LAPPEENRANTA UNIVERSITY OF TECHNOLOGY
Faculty of Technology
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BIOCHEMICAL MODIFICATION OF THERMOMECHANICAL PULP FIBERS

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ABSTRACT

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Title of the thesis

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Abstract

The first objective of this study was to find out reliable laboratory methods to predict the effect of enzymes on specific energy consumption and fiber properties of TMP pulp. The second one was to find with interactive software called "Knowledge discovery in databases" enzymes or other additives that can be used in finding a solution to reduce energy consumption of TMP pulp.

The chemical composition of wood and enzymes, which have activity on main wood components were presented in the literature part of the work. The results of previous research in energy reduction of TMP process with enzymes were also highlighted. The main principles of knowledge discovery have been included in literature part too.

The experimental part of the work contains the methods description in which the standard size chip, crushed chip and fiberized spruce chip (fiberized pulp) were used. Different types of enzymatic treatment with different dosages and time were tested during the experiments and showed. Pectinase, endoglucanase and mixture of enzymes were used for evaluation of method reliability. The fines content and fiber length of pulp was measured and used as evidence of enzymes' effect.

The refining method with "Bauer" laboratory disc refiner was evaluated as not highly reliable. It was not able to provide high repeatability of results, because of uncontrolled feeding capacity and refining consistency. The refining method with Valley refiner did not have a lot of variables and showed stable and repeatable results in energy saving. The results of experiments showed that efficient enzymes impregnation is probably the main target with enzymes application for energy saving. During the work the fiberized pulp showed high accessibility to enzymatic treatment and liquid penetration without special impregnating equipment. The reason was that fiberized pulp has larger wood surface area and thereby the contact area between the enzymatic solution and wood is also larger. Standard size chip and crushed chip treatment without special impregnator of enzymatic solution was evaluated as not efficient and did not show visible, repeatable results in energy consumption decrease. Thereby it was concluded that using of fiberized pulp and Valley refiner for measurements of enzymes' effectiveness in SEC decrease is more suitable than normal size chip and crushed chip with "Bauer" refiner.

Endoglucanase with 5 kg/t dosage showed about 20% energy consumption decrease. Mixture of enzymes with 1.5 kg/t dosage showed about 15% decrease of energy consumption during the refining. Pectinase at different dosages and treatment times did not show significant effect on energy consumption.

Results of knowledge discovery in databases showed the xylanase, cellulase and pectinase blend as most promising for energy reduction in TMP process. Surfactants were determined as effective additives for energy saving with enzymes.

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FOREWORD

This Master Thesis was written in the Department of Chemical Technology at Lappeenranta University of Technology during the spring of 2010.

First of all I would like to thank my supervisors Professor Andrzej Kraslawski, D.Sc. Markku Gummerus and M.Sc. Meri Ventola for suggested contemporary and interesting research topic, valuable advices and for supporting and guiding me through the whole process.

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Special thanks I want to address to my family, who have always supported me and believed in me.

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Dmitriy Kazymov

ABBREVIATIONS

CBD	Carbohydrate binding domain
CBH	Cellobiohydrolase
COD	Chemical oxygen demand
CSF	Canadian standard freeness
DP	Degree of polymerization
EG	Endoglucanase
G	Guaiacil phenylpropane unit of lignin
H	p – hydroxyphenyl unit of lignin
HPLC	High performance liquid chromatography
KDD	Knowledge discovery in databases
kDa	Molecular mass in kilo Daltons
LCC	Lignin carbohydrate complex
LiP	Lignin peroxidase
MnP	Manganese-dependent peroxidase
MW	Molecular weight
PEG	Polyethylene glycol
PG	Polygalacturonases
PME	Pectin Methyl Esterases
PL	Pectin Lyases
PSM	Polyoxyethylene sorbitan monooleate
S	Syringyl phenylpropane unit of lignin
SEC	Specific energy consumption
SOD	Superoxide dismutases
TMP	Thermomechanical pulp
VA	Veratryl alcohol
XIN	Xylanase

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1 Introduction

1.1 Background

Nowadays thermomechanical pulping (TMP) process is the dominating refiner-based mechanical pulping process /1/. Thermomechanical pulp is a type of high-yield pulp. This kind of pulp is comparatively cheap and provides good printing properties. Mechanical pulp mill requires much lower capital investment than chemical pulp mill. /2/ The major disadvantage of mechanical pulping processes is that they require large quantities of expensive electrical energy. /1, 3/ This is because mechanical pulp is produced by grinding and refining methods. Due to friction, the structure of the wood is softened and it is structure loosened, finally leading to the separation of the fibers from each others. However, only a small part of the energy brought into the system is used for separating the fibers; the major part being converted into heat. Therefore, the total effective energy amount is very low. /6, 13/

Enzyme treatment of wood and pulp attracts more and more attention of pulp producers. It is an attractive alternative to decrease energy demand in the refining process and to introduce novel functional properties of fibers. For instance different enzyme applications in pulp bleaching already works in a full-scale at many mills around the world. /2/

The greatest amount of enzymes of industrial interest is reproduced by fungi. For instance various oxidative extracellular enzymes like lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), laccase, and hydrogen peroxidase effused from ligninolytic fungi. /7/ The natural origin, non-toxicity, and mild operating conditions of fungi and their enzymes stimulate a plenty of researches on their applicability in the forest-based industry. /4/

An advantage of enzymatic treatment is that it takes only a few hours, which is a relatively short time, in comparison with the period of some weeks required by fungal treatment. It makes enzymatic delignification or modification industrially more usable. /8/

The effectiveness of enzymatic treatment depends on many factors, such as temperature, pH, dosage and molecular weight (MW) of certain enzyme and also application method. The enzymes can be applied to the surface of the wood chips by

spraying, or can be impregnated into the wood chips. The diffusion or impregnation of the enzymes into the wood chips is very important for their effective action. /9/

1.2 Content of the thesis work

This Master Thesis contains the literature part and the experimental part. The chemical composition of wood and information about properties of wood components are presented in the literature part. Enzymes which interact with wood components are overviewed in the literature part too. The literature part contains the information from previous researches, which are related to energy consumption decrease in TMP process by enzymes. The knowledge discovery system was used during the thesis work. Thereby literature part contains the explanation of the main principles of knowledge discovery.

The experimental part contains the laboratory methods descriptions, which were used during the work. Also the specification of equipment and major parameters of experiments are presented. Results of the work were analyzed and showed in the experimental part.

The final part of the work contains conclusions and summaries. The conclusions are based on knowledge from previous study and on data from experiments.

1.3 Objectives and potential utilization of results

The testing of enzymes in pilot scale is expensive and good alternative is reliable laboratory tests. Thereby the main target of the work was to find out the method in laboratory scale to predict the effect of enzymatic pretreatment on specific energy consumption (SEC) of TMP refining.

The interactive software with target to find possible enzymes, compositions of enzymes and additives which can reduce energy consumption during the chip refining was used during the work.

The results of this thesis can be used in UPM to test new enzymes quickly and with low expenses in laboratory scale. The developed method can be used with purpose to find out optimal dosages and conditions for enzymes in TMP process. The

results from the literature discovery can be useful in search of effective solutions for TMP and other processes development.

LITERATURE PART

2 Chemical composition of wood

The major constituents of wood material are cellulose, hemicelluloses, lignin and extractives. /10/ In Table 1 is showed in percents chemical compositions of some wood species.

Table 1. Chemical composition of some wood species. /10/

Constituent	**Scots Pine** ***(Pinus sylvestris)***	**Spruce** ***(Picea glauca)***	**Eucalyptus** ***(Eucalyptus camaldulensis)***	**Silver Birch** (***Betula verrucosa)***
Cellulose (%)	40	39.5	45.0	41.0
Hemicellulose				
-Glucomannan (%)	16.0	17.2	3.1	2.3
-Glucuronoxylan (%)	8.9	10.4	14.1	27.5
-Other polysaccharides (%)	3.6	3.0	2.0	2.6
Lignin (%)	27.7	27.5	31.3	22.0
Total extractives (%)	3.5	2.1	2.8	3.0

2.1 Cellulose

Cellulose is the major chemical component of fiber wall and contributing 40-45% of the wood's dry weight. The molecule of cellulose is linear homo-polysaccharide composed D-glucose units linked together by ß-1,4-glycosidic bonds (Figure 1) with the degree of polymerization from 10,000 in native wood to 1,000 in bleached kraft pulps. At C2, C3, and C6 positions each glucopyranose unit have a hydroxyl group. These groups are capable of undergoing the typical reactions known for primary and secondary alcohols. The molecular structure explains the characteristic properties of cellulose such as: hydrophilic properties, chirality, degradability, and broad chemical variability initiated by the high donor reactivity of hydroxyl groups. /10, 11/

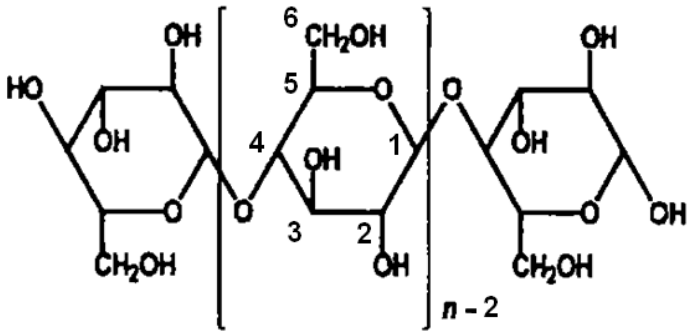

Figure 1. The structure of cellulose chain. /11/

The structure of the cellulose chain is highly stabilized by intra-molecular hydrogen bonds /14/. There are two intra-molecular hydrogen bonds between successive glucose residues, and the hydrogen bonding network consist of all polar functional groups except glycosidic oxygens, see the Figure 2-A. Intra- and inter-molecular hydrogen bonds promote the aggregation into a crystalline structure and give cellulose a multitude of partially crystalline fiber structures and morphologies, (Figure 2-B). The aggregates of cellulose molecules called microfibrils, which in turn can pack tightly together to form even larger fibrils and finally cellulose fibers. /11, 12, 14/

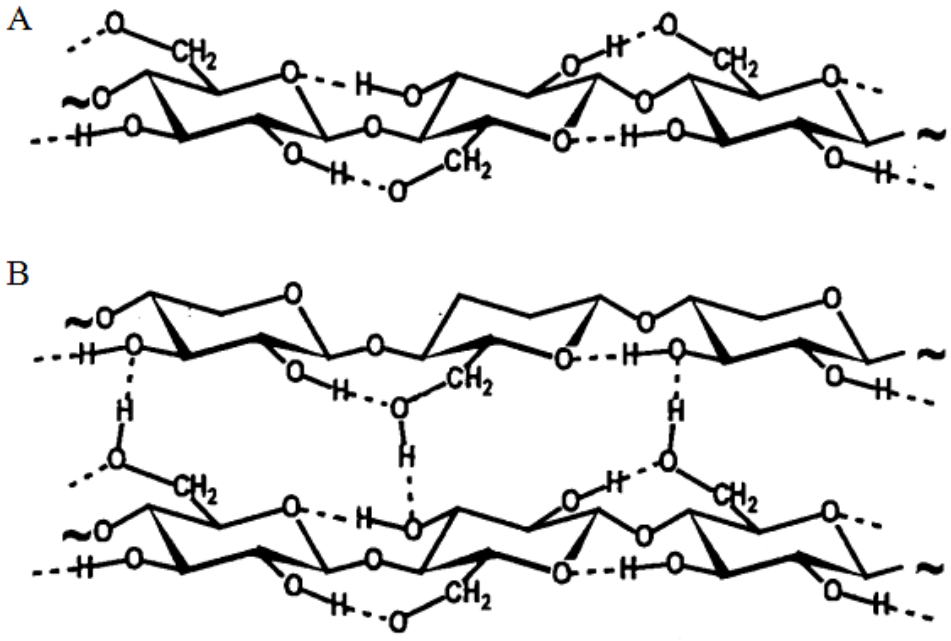

Figure 2. A) Inter-molecular hydrogen bonds and B) intra-molecular hydrogen bonds, (the hydrogen bonds are shown by dashed lines). /11/

There are four distinct crystalline cellulose structures named cellulose I, II, III and IV. /10, 11/ The ultra structure of native cellulose (cellulose I) has been discovered to possess unexpected complexity in the form of two crystal phases: I_α and I_β which differ in their inter-molecular hydrogen bonding patterns. The source of cellulose has influence on proportion of these crystal forms. Most natural cellulose contains both of them. /12, 15/ Cellulose II is obtained from cellulose I by sodium hydroxide treatment. Cellulose II is more stable than cellulose I because its chains are oriented in anti-parallel way with consequential formation of hydrogen bonds. From cellulose I or II can be obtained cellulose III by liquid ammonia treatment. This kind of cellulose reverts to its parent type at room temperature and more rapid in boiling water. Cellulose IV is the predominant form in plant primary cell walls and is thought to be poorly ordered form of cellulose I. It is produced from cellulose III by heating in glycerol. /13/

Even thought the cellulose is regularly structured and tightly packaged, it also contains less ordered, which called “amorphous” regions. The treatment of cellulose by chemicals firstly proceeds in amorphous area and than in crystalline. /11/

2.2 Hemicelluloses

Hemicelluloses are low molecular weight linear or branched polysaccharides, normally having degree of polymerization (DP) less than 200. Hemicelluloses have a heterogeneous composition of various sugar residues and substituted side chain, which makes them more soluble in water. /14/

The main sugars residues in the backbone determine the name of hemicelluloses, e.g. xylans (D-xylose units) and glucomannans (both D-glucose and D-mannose units). All hemicelluloses are separated into two groups for softwood and hardwood hemicelluloses according to the wood species (see the Table 2). /11/

Hardwood and softwood xylans have different side group patterns presented on Figure 3. Hardwood xylan is O-acetyl-4-O-methylglucuronoxylane, in which the xylan backbone is substituted at random intervals with acetyl and 4-O-methylglucuronic acid side groups. In hardwood the ratio of xylose to α-1,2-linked 4-O-methylglucuronic acid in twice higher than in softwood and consist of about 10:1. Approximate amount

of xylose units are acetylated at the C2 and/or C3 positions is 60-70%. Softwood xylan is mainly arabino-4-O-methylglucuronoxylan, in which L-arabinose units are α-1,3-linked to the xylan backbone. The ratio of xylose to 4-O-methylglucuronic acid is 5:1, and the xylose to arabinose ratio is 8:1. /11, 12/

