ABSTRACTS

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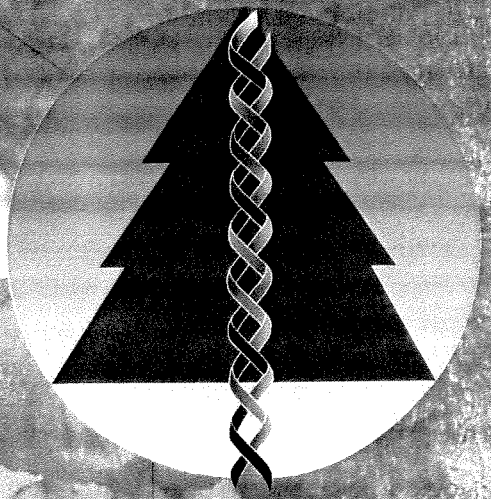

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SCREENING NEW ENZYMES FOR ENZYMATIC DEINKING

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Introduction

The deinking process involves the ink particles dislodgement from the fibre surface and the separation of the dispersed ink. The use of enzymes is generally related either to the fibre surface or the ink particles attack *(1,2,3,4)*. For that matter, enzymatic deinking is known to greatly depend on the pulp characteristics, namely the chemical composition of the fibre and ink particles and the printing process involved, which defines the fibre/ink interactions. Additionally, other variables influence the process (consistency, pH, temperature, mixing), which cannot be ignored *(3,5,6,7)*. In the present work several xylanolytic and cellulolytic enzymes with potential affinity to fibre constitutive were produced and preliminary characterised so that their contribution to the deinking of a mixed office wastepaper sample could be evaluated.

Methods and Materials

Enzyme production

Several organisms described as producers of xylanolytic and cellulolytic activities belonging to the Culture Collection of Industrial Microorganisms (CCMI) from INETI were preliminary used in this work: *Aspergillus oryzae* CCMI 32, *Aspergillus terreus* CCMI 498, *Trichoderma viride* CCMI 84, *Trichoderma harzianum* CCMI 482 and *Coriolus versicolor* CCMI 875. Stock cultures were maintained on potato dextrose agar slants at 4ºC, in the dark, and transferred into fresh medium once a month.

The culture medium used in this work consisted of (per litre of water): KH_2PO_4, 2.0g; $(NH_4)_2SO_4$, 3.0g; $MgSO_4 \cdot 7H_2O$, 0.3g; $CaCl_2$, 0.2g; $CoCl_2$, 1.7mg; peptone, 1.0g; Tween 80, 185µL; Vishniac solution, 0.25mL *(8)*; urea, 0.3g; and was supplemented with 10.0 g/L oat spelts xylan (Sigma), microcrystalline cellulose Avicel (Merck) or lactose (BDH) as the carbon source.The microorganisms were grown at 28ºC in Erlenmeyer flasks shaken at 150 rpm on an orbital shaker, and samples were collected during 15 days. The extracellular enzymatic activities produced were characterised in terms of pH and temperature profiles and thermostability. Enzyme crude preparations showing reasonable activity values were freeze-dried before application in deinking assays.

Analytical Assays

Endo-1,4-β-Xylanase was assayed using 1% (w/v) oat spelts xylan as substrate. Enzyme activity was expressed in international units (U) as the amount of enzyme required to release 1 µmol per minute of xylose reducing equivalent under the assay conditions. β-Xylosidase activity was determined using the synthetic substrate p-nitrophenyl β-D-xylopyranoside (Sigma) as described by Li *et al. (9)*. One unit of activity (U) was defined as the amount of enzyme which catalyses the release of 1 micromol per minute of p-nitrophenol under the assay conditions. Cellulase activity (FPase) was assayed using Whatman #1 filter paper as substrate. Enzyme activity was reported as glucose reducing equivalents released per minute (U) under the assay conditions.

Paper source

The paper pulp used in this work was kindly supplied by the paper company *Renova* and consisted of a mixed office wastepaper sample.

Deinking methodology

The process included enzymatic pulping and a fibre/ink separation stage as shown in Fig. 1. To accurately estimate the enzymatic action, control assays (in presence of denatured enzyme) were made in parallel. After preliminary trials using all the fungal enzymes previously produced and characterised, two enzymatic preparations were selected for further use as follows (Table I): from CCMI 498 grown on Xylan exhibiting an endo-1,4-β-xylanase activity of 164.8 U/g, a FPase activity of 3.4 U/g and no β-xylosidase activity; and CCMI 84 grown on Cellulose displaying an endo-1,4-β-xylanase activity of 315.2 U/g, a FPase activity of 16.5 U/g and a β-xylosidase activity of 91.6 U/g (on the deinking assay conditions: 50ºC and pH 5.0).

