Lignin transformation: role of a new versatile peroxidase from a *Bjerkandera* sp.

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INTRODUCTION

Attention has recently been paid to a novel class of ligninolytic peroxidases—versatile peroxidases, which combine the typical properties of both manganese peroxidase and lignin peroxidase, coupled to a broad substrate range. Most studies pertaining to versatile enzymes have focused on characterisation of their oxidation ability toward several compounds: however little effort has to date been devoted to the activity of those enzymes on what is thought to be their natural substrate lignin.

In this work, sulphur free lignin (obtained from straw) was used as model compound. This research effort was aimed at demonstrating that a versatile peroxidase from a *Bjerkandera* sp. can interact directly with said lignin preparations, and assessing the effects of the prevailing reaction conditions upon such decolourisation processes.

MATERIALS AND METHODS

Decolourisation of a lignin solution was used in attempts to assess occurrence of a reaction, catalysed by a versatile peroxidase isolated from *Bjerkandera* sp. (B33/3), in the absence of mediators and possible application to pulp and paper effluent decolourisation. Gel filtration allowed monitoring of lignin molecular mass distribution evolution.

Reaction conditions

The experimental conditions considered for the purified peroxidase reaction system were set according to a 2^3 full factorial design, as detailed in Table 1.

Table 1. Levels of processing variables tested in the decolourisation reactions initiated with the Peroxidase/ H_2O_2 system.

Variable	Peroxidase reaction	
	Level -1	Level +1
1 Reaction Time (h)	2.0	4.0
2 pH	4.0	5.3
3 Lignin: Enzyme ratio (mg/mg)	800	1500

RESULTS AND DISCUSSION

Calculated effects

Table 2. Calculated effects (and associated standard errors) for each variable and two-factor interactions.

Effect	Estimate ± Std error
Average	40.8 ± 1.5
Main effects	
variable 1	12.9 ± 3.1
variable 2	-49.8 ± 3.1
variable 3	20.6 ± 3.1
Two-factor interactions	
variable 1×variable 2	8.8 ± 3.1
variable 2×variable 3	9.5 ± 3.1
variable 1×variable 3	7.4 ± 3.1

Reaction characterisation

The molecular mass distribution of lignin was determined, by gel filtration chromatography, on a Sephadex G-100 column. Elution proceeded at 0.5 mL.min^{-1} with 100 mM NaOH (pH 13). The effluent was monitored spectrophotometrically at 280 nm.

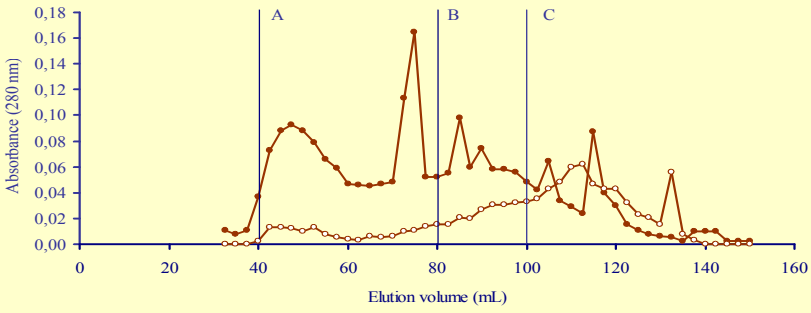

Figure 1: Gel permeation chromatogram of samples obtained from the control (pH 4.0) (•) and from the run in which the higher decolourisation level was obtained (o). A - excluded lignin fragments: (above 158000 Da); B - separated lignin fragments: (between 158000 and 44000 Da); C - separated lignin fragments: (below 44000 Da).

Conclusions

✓All variables tested affected the reaction responses; however the most important effect was that of pH—the higher the pH, the smaller the decolourisation extent.

✓ Reaction time also affected the reaction responses, hence suggesting that these reactions may occur to a great extent.

✓The relative amounts of lignin and enzyme affected the reaction responses as well; the higher the amount of enzyme added, the higher the decolourisation yield.

✓ Versatile peroxidase RBP is able to directly interact with lignin, in the absence of mediators.

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Acknowledgements
P. Moreira was sponsored by a doctoral fellowship (PRAXIS XXI/BD/15825/98) granted by FCT, Portugal.