

Effect of cellulase enzymes
on secondary fiber properties

Biotechnology in the paper industry

Dóra Dienes

Supervisor: Dr. Kati Réczey associate professor



Budapest University of Technology and Economics
Department of Agricultural Chemical Technology
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1. Introduction, aims

As mankind has been culturally developed the relationship between man and the main component of plant cell wall, cellulose has become more and more multilateral. Wood processing, paper and textile industry produce not only cellulosic stock materials and products utilized in many areas but also huge amount of cellulosic wastes.

Significance of paper recycling

Stock material of paper is chemical and/or mechanical pulp; however, waste paper has been increasingly important fiber-resource for paper industry besides primary fibers. Paper recycling reduces forest harvesting, energy consumption and emission of pollutants, moreover the problem of waste treatment. Some illustrative data: 1 ton paper manufactured of recycled fibers allows saving of 25-30 m³ water, 20-30 tree, appr. 4000 kWh electricity and results in reduced environmental pollution because of less consumption of chemicals.

The amount of world paper production and consumption has been increased to 30-fold in the XX. century (production was appr. 320 million tons in 2000). Composition of stock materials has been changed significantly as well: the ratio of primary fiber was appr. 90% in 1900 and even in 1950, whilst that was only 50% at the end of the last century with appr. 40% recycled fiber. Pattern of production shows that making of good quality fiber is concentrated in developed countries having wide-ranging woodlands. The ratio of paper recycling is different in every country. The proportion of waste paper in the processed stock is higher in Hungary than the average in the European Union (68% in 2003).

Utilization of enzymes

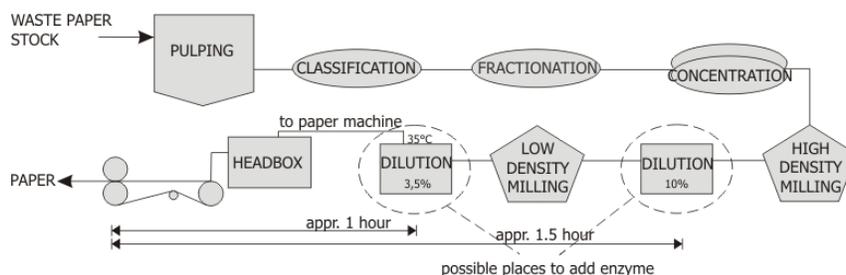
The using of enzymes (i.e. catalytic biomolecules, proteins) is based on their specific activity and environmentally friendly properties. The possibility of enzyme application is ensured by the development of biochemistry, microbiology, molecular biology and genetics. The most successful application of enzymes in pulp and paper industry so far is the biobleaching with xylanases. This process needs less chemicals, causes less pollution, furthermore it results in quality improvement.

The aim of the work

Although waste paper has been recycled for a long time, there are still problems to solve. Research area of present dissertation is improvement of this cellulose-waste utilisation technology in biotechnological way.

Our experiments aimed to improve drainage of secondary fibers using enzymatic treatment. In pre-experiments efficiency of cellulase, hemicellulase and amylase (in case of sized paper) enzymes have been tested. Based on our observations in these screening and previous results found in the literature, cellulase enzyme complex has been investigated further. The soft-rot fungus *Trichoderma reesei* is one of the most studied cellulolytic organisms because of its ability to produce a complete set of efficiently secreted cellulolytic enzymes. Effects of the cellulase complex and individual cellulase components especially from *T. reesei* were investigated.

The „Non-food” research group at Department of Agricultural Chemical Technology, Budapest University of Technology and Economics has been collaborating with Paper Research Institute since 1999 on the subject of enzymatic treatment of recycled fibers. Packaging paper products (sack paper among them) represent main part of paper manufacturing in Hungary. Present study was focused on waste paper based sack paper production, concentrating on improvement of secondary pulp drainage and air permeability of paper, with keeping physical properties of paper as good as possible. Commercial enzyme preparations produced for secondary fiber treatment and other industrial applications, as well as in-house fermented enzyme components were applied in treatment of recycled fibers. The investigation of enzymes that have not been used in this area before aimed to prove or deny assumptions based on our first results (Pergalase A40). Variability in the substrate composition results in difficulties; therefore general conclusions cannot be drawn.