Table 2. The major hemicelluloses components in softwood and hardwood. /10/

Wood	Hemicellulose type	Amount (% of wood)	Composition			DP
			Units	Molar ratios	Linkage	
SW	Galacto-glucomannan	5-8	β-D-Manp β-D-Glcp α-D-Galp Acetyl	3 1 1 1	1→4 1→4 1→6	100
	Glucomannan	10-15	β-D-Manp β-D-Glcp α-D-Galp Acetyl	4 1 0.1 1	1→4 1→4 1→6	100
	Arabino-glucurono-xylan	7-10	β-D-Xylp 4-O-Me-α-D-GlcpA α-L-Araf	10 2 1.3	1→4 1→2 1→3	100
HW	Glucuronoxylan	15-30	β-D-Xylp 4-O-Me-α-D-GlcpA Acetyl	10 1 7	1→4 1→2	200
	Glucomannan	2-5	β-D-Manp β-D-Glcp	1-2 1	1→4 1→4	200

In softwood xylan there are no acetyl groups. Mannans are typical hemicelluloses in both softwood and hardwood. They are divided into two groups: galactoglucomannans and glucomannans /11/. The first one are composed of β-1,4-linked glucose and mannose units, which are randomly distributed in the backbone, and galactose side groups attached to glucose or mannose units through α-1,6-linkages. There are also partially acetylated hydroxyl groups at the positions C2 and C3, dividing acetylated galactoglucomannans into two fractions according to their galactose content (Gal:Glc:Man, 1:1:3 and 0.1:1:3). Hardwood glucomannan contains glucose and mannose units in the ratio of 1:1-2 and neither galactose no acetyl groups are present. /13, 16/

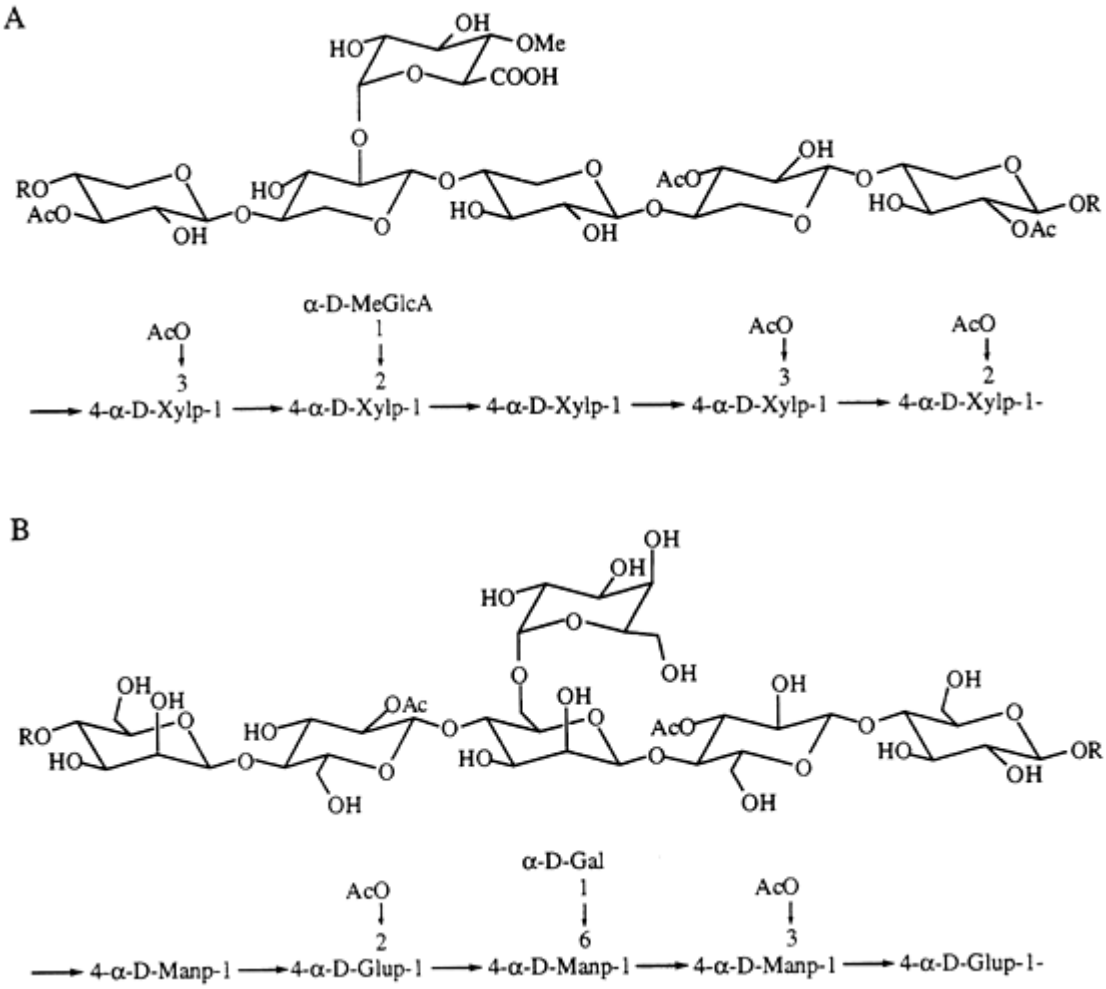

Figure 3. Typical structure of hemicelluloses A) hardwood O-acetyl-4-4methyl-glucuronoxylan and B) softwood O-acetyl-galactoglucomannan. /11/

In addition, all sugar components can take part in the formation of lignin carbohydrate complexes (LCC) by covalent linkages between lignin and carbohydrates in both wood and pulps. The most frequently suggested LCC-linkages in native wood are benzyl ester, benzyl either, and glycosidic linkages. However, the benzyl ester linkage is alkali-labile and may therefore be hydrolyzed during kraft pulping process. The latter two linkages are alkali-stable and would survive from the hydrolysis during kraft pulping process. /10/

2.3 Lignin

Lignin occurs in primary and secondary walls and middle lamella. It is chemically bonded with hemicelluloses and together with them forms a cementing component that envelops cellulose microfibrills and gives rigidity to the cell structure determined by microfibrills. Mass fraction of lignin in softwood in average is 27…30%, and in hardwood species – 18…24%. Unlike cellulose and other wood polysaccharides the lignin extracted from wood is not individual substance, and represents as a mixture of aromatic polymers with related structure. Therefore lignin is a complex, hydrophobic, cross-linked aromatic polymer. /10, 17/

2.3.1 Lignin structure

Lignin has a highly irregular three-dimensional structure, see the Figure 4. It is water insoluble high molecular mass compound. /10/

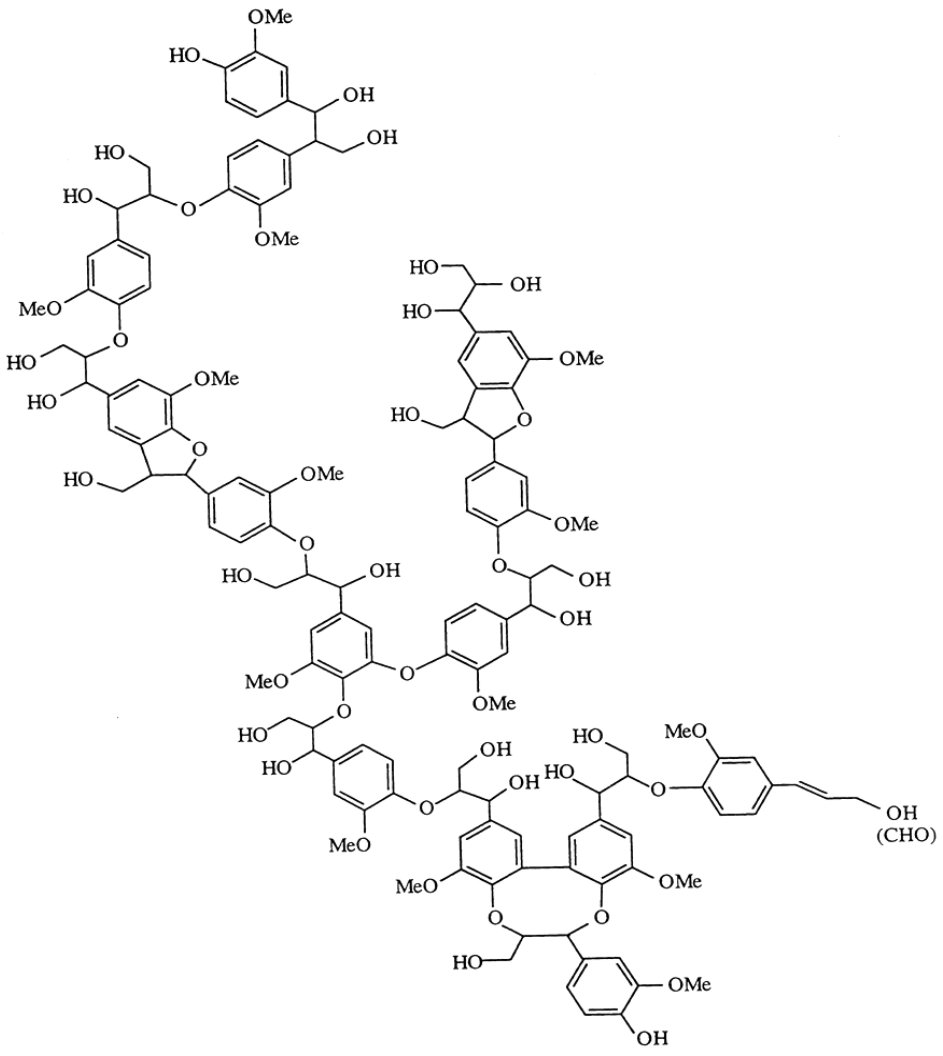

Figure 4. Part of softwood lignin. /14/

In lignin molecule, phanylpropane subunits linked together with ether and/or carbon-carbon bonds. /5/ Phenylpropane units of lignin are guaiacil (G) units from the precursor trans-coniferyl alcohol, syringyl (S) units from trans-sinapyl-alcohol, and p-hydroxyphenyl (H) units from the precursor trans-p-coumaryl alcohol, are presented on Figure 5. At least nine different kinds of ether and carbon-carbon bonds have been founded between phenylpropane subunits. /11/

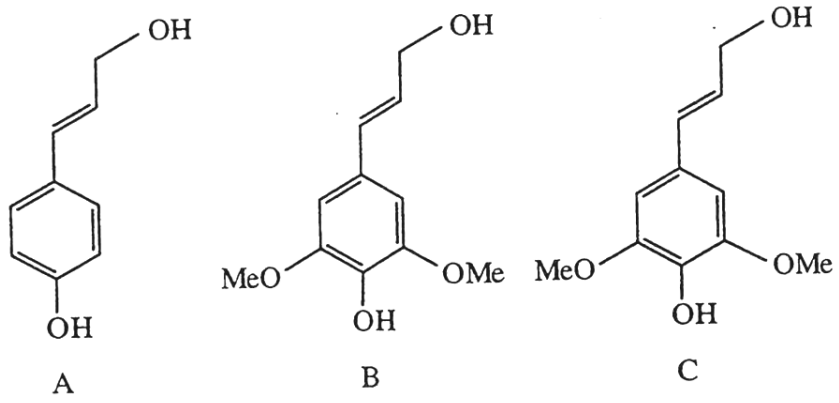

Figure 5. Structural components of lignin: A- coniferyl; B- sinapyl and C- p-coumaryl alcohols. /14/

The content of different units in lignin composition depends on wood species. Softwood contains mainly guaiacyl units while hardwood contains also syringyl units. For spruce (*Picea abies*) a ratio G:S:H is 94:1:5, and for pine G:S:H is 86:2:13. All lignin, even within the same cell wall are heterogeneous. Hardwood lignin are more heterogeneous than softwood and amount of syringil units can be from 20 till 60%, and even higher. For instance, for beech (*Fagus Sylvatica*) a ratio G:S:H = 49:46:5. /12/

Lignin is polyfunctional polymer. Its functional groups are different: methoxy, hydroxilic, phenolic and aliphatic, carbonyl-aldehydic and ketonic, carboxylic and also double links of alkene type. /11, 17/

Methoxy group (-OCH_3) in lignin are the groups with aryl-allyl simple ether bond. In softwood lignin (guaiacyl lignin) mass fraction of methoxy groups is 15…17%, in hardwood lignin (guaiacyl- syringil lignin) - 20…22%. Mass fraction is decreased during the lignin extraction. /11/

Hydroxylic group (-OH) are different in lignin-there are phenolic and alyphatic (alcohol) hydroxyl groups in unbound and bound state. Total content of unbound hydroxyl groups is 1, 1…1, 2 per one phenylpropane unit, that considered to their mass fraction 10…11%, which is also depends on method of extraction. /11/

2.3.2 Thermoplasticity of lignin

Native lignin in wood has properties of thermoplasticity. Thermoplasticity is ability to change the glass state to rubbery state (sometimes to viscous-flow state) under temperature action. Thermoplasticity of lignin has a big importance in processing of lignocarbohydrate materials with high lignin content. This property is taken into account in wood processes, production of wood plastics, boards, and different types of mechanical pulp (thermo mechanical pulp (TMP), chemi-thermo mechanical pulp (CTMP)) and so on. /10/

Softening of lignin, as in all polymers, takes place in certain temperature range. Softening temperatures (glass transition temperatures) of lignin samples, with close to native lignin structure, are in range from 130° to 190°C depending on wood species and extraction method. The molecular weight (MW) has a big influence on this value. For instance in extracted spruce lignin, softening temperature decreases from 176°C (MW=85000) to 127°C (MW=4300) /11/. The water content has also influence on softening temperature. Water has a plasticizing effect and decrease the softening point (to 80…130°C). This effect is achieved with small amount of water. With absolute moisture content near 2% the softening temperature of native lignin decreasing to 115°C, and to 100°C for sulfate lignin. Further increase of moisture content does not have any effect on softening temperature. /11, 12/