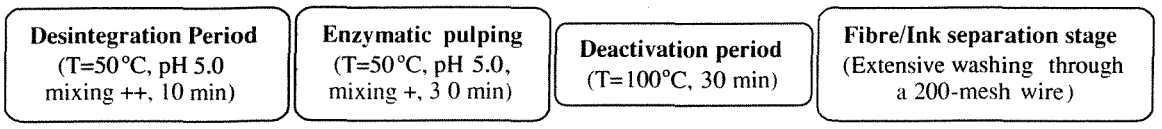

Fig. 1. Deinking methodology.

Table I. Enzyme dosage.

Enzyme Preparation	Endo-1,4-β-Xylanase activity (U/ g dry pulp)	FPase activity (U/ g dry pulp)	β-Xylosidase activity (U/ g dry pulp)
CCMI 498 (Xylan)	1.3	0.03	0.0
CCMI 84 (Cellulose)	1.3	0.07	0.4

Deinking evaluation

The process efficiency was evaluated by determining the physical and mechanical properties of pulp and paper (standard procedures) and by comparing the amount of ink present in paper sheets, before and after treatment, by image analysis.

Results and Discussion

As shown in Table II, the deinking operations contribute to a considerable ink removal in the mixed office wastepaper treatment. As a matter of fact, when the non-treated pulp and the control (no enzyme action present) are compared, a 42% decrease in residual ink is detected. This is probably the result of factors as mixing, temperature, washing, which enable the ink particles breaking down and allow their separation from the fibres *(3,5,6,7)*.

Nevertheless, the protocol can be improved by applying both tested enzymes. The preparation from CCMI 84 allows the best result since the ink area in the paper sheet decreases by 24% relatively to the control. Additionally, the particle statistics (average and median size) indicate that the amount of smaller particles decreases after the process. It is likely that this preparation is effective for the removal of smaller particles. The extract from CCMI 498 removes only 8% of the initial ink amount and produces only slight changes in particle features.

The enzymatic preparation from CCMI 84 is the only one which contains β-xylosidase activity and the cellulase dosage (measured as FPase) applied was slightly higher (Table I), whereas the endo-1,4-β-xylanase activity applied was similar for both enzymes. This seems to indicate that cellulase or, more probably, β-xylosidase activity is critical for the deinking process since the detected efficiencies were significantly different. Nonetheless, further studies of characterisation of enzyme activities should be performed in order to evaluate this hypothesis. At this stage, it can also not be excluded the presence of other unknown activities contributing to these results.

Table II: Effect of the enzymatic treatment on the removal of ink particles.

	Ink area (ppm)	% Variation	Average size (μm)	Median size (μm)
Non-treated pulp	5331	--	2065	746
Control	3109	--	1882	522
CCMI 498 (Xylan)	2850	8	2369	687
CCMI 84 (Cellulose)	2372	24	2755	1037

Another important feature regarding enzymes is their positive effect on the properties of the pulp and paper (Fig. 2). The enzymes either do not alter the strength properties (CCMI 84) or contribute to their improvement (CCMI 498). The pulp drainage rate has also varied according to the enzyme source. Enzyme extract from CCMI 84 was responsible for a 5% decrease, whereas CCMI 498 enzyme extract led to a 5% increase. These results suggest that the effective use of enzymes for deinking can also contribute to the pulp and paper properties improvement.

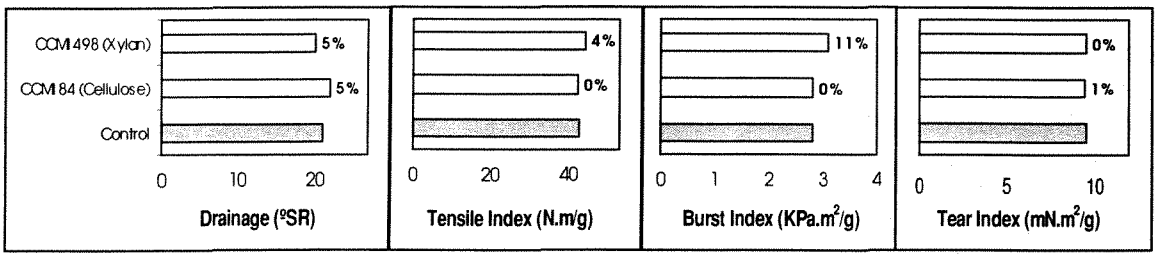

Fig. 2. Effect of the enzymatic treatment in the properties of pulp and paper.

Conclusions

These results demonstrate that the deinking methodology itself is responsible for removing 42% of the present ink. However, the action of both tested enzymes enhanced the ink removal by 24%, when using crude extracellular extract from *Trichoderma viride* CCMI 84 grown on microcrystaline cellulose Avicel, and by 8% when using the extract of *Aspergillus terreus* CCMI 498 grown on oat spelts xylan. Moreover, paper strength was not changed or it was even increased when these enzymes were included in the process.

Acknowledgments

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