Flowsheet of sackpaper production at Dunapack Ltd.

2. Applied substrate and tested enzymes

Substrate A commercial sackpaper (Dunakraft 90R, Dunapack Ltd., Hungary) manufactured of 8% virgin fiber, 25% once used secondary fiber, and 67% corrugated board waste paper was used in the experiments as the material to be treated.

Enzymes Commercially available enzyme preparations and in-house fermented enzymes were used to improve drainage of secondary fibers.

Pergalase A40 (Genencor International) is a commercially available *Trichoderma* enzyme preparation contains a mixture of cellulases and hemicellulases. Pergalase A40 is recommended for the treatment of secondary fibers. Optimal processing conditions for Pergalase A40 are reported by the producer as follows: 40-65°C, pH 4.5-6.5, pulp consistency 2-4%, reaction time 0.5–2.0 h. Light brown liquid.

IndiAge Super L (Genencor International) is produced by genetically modified microorganism, contains pure *T. reesei* Cel12A (EGIII) endoglucanase. IndiAge Super L is manufactured specially for textile finishing. It can be used in a broad pH range (4.5-7.5), within the temperature range of 30-50°C. Amber yellow liquid.

Ecostone N400 (AB Enzymes Oy) commercial enzyme preparation contains *Melanocarpus albomyces* Cel45A endoglucanase component. Ecostone N400 is manufactured for the textile industry (finishing of denim fabrics). Brown liquid.

The role of CBD was investigated in case of individual endoglucanase components. Culture broths containing only *T. reesei* Cel7B (EGI) and Cel7B core (catalytic domain of EGI) among cellulases were produced by genetically modified *T. reesei* strain QM9414 on glucose-containing medium. The target proteins were expressed under the constitutive promoter of the glyceraldehyde phosphate dehydrogenase gene (*gpdA*) from *A. nidulans*. Endogenous cellulase expression in the two recombinants (TrCel7B and TrCel7Bcore) was repressed by glucose.

Rhodothermus marinus Cel12A, a highly thermostable endoglucanase was expressed in *E. coli*. RmCel12A shows maximal activity at 90°C. Addition of enzyme before refining was investigated in model experiments with culture supernatant containing RmCel12A (temperature of pulp is higher than 90°C in this part of papermaking process).

3. Investigation of the treatment effects

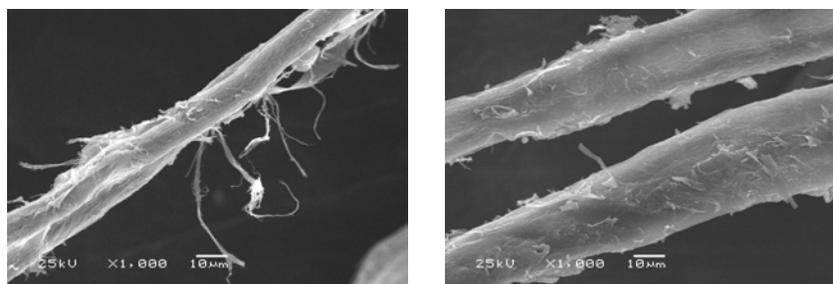
Fiber properties

Drainage property of pulp was determined by measuring its Schopper-Riegler freeness value ($^{\circ}$ SR) according to the standard procedure. Freeness is used in paper industry to characterize dewatering rate of fiber suspension.

During paper production (drying) structure of fibers is changed because of so-called hornification: fibers lose plasticity, become harder, strength properties are deteriorated. Pores of fibers become closed and flattened, therefore loss their ability to swell. The effect of enzymatic treatment on swelling ability was followed by measuring the water retention value (WRV) according to TAPPI method.