2.3.3 Chemical reactions of lignin

Aromatic nature, different bond types between phenylpropane units and functional groups makes lignin very reactive. However, presence of benzene ring, polyfunctionality, heteropolymeric nature of extracted lignin and three dimensional cross-linked structure of wood lignin complicate behavior in chemical reactions and complicate the study of chemical reactions. Chemical transformations of lignin can be divided into two groups: reactions of monomer units and macromolecular reactions. /10, 11, 13/

Lignin, as all polymers, has simultaneously occurrence of several chemical reactions, also competing reactions. The chemical composition of lignin is changing during the monomer units reactions (reactions of phenylpropane units), but the spatial structure (cross-linked in native lignin) and amount of units are not changed. These reactions are classified into three types: reactions of benzene ring, reactions of functional groups and intra-molecular reactions of chemical transformation. Benzene ring and different functional groups makes lignin capable to different reactions, which are typical for various classes of organic compounds. Unlike polysaccharides intra-molecular reactions takes very important place in chemistry of lignin. They can proceed in mild conditions, together with other reactions and have an effect on them. Intra-molecular transformations lead to the formation of intermediate (active intermediate substances in chemical reaction) from phenylpropane units. /13/

Macromolecular reactions of lignin divided into three groups: destruction, cross-linking action and terminal unit reactions. /11/

Destruction reactions in lignin lead to breaking the bonds between units and destroying the structural mesh. All types of destruction divided into chemical, physical and biochemical destruction. The most interesting is biochemical destruction. This destruction under the action of enzymes, produced by living organisms: fungi, bacteria, insect and so on. In comparison to other polysaccharides lignin is more stable to biochemical treatment. But some fungi, such as white rot fungi, have an intensive impact on lignin, and lead to hydrolytic and oxidative degradation. Therefore nowadays is very important to find out the fungi, which have selective action on lignin. It will help to develop environmentally friendly technology for pulp production, which gives the possibility to process in mild conditions without aggressive chemicals. /10, 11/

3 Enzymes

3.1 General information and classification

Enzymes are proteins that increase the rates (catalyze) of chemical reactions (see the Figure 6). In living cells all chemical processes are carried out by enzymes. The enzymatic action is very specific and usually only one specific reaction can be catalyzed by special enzyme. Catalysts increase the rate of a reaction, but are not consumed or produced by the reaction themselves. Therefore the equilibrium constant of reaction is not changing. It means that during the catalytic reaction catalyst catalyzes a reaction in both directions, so that the speed of reversion is also changed. /18/

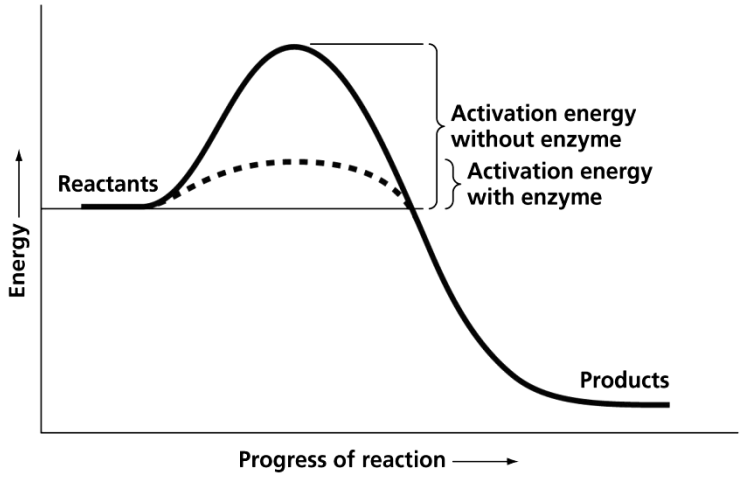

Figure 6. Effect of enzymes on reaction path. /18/

The reason of specific enzymes action is their structure. Enzymes are long chains of amino acid units held together by peptide bonds, looped and folded into secondary and tertiary (and often quaternary) structures by disulfide bonds, hydrophobic interactions, and salt bridges. Usually active enzymes involve "cofactors." These small molecules (sometimes inorganic ions) are necessary for complete the catalytically active structure of the enzyme. The enzymes, which don't have cofactor, are called an apoenzyme, and the apoenzyme-cofactor complex is called a holoenzyme. /18/

Enzymes classification is based on recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes by the reactions they catalyse. Therefore all enzymes divided into six big groups. /19, 20/

EC 1 – Oxydoreductases – enzymes which catalyze oxidation-reduction reactions, which mean the transfer of electron from one molecule to another during the reaction. The classical name is donor/acceptor oxidoreductase, but the name dehydrogenase is also acceptable. It is also possible to use name acceptor reductase. /18/

EC 2 – Transferases – the transfer of a chemical group from donor to acceptor are catalyzed by these enzymes. The standard names for transferases as folows: "donor:acceptor group-transferase" However, that is a lot to work with and when you are dealing with very long donors or acceptors, it becomes easier to use the common names which are just "donor group-transferase" or "acceptor group-transferase". /19/

EC 3 – Hydrolases – these enzymes catalyze the hydrolysis of various covalent bonds. The classical name is formed by the name of the substrate with an additional suffix – ase. /19/

EC 4 - Lyases - are enzymes which catalyze Carbon-Carbon (C-C), Carbon-Oxygen (C-O), Carbon-Nitrogen (C-N) and other bond cleavage not via hydrolysis or oxidation. The nomenclature for systematic names is based on the standard scheme: substrate group-lyase. In common names an additional suffix – lase is used. If lyase remove water molecule, then the common name can be dehydratase. /18/

EC 5 - Isomerases - these enzymes provide the catalytic changes within one molecule. The classification is based on the chemical reactions catalyzed by isomerase within substrate molecule. /18, 20/

EC 6 - Ligases - these enzymes catalyze the joining of two molecules with concomitant hydrolysis of the diphosphate bond in triphosphates. For common name it is possible to use ligase, synthase or synthetase. In some cases carboxylase is also acceptable. /19/

3.2 Carbohydrate modifying enzymes

Carbohydrate modifying enzymes are currently the main class of enzymes with industrial application in food and textile industry. In pulp and paper industry the first carbohydrate modifying enzyme was hemicellulase xylanase, which was effective in Kraft pulp bleaching. Nowadays cellulases have been used in energy saving in TMP refining, increase flexibility of fibers, improve deinking and drainage. /20/

Hemicellulolytic and cellulolytic microorganisms in nature can depredate the most part of polysaccharides. Degradation of hemicelluloses and celluloses is one of the numerous reactions, which are directed on destruction of wood structure. These organisms provide degradation by hydrolyzing glycosidic linkages mostly through the action of extraccelular enzymes. /21/

3.2.1 Cellulases

Cellulases are the group of enzymes which hydrolyse β-1,4-glycosidic linkages in cellulose. Cellulases have been categorized based on their structural properties into three classes. Exoglucanases (1,4-β-D-glucan-cellobiohydrolaze, E.C. 3.2.1.91) hydrolise cellulose from the free chain ends, producing mainly cellobiose as an end product. They are called cellobiohydrolase. Cellobiohydrolase makes the cellulase system able to solubilise crystalline cellulose effectively. /19, 23/ Endoglucanases (1,4-β-D-glucan-glucanohydrolaze, E.C. 3.2.1.4) attack randomly internal linkages within cellulose chain. The small oligomers can be hydrolised to monomers by β-glucosidases (E.C. 3.2.1.21). Total hydrolysis of crystalline cellulose is possible only through the cooperative action of endoglucanases and exoglucanases. Complete cellulose degradation (see the Figure 7) proceeds through the synergistic action of endoglucanases, exoglucanases and β-glucosidase. /25, 27/

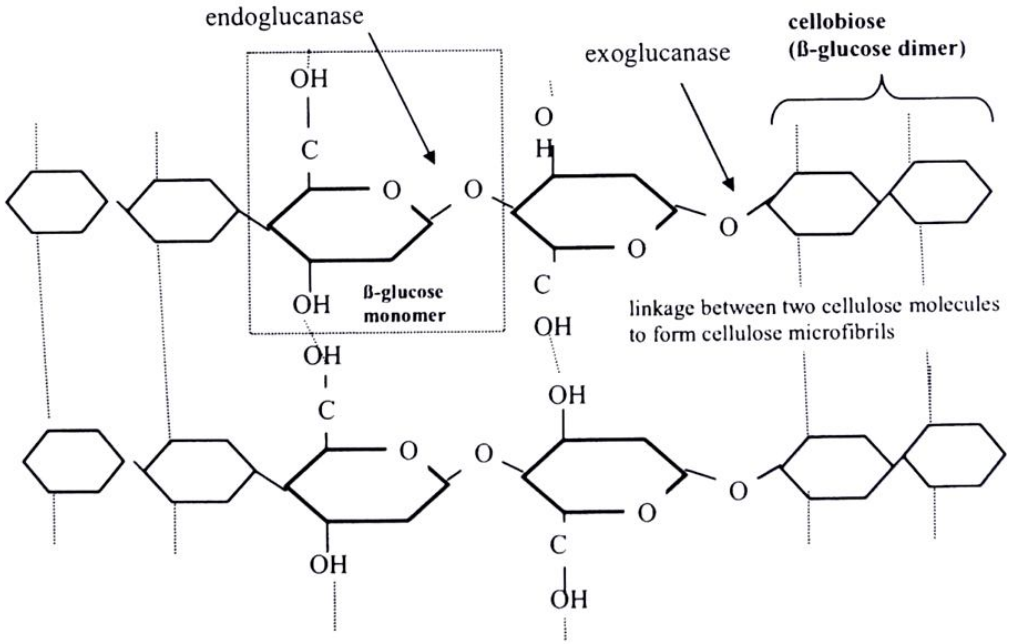

Figure 7. Simplified figure of cellulose degradation. /20/

By the one theory first attack on cellulose has an “endotype” and goes to amorphous regions of cellulose fibrils, which creates free chain ends available for “exotype” action. /25/

Efficient cellulase systems capable to degrade native cellulose are produced by white-rot and soft-rot fungi such as *Trichoderma, Fusarium, Humicola, Penicillum*, and *Schizophyllum*. These fungal cellulase systems usually contain various endoglucanases and exoglucanases and at least one β-glucosidase /26/. *Trichoderma reesei is* one of the producers of the most efficient cellulose degrading enzymes. It contains four major cellulases, two cellobiohydrolases, CBHI and CBHII, and two endoglucanases, EGI and EGII /20/. CBHI comprises 60% of the cellulases, secreted by fungi, and contains 497 amino acid residues. CBHII comprises 20% of the cellulases and contains 447 amino acid residues. EGI and EGII also differ from each other by cellulases content and amount of amino acids. /23, 25/

Most fungal cellulases show a common structure (Figure 8). They have relatively large catalytic active domain, a linker region and a small (~30 amino acid residues) cellulose binding domain (CBD) that binds to cellulose substrate. /19, 20/ The exact role and action mechanism of CBD have been under intensive research during the past years.

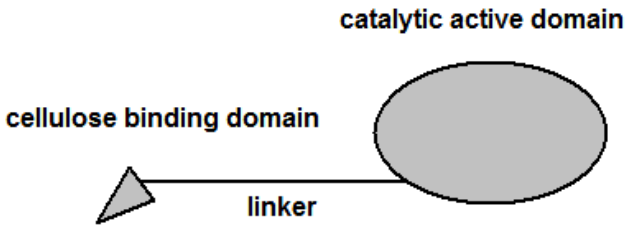

Figure 8. The common structure of cellulase. /20/

Functionally, cellulose binding domains (CBD’s) have been divided into three types: those which bind to the crystalline regions of solid polysaccharides and show weak, if any affinity for soluble carbohydrates (Type A); those which bind polysaccharide-chains, whether as soluble oligosaccharides or as amorphous regions of insoluble polysaccharides (Type B); and those which bind small sugar molecules (Type C). /21/ Actually, the CBD’s presence is important for binding of enzyme at insoluble and crystalline cellulose and for hydrolytic effects. /19/ It has been shown

that the ability of CBH's to degrade crystalline cellulose clearly decreases when the CBD is absent. /22, 49/

3.2.2 Hemicellulases

Hemicellulases are enzymes that degrade hemicelluloses by hydrolytic action on glycosidic linkages /22, 30/. Xylans and glucomannans are the most common hemicelluloses in hardwood and softwood respectively /30/. Thereby β-xylanases and β-mannases are the main endo-enzymes needed in hemicelluloses depolymerisation. The endoxylanases and endomannanases attack randomly at the internal linkages of xylans and glucomannans, respectively, releasing substituted oligomeric products. In the subsequent steps these intermediary products are further hydrolysed by a set of exo-enzymes (β-xylosidase and β-mannosidase) and side-group cleaving enzymes (α-arabinosidase, α-galactosidase, α-glucuronidase and esterase), resulting in the final monomelic end products. /23, 24, 25/

Xylanases

Endoxylanases (EC 3.2.1.8) catalyze the random hydrolysis of α-D-1,4-xylosidic linkages in xylans. Most xylanases belong to the two structurally different glycosyl hydrolase groups. /22/ Most characterized xylanases are able to hydrolyze different types of xylans showing only differences in the spectrum of end products. /20/ Some xylans contain xylan or a cellulose-binding domain, which have been found to increase the degree of hydrolysis. /22, 69/ Xylanase is industrially important enzyme, which is widely used in baking industry. Xylanase-aided bleaching of chemical pulp is the main application of xilanase in pulp and paper industry also. /20/

Mannanases

Endomannanases (EC 3.2.1.78) catalyze the random hydrolysis of β-D-1,4 mannopyranosyl linkages within the main chain of mannans and various polysaccharides consisting mainly of mannose, such as glucomannans, galactomannans, and galactoglucomannans. /19, 28/

The mannanase of T. reesei has been found to have a similar multidomain structure as several cellulolytic enzymes. The CBD has been found to increase the action of T. reesei mannanase on fiber-bound glucomannan even though the catalytic domain alone is also able to efficiently degrade crystalline mannan. /25/

Mannanase in pulp and paper industry are mostly used for paper machine runnability improvement and increasing of lignin extractability during the Kraft pulp bleaching. /20/

Other hemicellulases

α-glucuronidase (EC 3.2.1.131), α-arabinosidase (EC 3.2.1.55), and α-D-galactosidase (EC 3.2.1.22) can cleave the side groups connected to xylan and glucomannan main chains. Acetyl substituents bound to hemicellulose are removed by esterases (EC 3.1.1.72). /19, 30/

On Figure 9 the action of hemicellulases on hardwood and softwood xylan are showed.

