Potential of enzymes for wood debarking

Marjaana Rättö, Anne Kantelinen, Michael Bailey, and Liisa Viikari

ABSTRACT: The effect of enzymatic pretreatment on the energy consumption of wood debarking was studied on the laboratory scale using enzymes to degrade the cambial layer. The energy consumed in debarking spruce was decreased as much as 80% after pretreatment with pectinolytic enzymes. In addition to polygalacturonase activity, pectin lyase and xylanase activities were also present in the most efficient enzyme preparation. Due to the complex composition of the cambium and inner phloem, these and other enzymes that hydrolyze the various inner bark components are probably needed for efficient debarking.

KEYWORDS: Barking, degradation, energy consumption, enzymatic activity, enzymes, layers, Picea, pretreatment, wood.

The removal of bark is the first step in all wood-based processes. Debarking of pulpwood is mainly performed in debarking drums. Extensive debarking is needed for high-quality mechanical and chemical pulps because even small amounts of bark residues cause darkening of the product. The need for complete debarking leads to losses of raw material and to increased energy consumption from prolonged treatment in the mechanical drums.

The border between wood and bark is the cambium. In mechanical debarking, the bark is removed along the cambial layer. The cambial cells divide continuously and have a lower mechanical strength than that of other wood cells. Cambium characteristics include high pectin and protein content and the absence or low concentration of lignin (*1, 2*). The cambial tissue consists of intracellular material and primary cell walls. According to a model presented for the primary cell wall of dicotyledonous plants, the following carbohydrate polymers are present: cellulose, pectin, xyloglucan, arabinogalactan, and hydroxyproline-rich glycoprotein. The pectins in primary cell walls of dicotyledons are heteropolymers. In addition to galacturonic acid units, they also contain rhamnose linked to galacturonic acid units in interior chains, whereas galactose and arabinose are present as side chain structures (*3, 4*). The primary cell wall structure in coniferous trees has not been studied as closely as that of dicotyledons.

The content of pectin compounds in the cambial cells varies between the wood species studied. In *Betula platyphylla* (birch), *Fraxinus elatior* (ash), *Pinus ponderosa* (pine), and *Acer pseudoplatanus* (sycamore), the contents of pectic substances in the cambium are 18%, 6.6%, 8.5%, and 15%, respectively (*1*). The cambium of *Pinus silvestris* (pine) consists mainly of pectic material (partially esterified polygalacturonic acid, arabinan, galactan). In addition cellulose, glucomannan, and glucurono-araboxylan are present (*5*). In *Pinus silvestris*, 59% of the galacturonic acid units extracted from cambium were methylated (*6*). The cambial tissue of *Populus tremuloides* (quaking aspen) contains 40% pectins (rhamno-galacturonan, galactan, and arabinan), in addition to smaller amounts of arabinogalactan, xyloglucan, xylan, glucomannan, cellulose, and protein (*2*).

In this work, the possibilities of facilitating bark removal by using enzymes to hydrolyze the cambial layer were studied by measuring the energy needed to remove the bark in laboratory-scale experiments. On the basis of the available information on chemical composition of the cambium, pectinolytic, and hemicellulolytic enzyme preparations were chosen for the experiments.

Materials and methods

Enzymes

Three different pectinases were used: a commercial preparation Pectinex Ultra SPL (Novo) and two preparations produced at VTT Biotechnical laboratory: polygalacturonase produced by *Aspergillus niger* (Crude PG) (*7*), and a partially purified polygalacturonase obtained from *A. niger* (partially purified PG) (*8*). The xylanase was a commercial preparation, Pentosanase (MKC).

