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Assessing enzymatic deinking for secondary fibers paper recycling in the presence of flexographic inks

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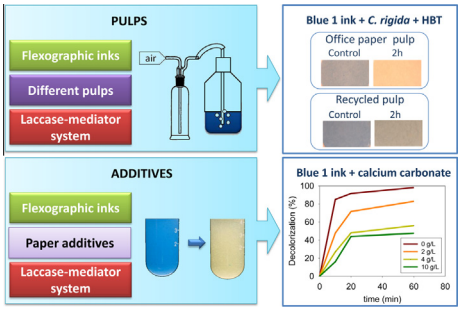
HIGHLIGHTS

- Flexographic inks biodeinking has been studied in the presence of different pulps.
- The influence of paper additives in biodeinking has been reported.
- The presence of some paper additives slowed the biodeinking process.
- Calcium carbonate strongly inhibited the enzymatic deinking.
- The best results were obtained with *Eucalyptus* bleached pulp in the absence of additives.

GRAPHICAL ABSTRACT



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ABSTRACT

Paper recycling systems based on flotation technologies are not efficient to deink secondary fibers from new printing technologies based on flexographic inks which, even being more environmentally friendly, still present some recycling problems. We have evaluated the efficiency of a biotechnological approach, based on the laccase-mediator system, for biodeinking of three flexographic inks (Blue 1, Magenta HX-E and Red 48:4) in the presence of several pulps (bleached pulp, deinked paper pulp from secondary fibers and pulp from commercial uncoated and coated office paper sheets). Two fungal laccases, one from the basidiomycete *Coriolopsis rigida* and the other from the ascomycete *Myceliophthora thermophila*, were used as catalysts in the presence of HBT (1-hydroxybenzotriazole) or methyl syringate, as models of synthetic and natural mediators, respectively. Higher decoloration rates were achieved for Blue 1 and Magenta HX-E than for Red 48:4. The best results were obtained using bleached pulps without additives. Then, to identify the chemical compounds that may hamper enzymatic biodeinking, the influence of several additives on ink decoloration and enzyme activity was tested. Only calcium carbonate slowed down ink decolorization by up to 40%, although it also decreased the mediator assisted enzyme deactivation. In this work, a system to evaluate the efficiency of the laccase-mediator system in biodeinking of flexographic inks in the presence of different pulps and papers has been developed.

1. Introduction

Recycled paper has become an important source of raw material for the pulp and paper industry. Generally, the industrial process for removing contaminants involves pulping, screening, cleaning and flotation. Although current flotation technologies are efficient for offset printed paper deinking, the use of environmentally friendly flexographic inks presents some recycling difficulties,

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due to the small size of the pigments and its hydrophilic character [1,2]. This fact poses a problem for the paper recycling process, since ink particles resettle in fibers causing waste waters issues.

Laccases are multicopper oxidases that catalyze the oxidation of phenolic compounds and aromatic amines using oxygen as its electron acceptor. The driving force of the enzymatic mechanism lays on a difference between the redox potential of the reducing substrate and that of the oxidation site in the enzyme's active center, the copper T1. Most laccases only allow direct oxidation of low-redox-potential compounds, but high-redox potential laccases produced by basidiomycetes ($E^\circ \geqslant 0.70$ V) are of great biotechnological interest due to their higher oxidative capabilities, including the oxidation of mediator compounds with high-redox potentials [3]. Both high- and low-redox fungal laccases catalyze the oxidation of a wide range of phenolic compounds, using molecular oxygen as the electron acceptor, but in the presence of low molecular mass compounds (acting as mediators) their substrate range is extended to non-phenolic substrates [3,4]. These compounds are oxidized by laccases to stable radicals, which act on compounds that are not directly oxidizable by these enzymes. It has been reported that the laccase-mediator system degrades not only lignin but also a great variety of recalcitrant and environmentally harmful compounds [5–7]. HBT (1-hydroxybenzotriazole) has been described as an efficient synthetic mediator for laccases in pulp delignification [8], pitch removal [9] and oxidation of polycyclic aromatic compounds [10]. Nevertheless, natural substances, as methyl syringate, are being tested as mediators since they are environmentally-friendly and could be obtained as by-products of the pulp industry or from industrial effluents [4,11–13]. In the last years, many studies have been conducted on laccase-producing strains and on the application of purified laccases, in both free and immobilized forms, for the removal of synthetic dyes [12,14–17], inkjet [18] and flexographic inks [4].

The industrial use of laccases from white rot fungi is currently restricted due to its limited heterologous expression [19–21]. However, a laccase from the ascomycete *Myceliophthora thermophila* has been successfully expressed in industrial heterologous hosts with high yields [22].

In a previous work we demonstrated the feasibility of decolorizing flexographic inks using laccases from three white rot fungi *Trametes villosa*, *Coriolopsis rigida,* and *Pycnoporus coccineus* and from one ascomycete, *M. thermophila*, in the presence of synthetic and natural mediators [4]. The highest decolorization values were obtained in reactions with the three basidiomycete laccases and HBT, but good results were also obtained using the *M. thermophila* laccase in the presence of syringyl-type natural mediators.