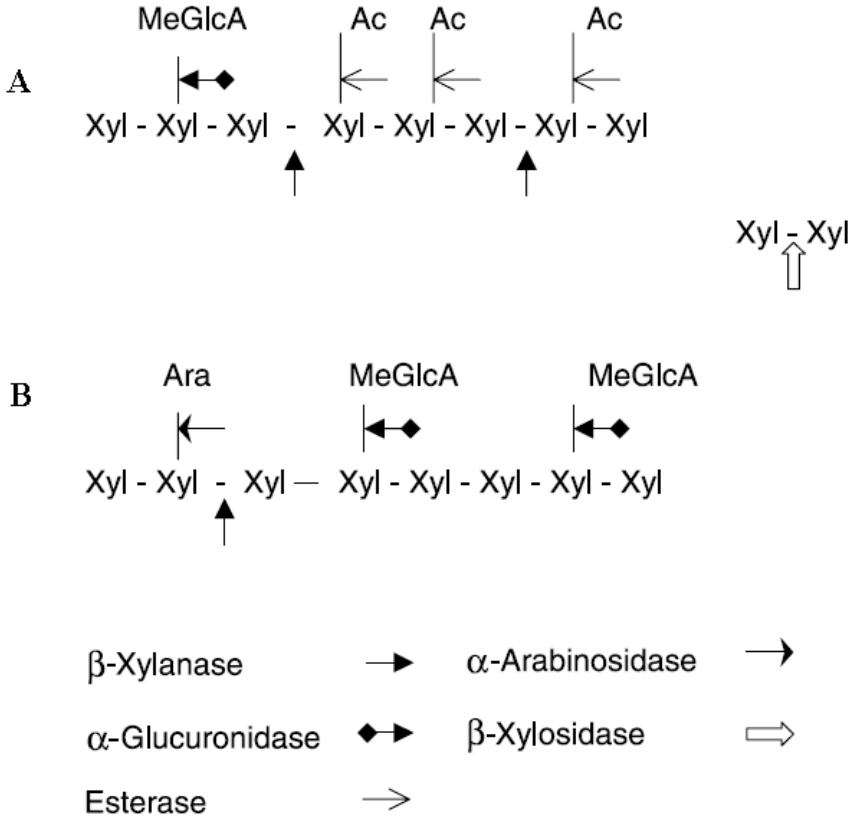

Figure 9. Action of hemicellulases on hardwood (A) and softwood (B) xylan. /20/

3.2.3 Amylases

Amylases hydrolyze starch. Starch contains about 15–30% amylose and 70–85% amylopectin. Amylose is a long linear polymer of α-1,4-linked glucose residues. Amylopectin is a branched polymer having both α-1,4 and α-1,6 linkages. /31/

Three types of amylases are involved in starch bioconversion: endo-amylase (α-amylase, EC 3.2.1.1), exo-amylases (glucoamylase or glucan 1,4-α-glucosidase, EC 3.2.1.3; β-amylase, EC 3.2.1.2), and debranching enzymes (pullulanase, EC 3.2.1.41; isoamylase, EC 3.2.1.68). /19/

α-Amylase cannot act on α-1,6 linkages and hydrolyzes internal α-1,4-glycosidic bonds of starch randomly and produces malto-oligosaccharides of varying chain lengths. Glucoamylase cleaves glucose units from the nonreducing end of starch and it can hydrolyze both α-1,4 and α-1,6 linkages of starch. β-Amylase hydrolyzes the α-1,4-glycosidic bonds in starch from the nonreducing ends. Pullulanase (pullulan α-1,6-glucanohydrolase) or isoamylase (glycogen α-1,6-glucanohydrolase) cleaves the α-1,6- linked branch points of starch and produces linear amylosaccharides of varying lengths. /19, 20, 31/ The Figure 10 summarize schematically the starch hydrolyses and further dextrins degradation to maltose and glucose.

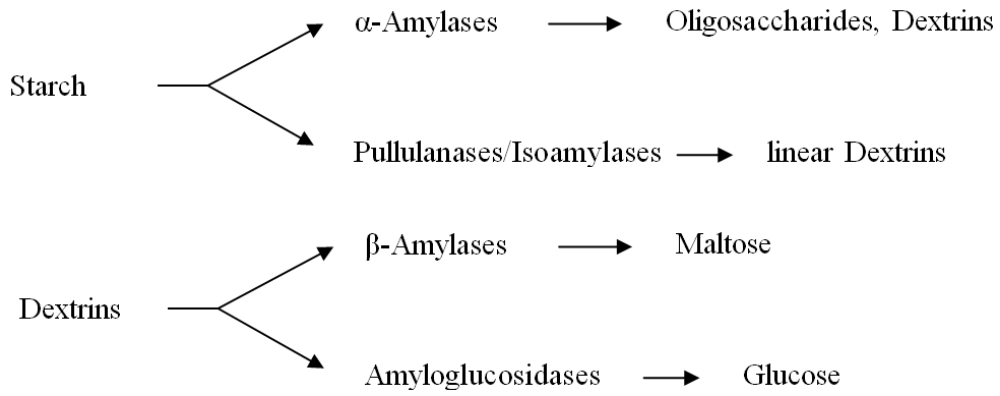

Figure 10. Schematic of enzymatic starch hydrolyses. /19/

In baking, brewing, detergent industries industry α-amylase is used for starch modification. It is also can be used in paper industry for coating starch. /20/

3.2.4 Pectinases

Pectin is a main component of the middle lamella and primary cell wall. /10/ The term pectin is used for group of components comprising rhamnogalacturonans, galactans and arabinans. Rhamnogalacturonan is a main component of pectin and has a backbone of α-(1,4)-linked D-galacturonic acid units and α-(1,2) or α-(1,4)-linked L-rhamnose. /19, 20/

Pectinases are the group of enzymes involved in depolymerisation of the pectic polymers. This group of enzymes mainly consists of polygalacturonases (PG, E.C 3.2.1.15), pectin methyl esterases (PME, (E.C 3.1.1.11) and pectin lyases (PL, E.C 4.2.2.10). /32, 33/

Polygalacturonases cleave the bonds between galacturonic acids of the pectin chain (Figure 11, A). It is a hydrolytic enzyme and exists in two forms: endo-PG and exo-PG. Endo-PG acts randomly on the α-(1,4)-polygalacturonic backbone, whereas exo-PG acts at the non-reducing end of the chain. /20/

Pectin methyl esterase provides demethylation of pectin and decrease the amount of esterified pectin (Figure 11, B). /20/

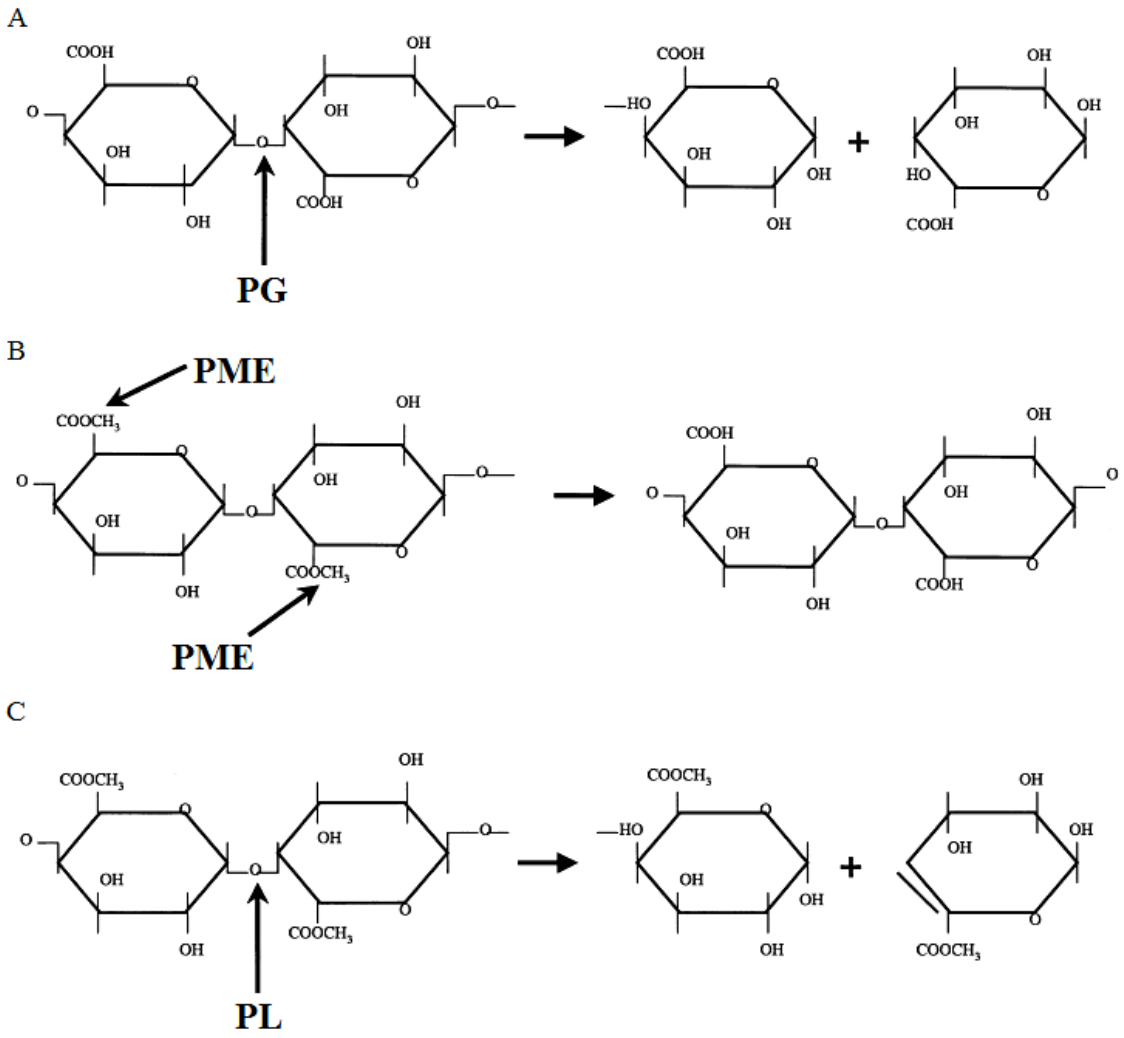

Figure 11. Different types of pectinases and their mode of action on pectic substances (A-polygalacturonase action, B-pectin methyl esterase action, C-pectin lyase action). /33/

Another type of pectinase is pectin lyase, which capable of depolymerising highly esterified pectin into small molecules without prior action of other enzymes (Figure 11, C). /32/

Pectinases are widely used in biotechnological applications especially in food industry, textile, pulp and paper industries and in waste-water treatment. /34/ Applications in pulp and paper industry include energy reduction in mechanical pulping and debarking, and anionic trash control at paper machine. /20/

3.3 Oxydoreductase

Reactions catalyzed by oxidative enzymes play significant role in complete degradation of cellulosic biomass. Lignin is a third major component of lignocellulosic materials. The enzymatic modification and degradation of lignin involve oxidoreductive and radical reactions. The enzymology of lignin identify three major enzymes systems participate in delignification: lignin peroxidase, (LiP, E.C. 1.11.1.14), manganese-dependent peroxidase, (MnP, E.C. 1.11.1.13), and laccase (E.C. 1.10.3.2). /19/

3.3.1 Laccase

Laccases (*p*-diphenol:dioxygen oxidoreductase) belong to the class of oxidases and catalyze the oxidation of phenolic compounds, aminophenols, polyphenols, polyamines, certain inorganic ions and aryl diamine compounds with the concomitant reduction of oxygen to water. /20, 35/ Laccase oxidizes lignin model compounds with phenolic hydroxyl substitutions to phenoxy radicals using O_2 as an electron acceptor (see the Figure 12). /35/

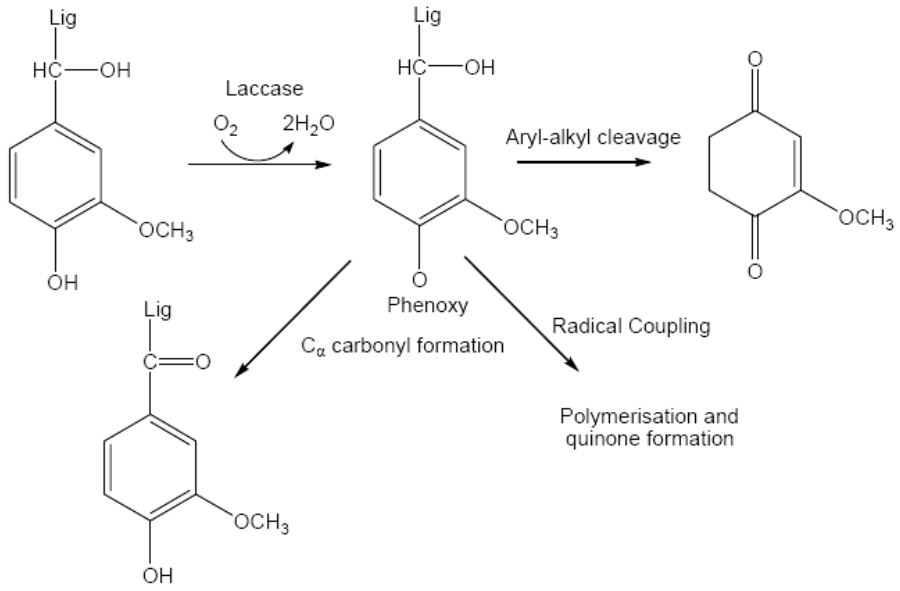

Figure 12. Schematic catalytic action of laccase. /58/

The catalytic active site of laccases has four copper ions, and their combined interaction couples the one-electron oxidation of donor substrates with the four-electron reduction of dioxygen. /36, 58/ The copper molecules are needed in the electron transfer reactions during the catalytic oxido-reductive actions of laccase. /20, 47/

