dye 75 UI/L at 353 h.

MnP activity time length of the higher concentration dye culture was also longer, it ended after more than 400 hours (16 days), double if compared with the one of the lower concentration dye cultures.

After this consideration it is possible to say that the presence of adsorbed dye did not inhibit the microorganism growth and MnP activity. On the contrary in this case, MnP activity time length and maximum activity value were promoted by the highest dye concentration.

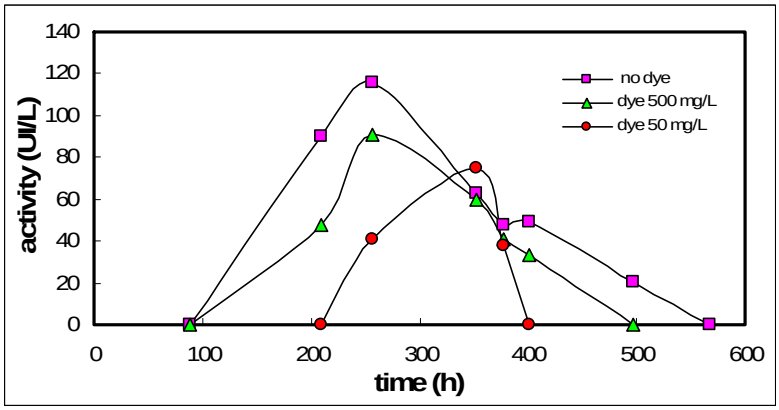

Fig. 5. Time course of MnP activity in Erlenmeyer flasks: effect of dye concentration.

At this point, in order to evaluate the dye degradation efficiency of the 500 mg/L culture, a methanol extraction was carried out to recollect undegraded Congo Red from hazelnut shells used in the biotic and in the abiotic cultures.

Examining the results obtained from the methanol extraction, reported in Table 2 and comparing them with the ones collected in Fig. 2 about adsorption test, it is possible to say that 160 mg/L on 187 mg/L adsorbed by hazelnut shells were recovered with methanol extraction from the abiotic culture. It is possible to see that more than 43% of adsorbed dye was degraded by the microorganism in biotic culture; the difference of 16% between the results obtained from culture "biotic1" and "biotic2" is attributed to biological variability.

Finally it is possible to say that taking into consideration these results, cultural conditions could be optimized to obtain not only higher but more persistent over time MnP activity in order to increase the amount of degradation of adsorbed dye. Future developments could be the set-up of a fixed bed reactor with *Phanerochaete chrysosporium* immobilized onto hazelnut shells for dyehouse effluent degradation.

Table 2. Results Collected from Methanol Extraction

	dye collected from each extraction (mg/L)			total of 3 extractions (mg/L)	total (mg/L)	% degraded
culture type	1	2	3			
abiotic	340	110	10	460	160	0
biotic "1"	160	90	40	290	100	37,50
biotic "2"	170	90	30	280	90	43,75

CONCLUSIONS

1. From this study it is possible to employ hazelnut shells, broken in small sizes, as a potentially inexpensive biosorbent without previous thermal treatment.
2. Shallow stationary immobilized cultures of *Phanerochaete chrysosporium* on an organic vegetable matter were not proposed until now, whereas in some other studies the cultures were made to grow on inert materials such as ceramic Rashig rings or ceramic Berl saddles (Bosco et al. 1999).
3. It was demonstrated that *Phanerochaete chrysosporium* is able to grow and to produce MnP in the absence of macronutrients (carbon and nitrogen sources) from the cultural medium and is able to take out them from the organic support.
4. After the evaluation of the best culture conditions, it was demonstrated that *Phanerochaete chrysosporium* is able to degrade more than 43% of Congo Red adsorbed on hazelnut shells from a solution of 500 mg/L.

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