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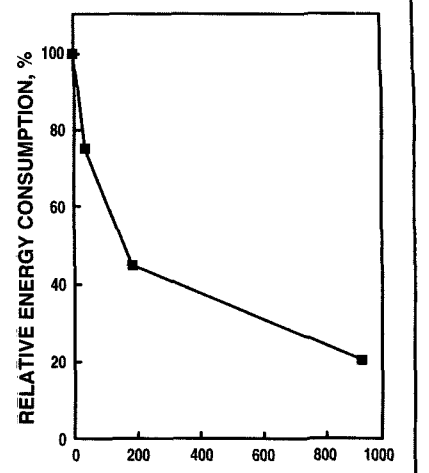
I. Effect of different enzymes on energy consumption in the debarking of spruce

Enzyme	Activity dosage, nkat/mL PG	PMGL	XYL	HEC	Energy consumed, as % of reference
Pectinex Ultra SPL	185	0.6	2.0	5.5	50
Crude PG	185	<0.1	1.1	1.6	77
Partially purified PG	185	<0.1	0.2	<0.1	87
Pentosanase	0.2	<0.1	100	1.6	82

PG = polygalacturonase.
PMGL = poly(methoxylgalacturonide) lyase.
XYL = xylanase.
HEC = endoglucanase.
Enzymatic treatment for 24 h at 20°C.

1. The effect of polygalacturonase dosage on energy consumption in the debarking of spruce (Pectinex Ultra SPL, 20°C, 24 h)



Activity measurements

Polygalacturonase [PG; poly (1,4,-α-D-galacturonide) glycanohydrolase, EC3.2.1.15], endoglucanase (HEC;1,4-ß-D-glucan 4-D-glucanohydrolase, EC 3.2.1.4) and xylanase (XYL; 1,4-ß-D-xylan xylanohydrolase, EC 3.2.1.8) activities were measured as described by Bailey and Pessa (*7*). Pectin lyase [PMGL; poly(methoxylgalacturonide) lyase, EC 4.2.2.10] activity was measured by the method of Manachini *et al.* (*9*). The activity was expressed as narokatals (nkat) using a molar extinction coefficient of 5500 L/(mol cm) for 4,5-unsaturated methylated oligogalacturonates.

Wood samples

Spruce (*Picea abies*) logs felled in October and November were used. Logs with a diameter of 15–20 cm were cut into discs with a thickness of 2 cm. Discs showing irregularities in shape or visually found to be injured either mechanically or microbiologically were discarded.

Spruce logs felled in May were used for analysis of cambial tissue.

Enzyme treatments

The discs were soaked in the enzyme solution in 10*mM* sodium citrate buffer, pH 5. Three discs from different logs were used in each treatment. The reference discs (from the same logs as the enzyme-treated discs) were soaked in buffer solutions.

Measurement of energy consumption in debarking

The energy consumption of bark removal was measured using a laboratory-scale debarking unit (*10*). In this apparatus, a blade removes the bark from a slowly rotating wood disc.

Measuring areas of 2 cm x 2 cm were created in the bark by removing 2–3 cm of the bark in front of each measuring area with a knife. The shear force needed to remove the 2-cm x 2-cm piece of bark was then measured and registered. The energy was calculated from the time-integral of the shear force.

Ten replicate measurements were carried out from each disc. The energy consumption in debarking of enzyme-treated wood discs was expressed as relative values (as a percentage of the energy consumption of the reference discs).

Enzyme stability

Enzyme stability was measured by first incubating Pectinex Ultra SPL (10 mL/L) at 30°C in process water from an industrial-scale debarker used for debarking spruce and then measuring the residual PG activity.