The action of laccase can be enhanced by mediators. /37/ Mediators are the small molecular weight compounds that can be oxidized with laccase. /20/ The mediators can provide the oxidative action at inner parts of the fibers, where the laccase cannot penetrate. /20, 37, 38/

3.3.2 Lignin peroxidase

Lignin peroxidase (LiP) plays a central role in the biodegradation of the plant cell wall constituent lignin. The first lignin peroxidase was isolated from white-rot fungi *Phanerochaete Crysosporium* in 1983. /40/

LiPs have high redox potential and can oxidize non-phenolic lignin model compounds, aromathic ethers, and polycyclic aromatics. LiP forms the cationic radicals, which oxidize the substrates in multi-step electron transfers and form intermediate radicals, such as phenoxy radicals and veratryl alcohol radical cations (see the Figure 13). These intermediate radicals undergo non-enzymatic reactions such as radical coupling and polymerization, side-chain cleavage, demethylation and

intramolecular addition and rearrangement that finally initiate the rings cleavages and lignin degradation. /19, 41, 42/

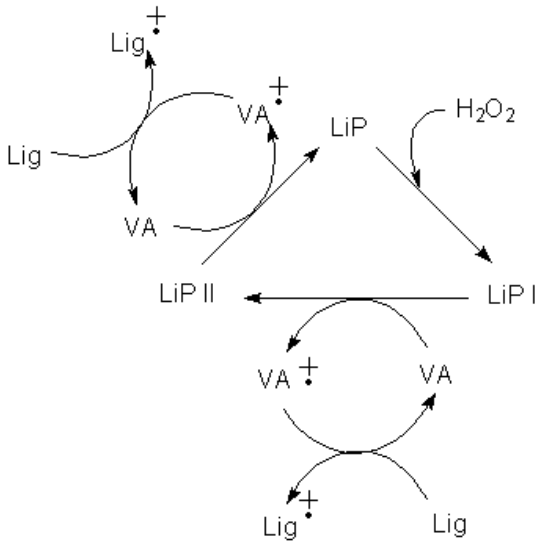

Figure 13. The oxidation states of lignin peroxidase. (Lig-lignin; Lip-lignin peroxidase; LipI and LipII-compounds I and II; VA-veratryl alcohol). /44/

After degradation of lignin on monomers (veratryl alcohol) peroxydases continue oxidation. And the major product of veratril alcohol oxidation is veratryl aldehyde. By this scheme the peroxydases oxydate the lignin compounds. /39, 43/

3.3.3 Manganese dependent peroxidase

Peroxydative enzyme dependent of Mn (II) and stimulated activity by lactate has been found from *Phanerochaete Crysosporium*. This enzyme designated manganese-dependent peroxydase. /42/ In enzymology this peroxydase catalyzes the next chemical reaction:

$$2\ Mn\ (II) + 2\ H^{+} + H_2O_2 \leftrightarrow 2\ Mn\ (III) + 2\ H_2O$$

Thereby MnP oxidizes Mn (II) to Mn (III), which is stabilized with organic acids like oxalic, malic, lactic or malonic acid after. /20/ Chelation of Mn (III) by organic acid is necessary to stabilize the ion and promote its release from the enzyme. Further, chelated Mn (III) oxidizes phenolic subunits in lignin. /19, 22, 40/

3.3.4 Versatile peroxidase

Versatile peroxidase (VP) has been recently described as a new family of ligninolytic peroxidases, together with LiP and MnP. /45/ The mechanism of VP is

the classical for peroxidases, where the substrate oxidation is carried out by a two-electron multistep reaction at the expense of hydrogen peroxide. VP oxidizes Mn (II) to Mn (III), degrades the non-phenolic lignin model dimmers, and oxidizes veratryl alcohol, p-dimethoxybenzene and high redox potential dyes. /46/

3.4 Cell wall loosening enzymes

Cell wall loosening appears during the growing of young plant cells. It contains loosening of hydrogen bonds between xyloglucan and cellulose microfibrils, the enzymatic cleavage of xyloglucan chains and an internal osmotic pressure that pushes the microfibrills apart. The process of cell wall loosening is catalyzed by expansins, endo-β-glucanases, and xyloglucan-endotransglycosylases (XET). /20/

3.4.1 Expansins

Expansins belong to a large group of cell wall loosening proteins involved in cell enlargement and in a variety of other developmental processes of cell wall modification. /51/ The proposed model of expansins action is that these proteins modify the cell-wall matrix to enable growth and development of plant cells. /53/

Nowadays the expansin family can be divided into four groups: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB). /50, 51/ EXPA and EXPB are proteins which cause cell wall loosening, whereas EXLA and EXLB proteins are known only from their gene sequences. /52/

Have been proposed that expansin cleave hydrogen bonding between cellulose microfibrils or between cellulose and other polysaccharides without having hydrolytic activity. /50/

On the Figure 14 cellulose microfibrils (shaded areas) to which are bound various glycans such as xyloglucan or xylan (thin strands) by hydrogen bonds (indicated by rows of short lines) are presented.

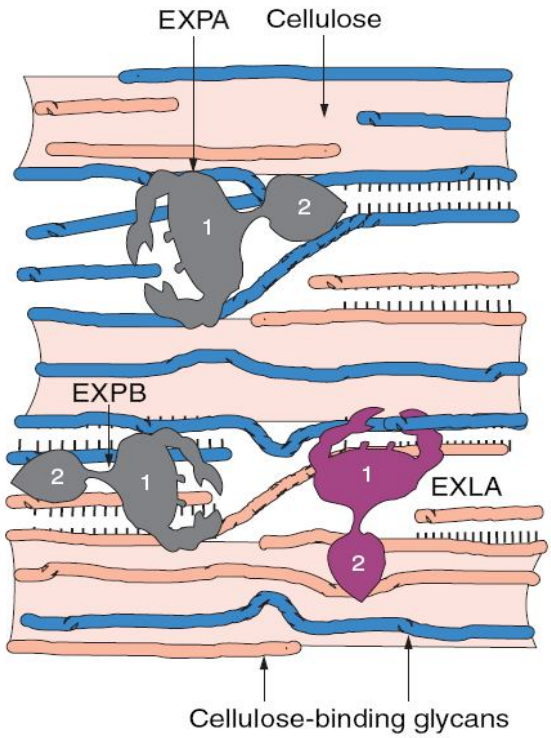

Figure 14. A simplified model of the plant cell wall and its loosening by expansins (α-Expansins (EXPA), β-Expansins (EXPB), Expansin-like A (EXLA)). /52/

Extension of the cell wall entails movement and separation of the cellulose microfibrils by a process of molecular creep. EXPA may promote such movement by inducing local dissociation and slippage of xyloglucans on the surface of the cellulose, whereas EXPB work on a different glycan, perhaps xylan, for similar effect. EXLA and EXLB proteins are predicted to be secreted to the cell wall, but their activity has not yet been established. /52/

Expansins can be used in bioengineering of cell walls, for cell wall structure and properties modification in such as wood, textiles and polymers industries. /53/

3.4.2 Swollenin

Swollenin is a protein which has an N-terminal fungal type carbohydrate-binding module domain connected by a linker region to the expansin-like domain. /54, 55/ The protein was found from cellulolytic fungus *Trichoderma reesei* and named swollenin due to its ability to swell cotton fibers without producing detectable amounts of reducing sugars. /55/

3.4.3 Xyloglucan endotransferases

Cell wall expansion and elongation is described by temporary loosening followed by rapid reinforcement of wall structure. Xyloglucan endotransglycosylases (XETs) are enzymes, which capable of modulating the chemistry of the matrix and therefore performing both of these functions. /56/ XETs catalyze cleavage of a xyloglucan chain and with reattachment of the xyloglucan to another xyloglucan chain in the cell wall. /20/ These enzymes belong to a larger family of enzymes known as glycoside hydrolases, which catalyze cleavage of glycosidic bonds using general acid catalysis. /56/

3.5 Lipases

Lipases (E.C. 3.1.1.3) catalyze the hydrolysis and synthesis of long-chain acylglycerols with trioleoylglycerol being the standard substrate. /57/ The lipases are a versatile group of enzymes and such activities like phospholipase, lysophospholipase, cholesterol esterase, cutinase, amidase and other esterase type of activities can be expressed. /59/

Also lipases can be defined as lipolytic enzymes. They are capable to hydrolyze lipid substrates, such as phospholipases, cutinases or enzymes hydrolyzing ester substrates of lipid character. /59/

Lipases are used in different industries e.g., detergents, oil and fats, baking, organic synthesis, hard surface cleaning, leather industry and paper industry. /57/ In paper industry lipases are used to reduce pitch problems, improve the strength properties of mechanical pulp and runability of paper machines. /20/

3.6 Esterases

The esterases (E.C. 3.1.1.72) hydrolyze the ester linkages between xylose units of the xylan and acetic acid (acetyl xylan esterase) or between arabinose side chain residues and phenolic acids such as ferulic acid (feruloyl esterase) and p-coumaric acid (p-coumaroyl esterase). /19/

Esterases remove acetyl side groups, thereby deacetylates the polymers to a less adhesive alcohol form and it leads to more hydrophilic particles. By the way

esterases can reduce the stickies problems in deinking process. Esterases are also useful in pitch control together with lipases. /20, 60/

3.7 Proteases

The catalytic function of proteases (E. C. 3.4.21.62) is hydrolyzing of peptide bonds in proteins. /19/ Proteases have been categorized, depending on their site of action, into two major groups: exoproteases and endoprotease. Exoproteases act only near the ends of the N- or C-termini of the polypeptide chains. Endoproteases attack peptide bonds in more central locations of the polypeptide chain more remote from the N- and C-termini. /18, 19/

Proteases find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes. In pulp and paper industry proteases are used in biofilms degradation together with other enzymes. /20/

4 Effect governing enzymes activity

The enzymes activity depends on many factors such as inhibitors, activators, chemical environment (pH value), temperature and concentration of substrate. /18/ Temperature, pH value and additives are the main factors, which can be controlled, and effect on enzymes activity during the mechanical pulping process. /1, 17, 19/

4.1 Temperature and pH value

The enzymes-catalyzed reactions depend on temperature and have an optimum temperature range. The initial boundary of interval is determined by thermodynamic increase of reaction rate (see the Figure 15 A-1). The final boundary of of temperature interval for maximum enzymes activity is determined by thermal denaturation of enzyme (see the Figure 15 A-2). The optimum is generally between 40 and 60°C, but some temperature –intensive enzymes show the highest activity even under 100°C. /19/

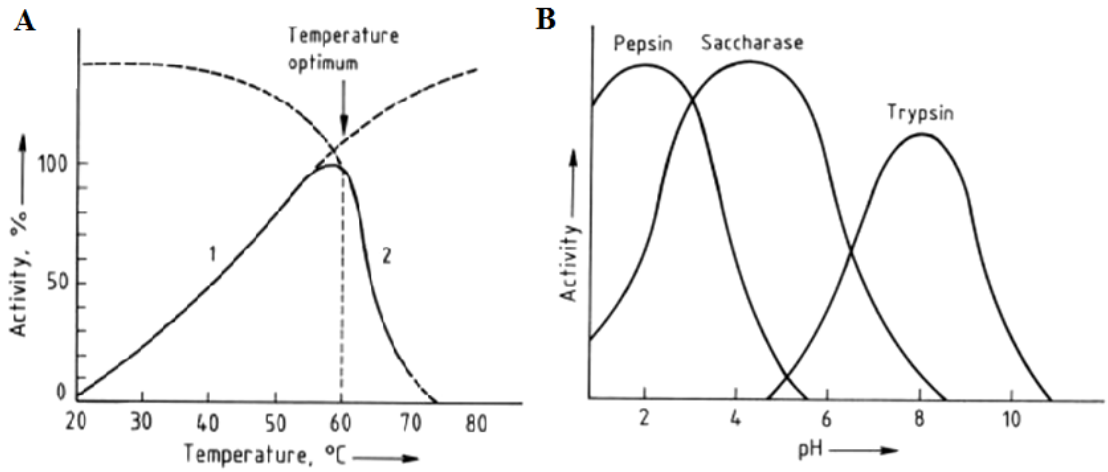

Figure 15. A-temperature optimum for enzymes activity; B-activity of various enzymes as function of pH. /19/

All enzymes have optimum pH range for activity. The ionic strength and type of buffer have a strong influence on optimum. It may also be influenced by temperature, substrate and coenzyme concentrations. The most of enzymes have optimum pH in range from 5 to 7, but some enzymes have optimum in extreme values of 1.5 and 10.5 (see the Figure 15 B). /19/

Data about optimal temperature and pH ranges for wood component hydrolyzing and modifying enzymes from different sources have been gathered and summarized in Table 3.