In this paper a new method for biodeinking commercial pulp pre-dyed with flexographic inks at lab scale is proposed. Decolorization by *C. rigida* and *M. thermophila* laccases has been assayed in the presence of HBT or methyl syringate as synthetic and natural mediators. Moreover, influence of different paper additives commonly present in secondary fibers has been evaluated.

2. Material and methods

2.1. Flexographic inks, laccases and mediators

The flexographic inks, Blue 1 (B1), Red 48:4 (R48) and Flexiprint Magenta HX-E (MG), were supplied by Quimovil SA and Flint Group Iberia SA (Spain). Laccase from *M. thermophila* (Novozym 51003) was supplied by Novozymes® (Denmark) and laccase from *C. rigida* was produced in a basal medium with glucose supplemented with copper plus ethanol [23]. The mediators used were HBT (Fluka) and methyl syringate (Novozymes®).

Laccase activity was determined at 25 °C by measuring the oxidation of 5 mM 2,6-dimethoxyphenol (Merck) to coerulignone, in 100 mM sodium tartrate, pH 4 for *C. rigida* or 100 mM sodium phosphate, pH 6 for *M. thermophila* laccase ($\varepsilon_{469} = 27{,}500\ M^{-1}\ cm^{-1}$, referred to substrate concentration [24]). One unit of laccase was defined as the amount of enzyme oxidizing 1 μmol of substrate per min.

2.2. Biodeinking treatments

Bleached pulp from *Eucalyptus globulus* and deinked paper pulp from secondary fibers were obtained from the ENCE (Pontevedra, Spain) and Holmen (Madrid, Spain) mills, respectively. Other pulps were obtained in the laboratory by using highly refined paper sheets without fillers (containing alkyl ketene dimer binder and a yellow dye) and commercial 300 g/m^2 coated office paper sheets (containing 1.3% latex and 45.5% fillers), both from TORRASPAPEL S.A. (Barcelona, Spain). Paper sheets were firstly soaked during 24 h at 4 °C. 250 godp (grams of oven dry pulp) of both papers were disintegrated at 15% consistency (weight percent of air-dry pulp in suspension) for 30 min and 50 °C, in a modified blender (a new blade for a Thermomix Vorwerk® was designed to assure a proper disintegration).

Biodeinking treatments were performed with 3 godp ENCE bleached pulp at 2% consistency, previously dyed with 0.1% ink (B1 and MG inks) or 0.05% ink (R48 ink). Decolorization of B1 and MG inks was also assayed in the presence of the other three pulps. Treatments were carried out in 500 mL bottles containing 150 mL of dyed pulp placed in an orbital shaker at 28 °C and 280 rpm with 5 U/mL of laccase and 500 μM of mediator in 50 mM of sodium acetate, pH 4 for *C. rigida* laccase or 50 mM of sodium phosphate, pH 6 for *M. thermophila* laccase. Tween 80 at 0.01% was added in all cases [4]. Since deinked and office paper pulps were strongly alkaline, before adding chemicals the pH value was adjusted with HCl to 4 in reactions catalyzed by the laccase from *C. rigida*, or to 6 for that of *M. thermophila*. During biodeinking, air (3 L/min) was bubbled into the suspension through a porous diffuser. Control reactions, without enzyme and mediator, were treated in the same conditions. Each experimental condition was assayed twice. At the end of the treatment (time range from 1 h to 24 h) the pulps were filtered with the aid of a vacuum pump in a Bückner funnel and pressed to obtain a handsheet (Tappi method T218 sp-029). The filtrate was collected to be subsequently analyzed.

2.3. Influence of paper additives on ink decolorization

Additional ink decolorization assays without pulp were performed using *C. rigida* laccase and HBT during 24 h, as described above. Different concentrations (0–10 g/L) of calcium carbonate or starch were added to the suspensions before addition of laccase and mediator, as well as 1% of optical brightener derived tetrasulfonic estilbene, alkyl ketene dimer binder, styrene acrylic binder or carboxylated styrene butadiene latex. Paper additives were kindly supplied by TORRASPAPEL S.A. (Barcelona, Spain). In treatments containing calcium carbonate it was necessary to adjust the pH value to 4 with HCl before the enzyme addition.

2.4. Color analysis of pulps and filtrates

To evaluate the chromatic changes of the handsheets obtained, the color coordinates CIE $L^*a^*b^*$ were measured using a Datacolor Elrepho 2000 spectrophotometer, according to Tappi T 527 om-07. This measurement system is based on lightness and opposite colors on a three-dimensional space, whose coordinates are lightness ($L^* = 0$ yields black and $L^* = 100$ indicates diffuse white), position between red/magenta and green (a^*), and position between