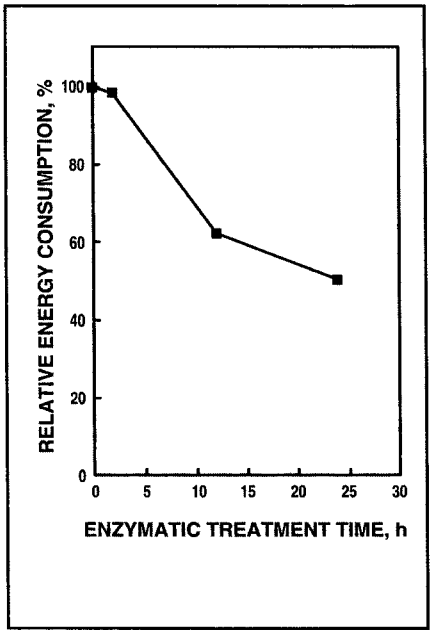
Enzymatic hydrolysis of cambial tissue

Cambial tissue was isolated from spruce logs felled in May. The bark was stripped off, and the cambium was removed by gentle scraping. The cambial tissue was stored frozen and was used in hydrolysis experiments without drying.

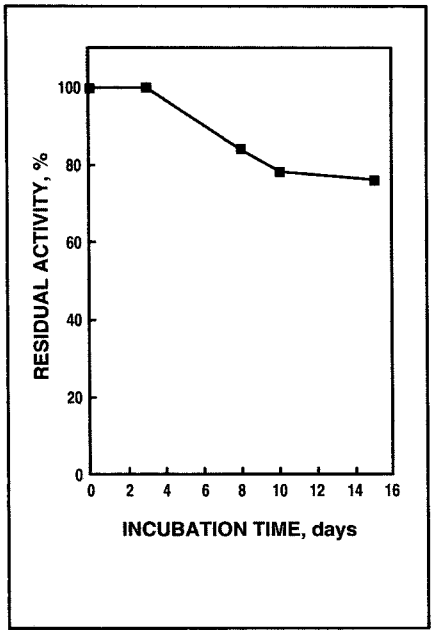
The enzymatic hydrolysis of isolated cambium (20 mg/mL) was studied in 0.07*M* KH_2PO_4–Na_2HPO_4 buffer, pH 4.9. As a reference, the cambium samples were incubated in buffers. The hydrolyzates were assayed for reducing sugars using the DNS method (*11*) and for galacturonic acid by HPLC (high-performance liquid chromatography).

The HPLC instrument consisted of: a M-6000A pump, a M-710B automatic injector, two fast fruit juice columns

2. The effect of enzymatic treatment time on energy consumption in the debarking of spruce (Pectinex Ultra SPL, PG dosage of 185 nkat/mL, 20°C)



3. The stability of Pectinex Ultra SPL in the debarking water



II. Hydrolysis of isolated cambium with different pectinase preparations

Enzyme	Hydrolysis products, % of substrate: Reducing sugars	Hydrolysis products, % of substrate: Galacturonic acid
Pectinex Ultra SPL	42	12.3
Crude PG	38	3.9
Partially purified PG	38	5.4
Pentosanase	35	0.8
Reference	31	0.4

PG = 1000 nkat/g substrate.

(7.8 x 150 mm) connected in series in a column oven, a M-490 UV detector monitoring at wavelengths of 215 nm and 255 nm, and a M-411 refractive index detector. (All instruments from Millipore/Waters.)

The mobile phase was 0.2% aqueous phosphoric acid at a flow rate of 0.5 mL/min. The analyses were carried out at 40°C.

Results

Effect of enzymatic treatments on energy consumption in debarking

The possibilities of facilitating wood debarking by enzymatic pretreatment were studied by measuring the energy consumed in the debarking of spruce samples treated with three different pectinase preparations and a hemicellulase preparation. The pectinases were dosed according to their polygalacturonase activity (185 nkat/mL in the soaking solution), and the hemicellulase was dosed according to its xylanase activity (100 nkat/mL).

All the enzymes studied decreased the energy consumption to some extent (**Table I**). The best result, a 50% decrease in energy consumption, was obtained with Pectinex Ultra SPL. Of the three pectinases, this preparation showed the widest spectrum of the activities of enzymes that hydrolyze the various cambial components. In addition to polygalacturonase, pectin lyase, xylanase, and endoglucanase activities were also detected. The partially purified polygalacturonase, with the lowest xylanase and endoglucanase activities, was the least efficient of the three pectinases. An 18% decrease in energy consumption was obtained with the xylanase preparation.