Table 3. Optimum pH and temperature ranges for some wood affecting enzymes. /19, 25, 26, 29, 31, 42/

Enzyme	Optimal pH	Optimal temperature, °C
Cellulase	4-5	50-60
Cellobiohydrolases (CBH I, CBH II)	4-10	60-70
Endoglucanase (EG I, EG II)	5-7	50-60
Amylase (Novozyme)	6-9	70-90
Pectinase (Novozyme)	4.5-5	50
Laccase	3-5	40-60
Lignin Peroxidase (*Phanerochaete Chrysosporium)*	5.5	40
Manganese Peroxidase (*Phanerochaete Chrysosporium)*	4-6.5	40
Lipases	6	40
Protease Thermo stable protease (Novozyme)	8-11	50-60 75-100
Esterases (*Aspergillus niger*)	5	55

4.2 Surfactants as enzymes activators

Interactions between surfactants and enzymes in aqueous solutions have been extensively studied for technical applications, such as drug delivery, cosmetics and detergency, and for studying interactions between membrane proteins and lipids, and then both components must be compatible with each other. /61, 62/

In literature have been reported the effect of surfactants on enzymatic hydrolysis of rice straw. The experiment was carried out under the temperature 40 and 50°C with cellulase and polyethylene glycol (PEG) and polyoxyethylene sorbitan monooleate (PSM) as surfactants. The amount of surfactants was 0.2 g per g

of wood. The results show that samples with surfactants addition had higher conversion. (Figure 16) /63/

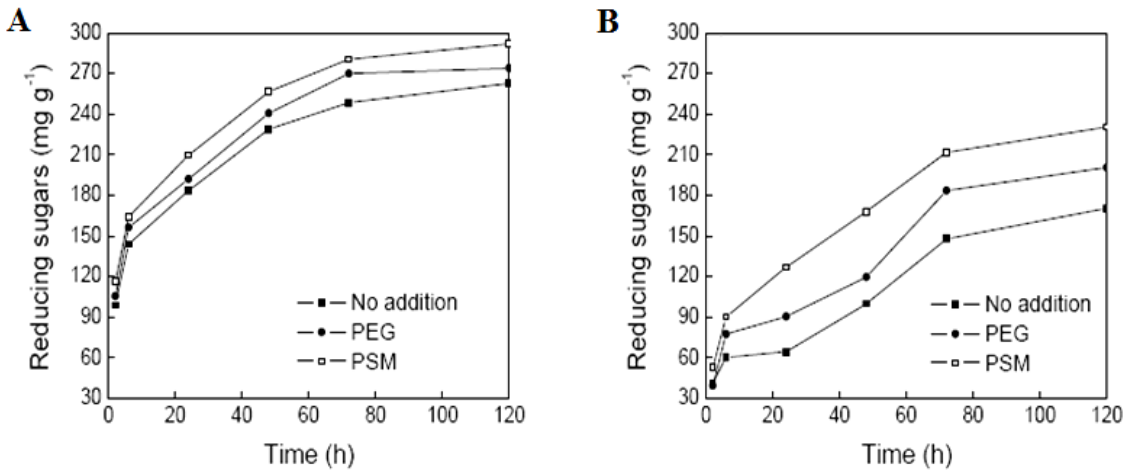

Figure 16. Relationship between reducing sugars to time of hydrolysis with different temperature, A-40°C, B-50°C. /63/

As we can see addition of surfactant can effectively improve the enzymatic hydrolyses of rice straw, as lignicellulosic material. /63/

5 Enzymes in thermomechanical pulp production

The production of mechanical pulp need large amount of energy. Study of fungi and their action on wood components showed the possibility of using enzymes for wood treatment. Therefore new functional properties of wood can be introduced by enzymes. /65, 66/

Nowadays a lot of laboratory experiments which show reducing of specific energy consumption for chip refining have been already done. Parallel with this was checked the main properties of pulp, such as strength, light scattering, opacity and so on, which also have been improved by the treatment. /64/

Manganese peroxidase (MnP), laccase-mediator system, pectinase, endoclucanase and cellulase mixture have been used in different researches for finding the most effective and suitable combinations for reducing energy consumption in refining. /19, 20/ Cellulases mainly affect on crystalline parts of cellulose and forms cavities in wood structure, that increasing the fibrillation and fiber flexibility. But these processes also have a negative effect on yield loss, because some part of cellulose destructed by cellulases. /67/

Manganese peroxidases have affects on lignin structure and mostly have a part in lignin degradation processes. The treatment by pectinase enzymes are commonly used in processes involving the degradation of plant materials, and they activates in 4-5 pH range, that makes their usage possible in mechanical pulp production process. The less aggressive pectinase was more selective and resulted in thermomechanical pulps of higher pulp strength and scattering coefficient than the aggressive pectinase. Pulps produced using the multiple component enzyme blend developed the highest pulp strength properties. For instance, pulps produced from the pectinase pretreatment achieved similar pulp strength with higher scattering coefficient and lower COD generation as compared to bisulfite pulp. An optimal performance range between 40°C to 55°C for most enzymes, which have been tested for wood fiber treatments, also provides their applicability. /68, 70, 72/

The physical–chemical mechanism during the production of mechanical pulp is not known sufficiently. Especially effects of different components of fiber wall structure on either biopulping or development of pulp properties is not known well in industrial processes. Biological techniques makes possible to achieve more controlled effect to different fiber cell wall components. This opens new abilities to tailor-made TMP pulp and also makes it possible to widen the range of resources for production. By researches of biochemical pulping becomes more likely progress in efficient energy usage, without harm for final product. /73/

5.1 Effect of cellobiohydrolase treatment

Cellobiohydrolase (CBH I) with molecular weight 64 kDa was taken to analyze the impact of cellobiohydrolase on specific energy consumption. In three independent series, coarse once refined TMP pulps, with freeness values (CSF) of 450—550 ml, were treated with CBH I enzyme. The consistency of the pulp suspension during treatment in each experiment was 50 g o.d.p./l of tap water, the treatment time 2 h and temperature 45—50° C. The amount of treated pulp was 1 kg of dry pulp and the enzyme dosage 0.5 mg/g of pulp. The pulps were further refined using a single rotating disk atmospheric refiner using decreasing plate settings. The refining was followed by determining the freeness values of the intermediate samples and stopped when the freeness values were below 100 ml. /81/

The energy consumption in each refining experiment was measured and the specific energy consumption was calculated and reported as kWh/kg oven dry weight basis. The results are presented in Table 4. /81/

Table 4. The specific energy consumption with CSF level of 100 ml for treated with CBH I and CBH I/CBH II samples. /81/

Treatment	Test 1 kWh/kg	Test 2 kWh/kg	Test 3 kWh/kg	Test 4 kWh/kg
CBH I	1.73	1.64	2.04	1.81
CBH I/CBHII	-	-	-	1.77
Controls	1.97	2.05	2.39	2.08

It can be observed from results that by using the CBH I is possible to reduce the energy consumption by 15—20% as compared with the reference sample. The same effect was also obtained, when the preparation contained both CBH activities.

5.2 Effect of cellobiohydrolase and mannanase blend treatment

To evaluate the effect of mannanase on SEC level and on paper properties several researches have been done. Mannanase with molecular weight 51-53 kDa and CBH I with MW 64 kDa were used in experiment. In the experiment the enzymes dosages were as follows: 1) CBH I 0.2 mg/g o.d.p.; 2) CBH I 0.1 mg/g o.d.p. + mannanase 0.1 mg/g o.d.p. Spruce TMP pulp samples (CSF 640 ml) were treated for 2 hours at 45-50°C After treatment pulp was refined in Sprout-Waldron single rotating disk refiner to obtain CSF values about 150-160 ml. From refined pulp handsheets were also made and tested. Results of the experiments are presented in Table 5. /74/

Table 5. Specific energy consumption (at CSF level of 120 ml) and optical properties of handsheets. /74/

Treatment	SEC, kWh/kg	ISO brightness, %	Light scattering coef., m^2/kg	Light absorption coef., m^2/kg	Opacity, %
Control	2.25	58.0	50.1	2.87	92.3
CBH I	2.15	58.2	50.2	2.73	91.0
CBH I + mannanase	2.0	59.8	52.5	2.46	91.0

According to the results it can be concluded that the treatment with CBH I + mannanase gives a lower energy consumption and improves ISO-brightness and light scattering as compared with control and treated with CBH I samples. /74/

5.3 Effect of lignin peroxidase, manganese peroxidase and laccase treatment

Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase were used to test their effect on specific energy consumption (SEC) for refining TMP and CTMP pulp from sugarcane bagasse. The enzymes were extracted from *Phanerochaete Chrysosporium* fungi. The residence time for bagasse treatment was 12, 36 and 48 hours with enzymes, at 25-29°C. Water to bagasse ratio was 8:1 and 5 ml of hydrogen peroxide were added to activate peroxidise enzymes. /73/

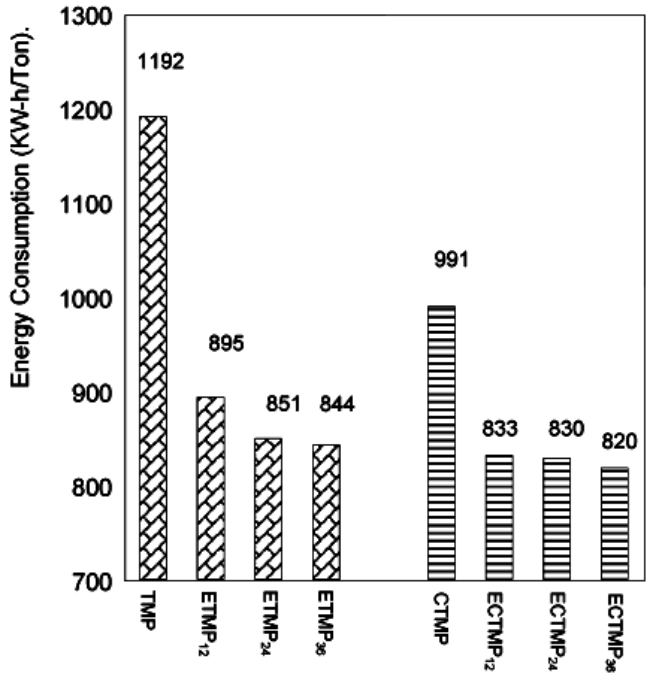

Figure 17. Energy consumption for TMP and CTMP pretreated with enzymes (refining degree for all of the pulps was 70°SR). /73/

The results, which are presented on Figure 17 show, that enzymes from *Phanerochaete Chrysosporium* fungi reduces the energy consumption by 29.2% with 36 hours treatment for TMP processing and by 17.3 % with 36 hours treatment for CTMP processing. /73/

During another research pretreatment by MnP and laccase separately has been evaluated. The refining energy curves the initial refiner loads of wood chips were

measured to determine enzymatic effect. The higher initial load means more effective chip refining. The initial refiner load of compressed and MnP enzymes treated spruce wood was slightly higher than of the fresh wood. It means that MnP treatment softened the wood material. /3, 48/

On the Figure 18 we can see that MnP treatment decrease SEC in refining was of spruce chip, at the same time laccase treatment did not show significant effect on SEC. Energy savings by MnP treatment of spruce chips was indicated at 6%. This was reached at CSF level of 350 ml. /3/

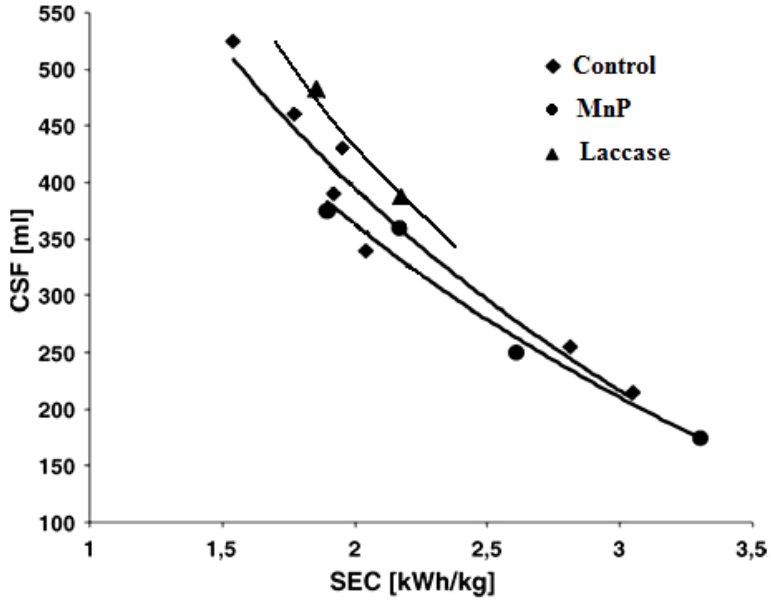

Figure 18. Canadian standard freeness as a function of specific energy consumption for the spruce pulp treated with laccase and manganese peroxidase enzyme. /3/

In literature has been also noticed that laccase treatment alone reduces energy consumption by 5%. /83/

5.4 Effect of pectinase treatment

Some studies were conducted to determine the influence of pectinases on energy consumption during refining. Pretreatment process was carried out by compression of chip in screw compression device or by twin roll press. Compression ratio was from 1:1 to 8:1 and also pre-steaming process was included in the chip preparation. Impregnation followed immediately after the compression and/or thermal pretreatment. The retention time of treatment was 120 min at 50°C. /79/

To reach a given pulp freeness of 100 ml CSF, the energy reduction was 400 kWh/t. This means from 2500 kWh/t without pectinase treatment to 2100 kWh/t with pectinase treatment, or by about 16% (see the Figure 19) /79/

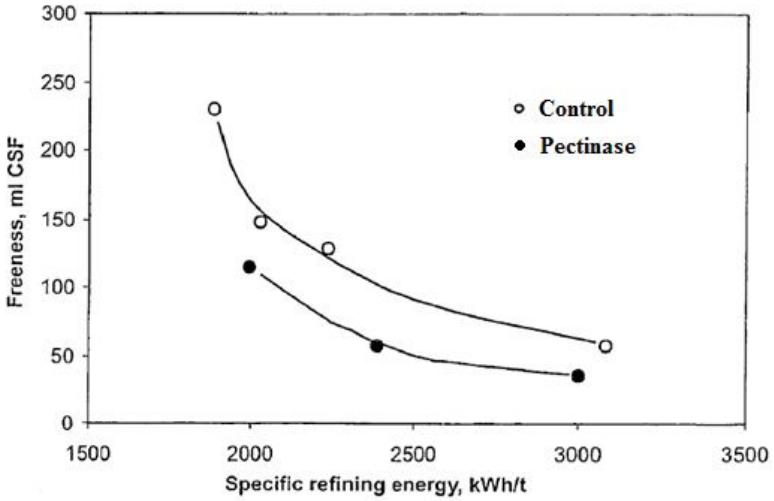

Figure 19. Canadian standard freeness as a function of specific energy consumption for TMP pulp treated with pectinase enzyme. /79/

Another set of experiments with target to reach better results in energy savings was made. It was done under the high intensity conditions (HI-TMP), which means preheating steam pressure 5.9 bars instead of 2.8 bar pressure in normal conditions and time of pre-steaming 12 sec instead of 3-4 min.