The pulp was fractionated using a Bauer–McNett classifier. The fiber length averages and distribution were determined for the untreated and enzyme treated pulps with a Kajaani FS-100 fiber analyzer.

Fiber samples were prepared from untreated and treated pulps to scanning electron microscopic analysis (JSM-5600 LV type) for surface characterization. The specimens were dried onto the sample holder and than coated with platinum.



Electron micrographs of untreated (A) and enzyme treated (B) secondary fibers.

Physical and mechanical properties of paper

Laboratory handsheets were prepared on Rapid–Köthen equipment. Air permeability (Gurley) and mechanical properties were determined on the final sheets, such as burst index, tensile index and tear index according to standard procedures.

4. New scientific results

New scientific results achieved in improvement of secondary fiber drainage performed in biotechnological way applying commercially available and in-house produced enzymes are as follows:

1. IndiAge Super L, commercial enzyme (Genencor International) contains *Trichoderma reesei* Cel12A (EGIII) endoglucanase component, manufactured for textile finishing, was found highly effective in improvement of secondary fiber drainage.
2. Amongst endoglucanases from *Trichoderma reesei*, the low molecular weight Cel12A (EGIII) -which is produced in only 0.1-0.2% of total amount of cellulases and lacks the cellulose-binding domain- is more effective, than the two main endoglucanases i.e. Cel7B (EGI) and Cel5A (EGII) in increasing of secondary pulp dewatering.
3. Air permeability of paper manufactured of mainly secondary fibers (Dunakraft 90R, Dunapack Ltd.) can be significantly enhanced by both cellulase complex (Pergalase A40) and Cel12A (EGIII) endoglucanase component (IndiAge Super L) from *T. reesei*. This property is important in case of sack paper production.
4. Effect of a given cellulase enzyme on secondary fiber properties can not be predicted from that of another enzyme even in case of similar origin (fungal/bacterial), molecular size, and molecular structure (having/lacking CBD).
5. Culture supernatants containing individual TrCel7B (EGI) and TrCel7B core produced in the presence of glucose (repressing endogenous cellulase expression) by genetically modified *T. reesei* QM9414 were found to be suitable for direct application, i.e. without any purification in upgrading of secondary fiber properties.
TrCel7B (EGI) and its catalytic domain have not caused deterioration of paper strength when those were applied in treatment of secondary pulp (Dunakraft 90R).
6. Culture supernatant containing individually the low molecular weight RmCel12A endoglucanase -that has no cellulose-binding domain- was found to be suitable for direct application in improvement of secondary fiber drainage (in experiments modeling high density milling and following process steps).

5. Application of results

Cellulases have been studied for improved paper recycling since the 1980s. Secondary pulp contains great amount of fines (small cellulose fibers) having high relative surface, therefore these fibers adsorb water in large extent. This effect of fines is enhanced by microfibrils and colloidal layer located on the surface. Thus dewatering rate on wire section is lower compared to primary pulp. The result is considerably decreased productivity of the paper making process compared to operation using virgin fibers.

Advantage of higher freeness achieved by hydrolysis of excess fines and microfibrils can be taken for enhancing the operation rate and/or greater dilution can be applied in the headbox of the paper machine. Latter one allows better sheet formation, thus improved quality of the product. Moreover, by adding enzymes to the pulp before refining the power consumption of the refiner can be lowered, or in case of same power consumption the refining rate can be improved, which gives better strength properties to the end product. In this way enzymatic treatment makes possible using poor quality raw material.

Productivity of paper processing can be significantly increased through improvement of pulp drainage; for instance 10% enhancement in dewatering rate results in 5-10% higher rate of paper machine. Considering that costs of commercial enzyme preparations are still unaffordably high for large-scale application in Hungary, in-house produced enzymes without requiring purification can be potential alternatives. In the future, scaling-up i.e. investigation of tested enzymes on paper machine is needed for confirmation of presented promising laboratory experiment results.

6. Publications and presentations

Research papers published in international and Hungarian journals

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