The effects of enzyme dosage and treatment time were studied using Pectinase Ultra SPL. As much as 80% decrease in energy consumption was obtained with a polygalacturonase dosage of 900 nkat/mL in a 24-h treatment. A moderate effect, a 25% decrease in energy consumption, was obtained when only 4% of this activity (40 nkat/mL) was used. For a 50% decrease in the energy consumption, a polygalacturonase dosage of 185 nkat/mL was needed (**Fig. 1**). With this dosage, a 40% decrease was obtained in 12 h, whereas only a slight effect was observed after 4 h of treatment (**Fig. 2**).

Enzyme stability

The stability of the enzyme in process waters containing various components dissolved from wood and bark was studied in order to evaluate the possibilities for enzyme recycling. Pectinex Ultra SPL was incubated in process water from an industrial-scale debarker used for debarking spruce, and the residual activity was measured.

The polygalacturonase activity was relatively stable, showing 70% residual activity after 15 days at 30°C (**Fig. 3**). Thus, it would seem possible to decrease the costs of the enzymatic pretreatment by repeated use of the same enzyme solution. Only the effects of the soluble components were considered here. If the system contains large amounts of dry matter (bark), an additional decrease of activity may occur as a result of absorption of the enzymes.

Enzymatic hydrolysis of isolated cambium

The efficiencies of the enzymes in the hydrolysis of isolated cambial tissue were also compared. Cambial tissue was isolated from spruce felled during the spring. The polysaccharides in iso-

lated cambium were partially degraded during the isolation, as indicated by the high content of reducing sugars (31% of dry weight) in the reference sample. This effect was probably caused by endogenous enzymes.

However, galacturonic acid was not detected in the reference samples. When the substrate was hydrolyzed with the three pectinases dosed to the same polygalacturonase activity, the most efficient hydrolysis of cambial pectin to galacturonic acid was obtained with Pectinex Ultra SPL (**Table II**). The amounts of galacturonic acid released by crude polygalacturonase and partially purified polygalacturonase were less than half of that released by Pectinex Ultra SPL. In addition to polygalacturonase, Pectinex Ultra SPL also contained the highest pectin lyase activity. Furthermore, other enzymatic activities not measured here, such as enzymes cleaving the neutral sugar side chains and acetyl and methyl groups connected to pectin, may have further facilitated the overall hydrolysis of pectin.

Discussion

Enzymatic treatments cause significant decreases in the energy consumption during debarking. With the highest enzyme dosage, as much as 80% of the energy needed for bark removal could be saved. The target of the enzymatic treatments is the cambial layer, with its high content of pectins.

In this study, enzyme preparations with polygalacturonase or xylanase as the main activity were used. In addition to these, other enzymes that act on the various components of the cambium may also have an effect. The role of each enzyme and the optimal composition of the enzyme mixture for debarking remains to be determined.

In these experiments, the wood was cut into relatively thin discs before the enzymatic treatment, and the enzyme probably diffused mainly tangentially. In logs, the bark forms an effective barrier to the enzyme and may exclude enzyme diffusion. This barrier could be overcome in practice by applying the enzymatic treatment to poorly debarked logs selected after preliminary mechanical debarking. In the case of poorly debarked wood, the major portion of the bark would already be removed, and diffusion of the enzyme would therefore be facilitated. In a mill, it might be advantageous to spray poorly debarked logs with enzyme solution before repeating the mechanical debarking.

A substantial disadvantage of the current mechanical debarking methods is that the process has to be continued well beyond the time required to remove most of the bark in order to remove the few pieces of bark that hold tenaciously to the logs. The enzymatic treatment applied after preliminary debarking could reduce the wood losses that normally occur. Thus, the enzymatic treatment could lead to substantial savings in raw material in addition to the savings in energy. ■

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