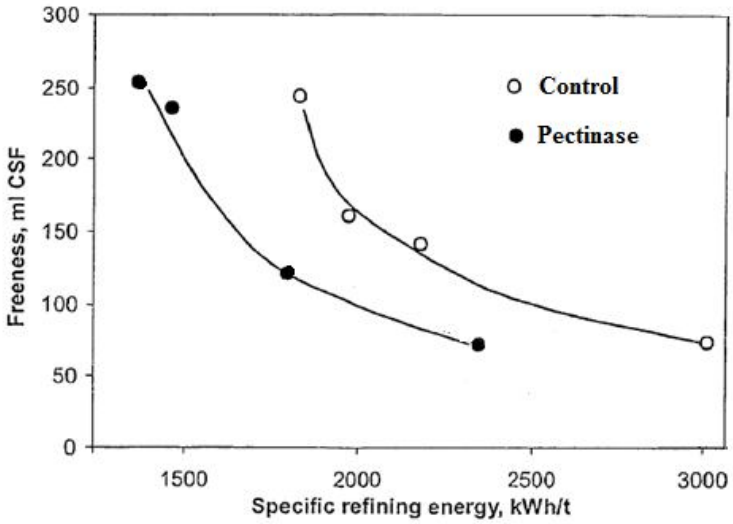

Figure 20. Canadian standard freeness as a function of specific energy consumption for HI-TMP pulp treated with pectinase enzyme. /79/

Results of experiments are presented on Figure 20 and show, that energy reduction was about 500 kWh/t. This means from 2500 kWh/t without pectinase treatment to 2000 kWh/t with pectinase treatment, or by about 20%. /79/

6 Knowledge discovery in databases

Knowledge discovery in databases (KDD) is the non-trivial process of identifying valid, novel, potentially useful, and ultimately understandable patterns in data. At the core of KDD is the application of "Data Mining" methods for pattern recognition and discovery. /84/

6.1 Data mining

It is a step in the KDD consisting in applying data analysis and discovery algorithms that, under acceptable limitations of computational efficiency, produce a particular enumeration of patterns (or models) over the data. /84, 85/

Data Mining differs from the traditional techniques in that it does not recover from a collection a subset of documents which are hopefully relevant to a query, based on keyword searching. Instead, the goal is to extract from the documents (which may be in a variety of languages) salient facts about pre-specified types of entities and relationships. Data Mining functions can be divided into two categories: supervised (directed) and unsupervised (undirected). Supervised functions are used to predict a value; they require the specification of a target (known outcome). Unsupervised functions are used to find the intrinsic structure, relationships, or affinities in data. /84/

There are two main models of knowledge discovery process: a) open - for generation of a hypothesis b) closed - for testing of a hypothesis. The main principles of open and closed discovery processes are presented on Figure 21A. /84/

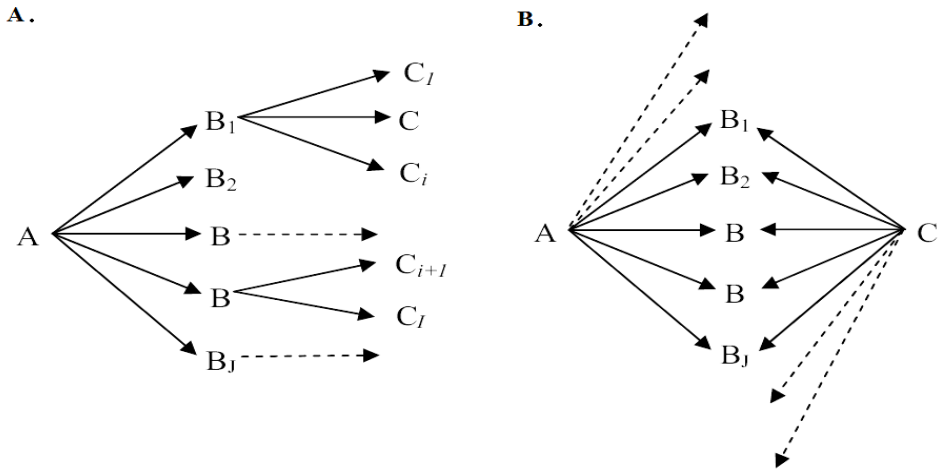

Figure 21A. Schematic principle of A-open knowledge discovery process; B-closed knowledge discovery process. /84/

Usually first is used an open approach for the generation of a hypothesis and next a closed approach for testing of the generated one. There exists also a semi-open discovery process. It is a procedure similar to open process, but performed with the specific, a priori determined, selection criteria for the hypothesis. The closed discovery process seems to be the most promising for knowledge discovery related to a search for new products to be produced from the known raw materials or the identification of new raw materials for the synthesis of the known products. /86/

6.2 Development of search paradigm

During the past two decades there was developed by Smalheiser and Swanson a set of interactive software and database search strategy for knowledge discovery, called "Arrowsmith". They suggested to combine existing bibliographical information for discovery of knowledge. The approach proposed by Smalheiser and Swanson is based on the implementation of the following transitivity rule: term A is related with term B, and term B is related with term C. Simultaneously we assume that there is a relation between A and C and we search for this relation-term B (see the Figure 21B). /84/

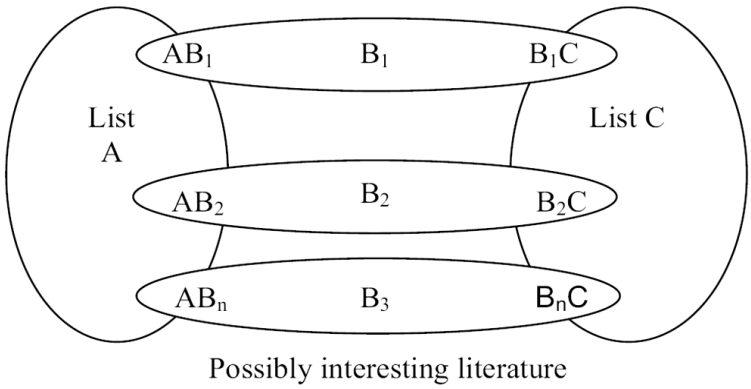

Figure 21B. "Arrowsmith" principle. /84/

The discoveries obtained when using the "Arrowsmith" approach and software, have been confirmed experimentally and clinically in the field of medicine. The "Arrowsmith" method is an example of the closed approach. "Arrowsmith" is also an interactive software for the discovery of plausible hypotheses linking findings across specialties in biomedical literature (e.g. Medline). It contains a pre-compiled `stop-list' of 5000 words, which are definitely noninteresting (e.g. `the', `for'). /84/

6.3 Use of semantic analysis in closed discovery process

Approach to the analysis of title, abstract and full text (articles, patents, web-sites) is based on the use of semantic analysis. There are developed the specific semantic rules to analyse the text. For instance, if we are looking for material with the specific property, there should be in the analysed text at least one phrase containing the name of this property, e.g. bacterial. In this case, any substance in such phrase, with high probability, could be a sought material. The application of the semantic analysis for the searching of products will considerably simplify and speed up the process of the text analysis. /85/

Very often, the researcher has to identify a new material with more than two priori specified properties. For this case, a multi-level approach has been developed. A new method of knowledge discovery have been developed and applied in the research of the biochemical reactions. The Data Mining techniques and semantic rules allow a huge number of literature sources to be treated and reduce the amount of routine work in finding new knowledge. Therefore, it can significantly reduce the development time for the design of a new product. /84, 86/

7 Conclusions of the literature part

In the first part of literature review the main wood components and enzymes, which affect on them, are presented. The second part contains the optimal temperature and pH for enzymatic action. The most effective enzymes in question of energy saving in TMP processing were described in third part of literature overview. Finally in the literature part the information about knowledge discovery methods and their applications are presented.

Based on previous researches we can conclude that pectinase and cellobiohydrolase showed the significant effect in energy saving of TMP process for spruce chip. Manganese peroxidases also show some small effect on energy decrease during mechanical pulp production. This information from previous studies can be used during experiments. The approximate expectations, which based on literature part can give the information about reliability of tested method. The information about the optimal condition for enzymes can be also used during the experiments.

Based on knowledge discovery methods review we can conclude that "Arrowsmith" software can be useful technique to find out effective mixtures of enzymes and additives, which can activate the enzymatic action.

EXPERIMENTAL PART

8 Target of experimental part

The main target of experimental part was to find out reliable method to predict the effect of enzymes on specific energy consumption for refining of spruce chip.

The first part of the experiments contains the laboratory method evaluation. It is based on verification of enzymes, which have been previously used.

The second part of the experiments is knowledge discovery in databases with target to find out interesting mixture of enzymes or additives for enhancement of enzymatic activity.

9 Materials

Three different size raw materials: normal size chip, crushed chip and water impregnated, instantly preheated, pressed and then fiberized at 400 kWh/t chip – further named fiberized pulp, were used during the experiments. The usage of smaller size chips was done with the purpose to improve the penetration of enzymatic solution into the wood structure. The impregnation into the wood is difficult without special equipments and increased contact area can give better enzymes penetration. Dimensions of wood raw materials can be seen in Figures 22 and 23.

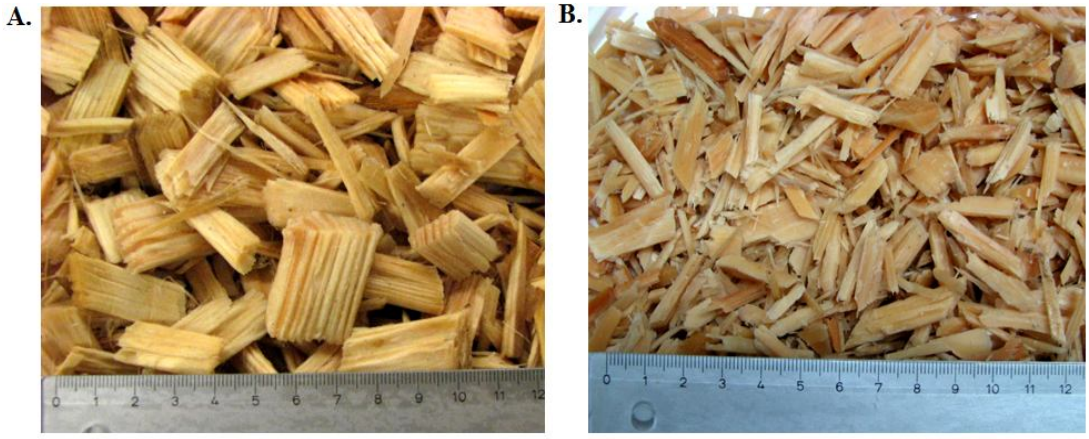

Figure 22. The chip dimensions (A – normal size chip; B – crushed chip).

Figure 23. The fiberized pulp dimensions.

The pectinase, endoglucanase and mixture of enzymes were used in this study. Dosages of enzymes varied from 1.5 to 30 kg/t chips. The high enzymes dosages were used to achieve the same effect as in the previous studies. The enzymes were diluted with tap water at 24°C.

10 Experimental methods

10.1 Chip Treatment

In the experimental part two different cookers were used. The first one was big digester with continuous circulation system (see the Figure 24 A). It had the heat exchanger to control the cooking temperature. The capacity of this digester was 5 kg of o.d.w. and it was used for normal size and crushed chip. There was no mixing of chip during the cooking. The cooking temperature was 50 – 60 °C. The chip:water ratio in digester was 1:4.

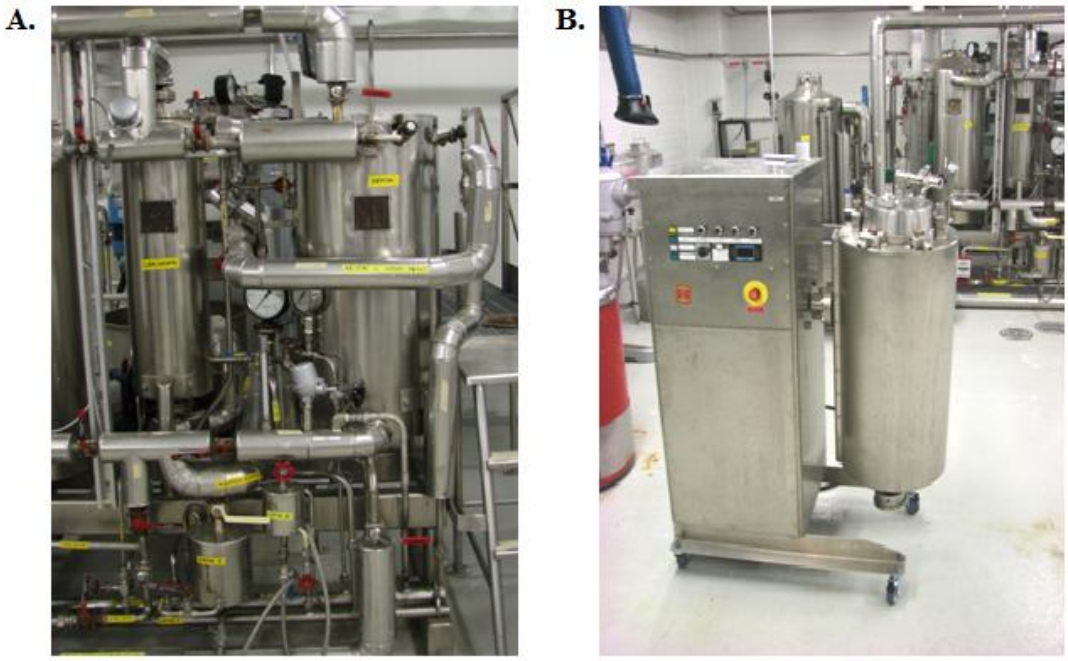

Figure 24. A-big digester with continuous circulation system; B-small rotating digester.

The second cooker was small rotating digester (see the Figure 24 B) with capacity of 1 kg and it was used for crushed chip and for fiberized pulp. Rotating mechanism in digester provided efficient chip mixing and heating system controlled the cooking temperature. The cooking temperature was 50 – 60 °C. The chip:water ratio was 1:4 and 1:10.

The treatment time in both digesters was 2 hours and the chip was pre-steamed before treatment for 10 min. The pre-steaming was used with purpose to remove the air and to improve the cooking liquid penetration into wood chip. Steaming was also used after treatment with target to stop the enzymatic reaction.

10.2 Refining

In the experimental part two different refiners were used. There was Bauer refiner and Valley Hollander.

10.2.1 Refining in Bauer refiner

The normal size chips and crushed chips were refined in standard single disk 8" Bauer refiner. The chip was refined in four stages to achieve desired freeness level. The amount of water for refining was 10 l/min for first refining stage and 5 l/min for remaining stages. The refining consistency was about 2%. Two methods

with drying in centrifuge dryer between refining stage and without drying were used. The pulp dry matter content after drying in centrifuge was measured after each refining stage. And the freeness level after each stage was measured after hot disintegration. The method without drying was used with target to reduce the time required for refining and to reduce the total time of experiment. The water amount for refining in both methods was the same. The specific energy consumption was measured in kWh/kg by energy meter, which was installed on Bauer refiner. The energy meter measured active power energy and energy of free running.

10.2.2 Refining in Valley Hollander

Valley Hollander was used to refine fiberized treated pulp. Refining was done according to standard (SCAN-C 25:76) for Kraft pulp. The pulp consistency was 2% (460 g o.d.p. and 23l of water) and the load weight was 8 kg and 7 kg. The total refining time was 90 min. The energy meter from Bauer refiner was used for energy consumption measurement.

10.3 Trials descriptions

Different methods for normal size chip and for crushed chip and on the other hand for fiberized pulp processing were used during experimental part. The information about main parameters of different trials is shown in Table 6.

Table 6. Trials parameters.

Raw material	Pre-steaming	Cooking	Steaming	Refiner	Digester
Normal size chip	10 min	2 hours	10 min	Bauer	Big
Crushed chip	10 min	2 hours	10 min	Bauer	Big
Crushed chip	10 min	2 hours	10 min	Bauer	Small
Fiberized pulp	-	2 hours	-	Valley Hollander	Small

The refining parameters for Bauer refiner and for Valley Hollander are presented in Appendix I, II, III, IV, V, and VI.

10.4 Morphological study of fibers

Straight after refining (with Valley beater) the fibers were examined with L&W Fiber Master STFI tester. The following data was collected: average fiber length, average width, length distribution and width distribution. Also fines content was determined. Fines by definition are materials that are small enough to pass a 200 mesh screen (with diameter approximately 76 micro-meters). But in the L&W fiber tester, fines are expressed as the percentage of material shorter than 0.2 mm in relation to the number of fibers longer than 0.2 mm. Two or three parallel tests were done in order to receive accurate data.

10.5 Hot disintegration of pulp

For the hot wet disintegration the L&W wet disintegrator was used. The equipments for the method includes vessel with certain size, measuring cylinder and thermometer. The pulp disintegration was done according to standard ISO 5263:1995 (E). The freeness measurements of disintegrated pulp by Canadian standard method were done.

10.6 Bulk density measurement

The bulk density was measured for normal size chip and crushed chip according to standard (SCAN-CM 46:92). The bulk density is important in the trade of wood chips on a volume basis, as well as in pulp production where it influences the inflow of wood to a digester or to a refiner and can show also the softness of wood chip. This in turn could make possible to see the effect of enzymes on wood.

The bulk density is calculated on the basis of the measured weight and the bulk volume (See the Equation 1):

$$X = \frac{10 \cdot w \cdot y}{h \cdot A} \quad (1)$$

Where: X is the bulk density, in kilograms oven-dry wood per cubic meter;

w is the mass of the sample, in grams;

y is the dry matter content, as a percentage;

h is the mean height of the chips in the tube, in centimeters;

A is the cross section area of the tube, in square centimeters.

11 Results and discussions

11.1 Liquid penetration into the wood and bulk density

Dry matter content of chip was measured before and after treatment and results of several measurements for normal size and crushed chip are presented in Table 7.

Table 7. Results of dry matter content of the chip.

Raw material	Treatment	Dry matter content, %
Normal size chip	Untreated	46
	Steamed	43.4
	Cooked	31.2
	Steamed-cooked-steamed	32.7
Crushed chip	Untreated	46.4
	Steamed	42.3
	Cooked	31.0
	Steamed-cooked-steamed	31.6

The results show how the treatment and type of chip influence on liquid penetration inside of wood. We can see that for crushed chip dry matter content decreased more than for normal size chip. It is because the liquid penetration is better.

The results of bulk density measurements for normal size chip and for crushed chip are presented in Table 8.

The method did not show high reliability because repeatability was poor. As we can see from Table 8 the bulk density value was increased by enzymatic treatment for both types of chip. According to results it can be assumed that mixture of enzymes has softening effect on wood chip, and in case of better impregnation for crushed chip the softening effect is higher.

Table 8. Results of bulk density measurements.

Raw material	Treatment	Bulk density, kg/m^3
Normal size chip	Untreated	147.4
	Cooked with water	151.8
	Cooked with Mix at 2.5 kg/t dosage	153.5
Crushed chip	Untreated	136.3
	Cooked with water	138.1
	Cooked with Mix at 2.5 kg/t dosage	154.7

11.2 Results for normal size chip and for crushed chip

The several variations of experiments in order to estimate the method reliability were done. The results of first set of experiments are showed on Figure 25. It was done in big digester. It can be seen the differences in energy consumptions for refining of chips with different treatment. The line 1 shows the energy consumption for normal size chip refining. The chip was steamed with direct steam for 10 min. The line 2 shows the energy consumption which is required for the refining of crushed chip, which was steamed for 10 min. The energy consumptions for refining of chips which were steamed and then cooked in a big digester under the conditions, which is described above (Table 6), are showed by lines 3 and 4. The line 4 shows the energy consumption for second time steamed chip.

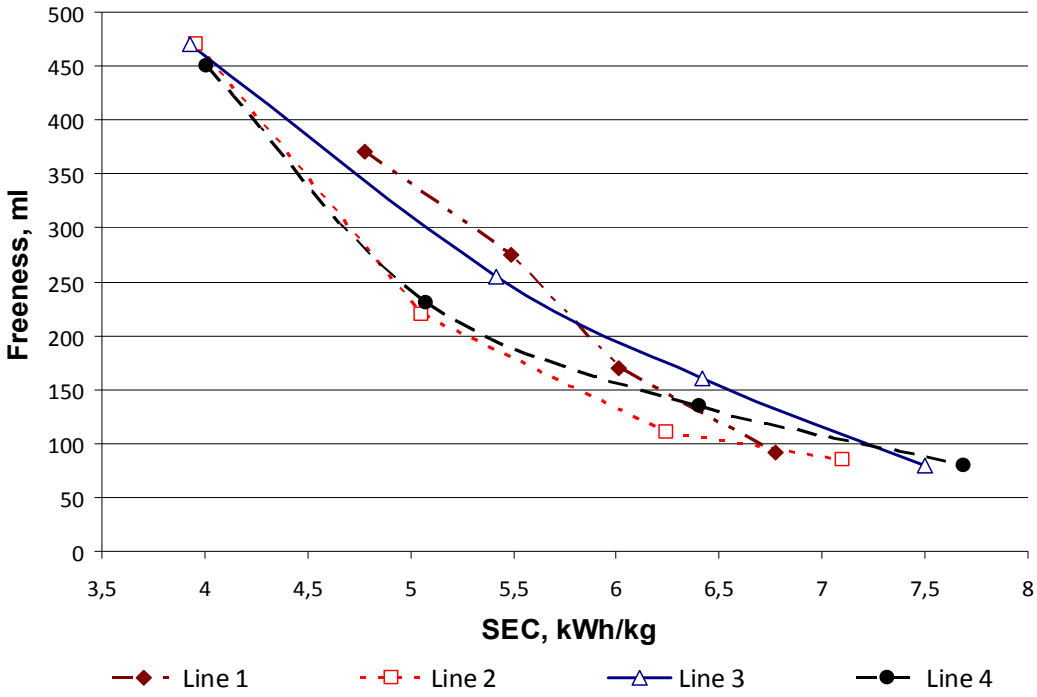

Figure 25. The relationship between specific energy consumption and freeness level (♦ - line 1 – steamed normal size chip; □ – line 2 – steamed crushed chip; Δ - line 3 – cooked, steamed crushed chip; ● - line 4 – cooked, 2 times steamed crushed chip).

On the Figure 25 we cannot see clear differences between the lines, especially at the end of refining. Therefore it questioned the reliability of the method. Because the energy consumption for crushed chip, which was steamed once, too much differ from energy consumption for refining of steamed and cooked chip. At the same moment when two times steamed and cooked chip refining consumed almost the same amount of energy as one time steamed crushed chip.

In second set of experiment the mixture of enzymes was used. The mixture of enzymes was taken with dosage 2.5 kg/t. The results of experiment are showed on Figure 26.

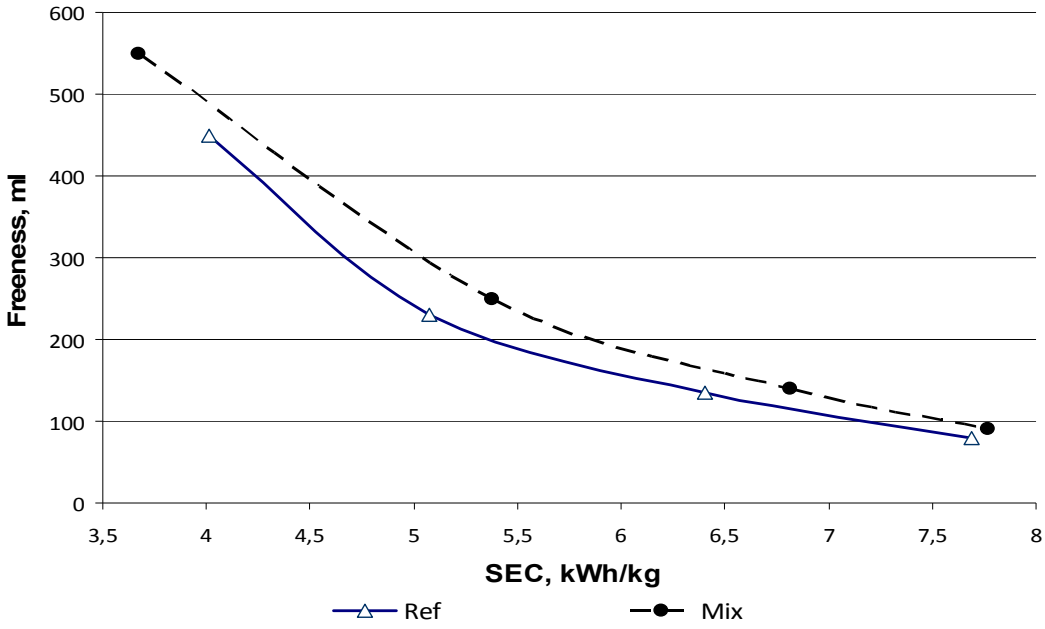

Figure 26. The relationship between specific energy consumption and freeness level (Δ - Ref – untreated chip; ● - Mix – treated with mixture of enzymes (2.5 kg/t)). The chip was pretreated in big digester and dried between refining stages.

As we can see on the Figure 26 the treated chip requires more energy for refining than untreated chip. This contradicts with previous researches.

Higher enzyme dosage

The next Figure 21 shows the measured specific energy consumption for pulp refining, which was not dried between refining stages. The lines, the same as on the previous figure, show the relation between specific energy consumption and freeness level for untreated and treated with enzymes chip. The mixture of enzymes was used with the higher dosage, 5 kg/t.

On the Figure 27 we can see that enzymatic treatment has a decreasing effect on energy consumption, when the dosage is higher than previously.

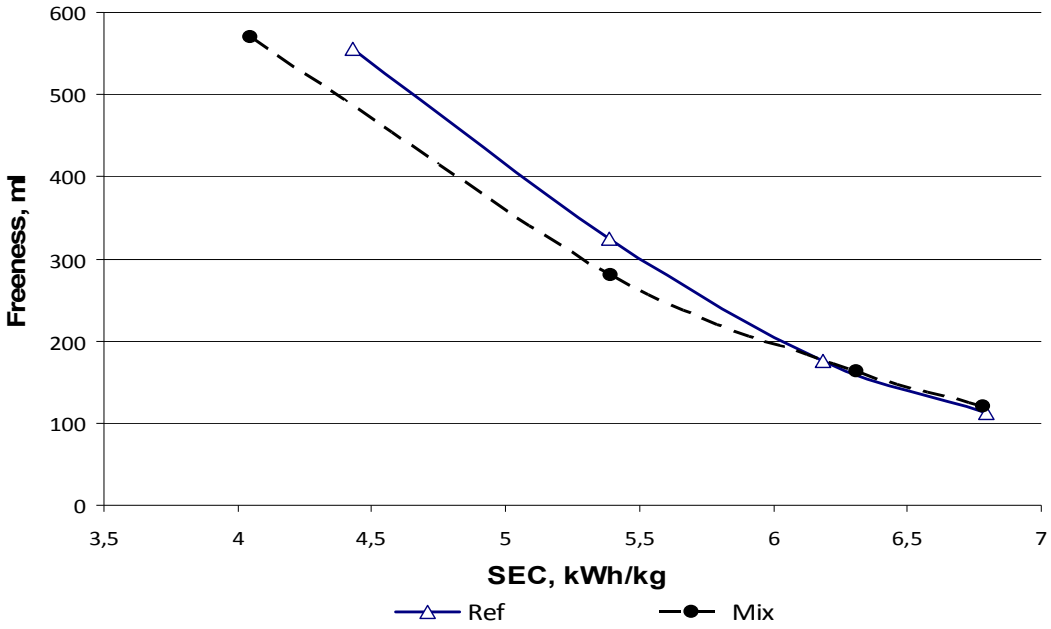

Figure 27. The relationship between specific energy consumption and freeness level (Δ - Ref–untreated chip; ● - Mix–treated with mixture of enzymes (5 kg/t)). The chip was pretreated in big digester and not dried between refining stages.

Small rotating digester

There was also thought that the chip mixing during the cooking in big digester is not sufficient and therefore with the purpose to improve it the small rotating digester was used. The chip was treated with the same conditions as in big digester. The effect of the cooker type on the specific energy consumption for refining is showed on the Figure 28. As we can see the chip took more energy for refining after enzymatic treatment. This result cannot be compared with results of treatment in big digester and gives the possibility to conclude that the method is not sufficient and not reliable for estimation of enzymatic effect on specific energy reduction.

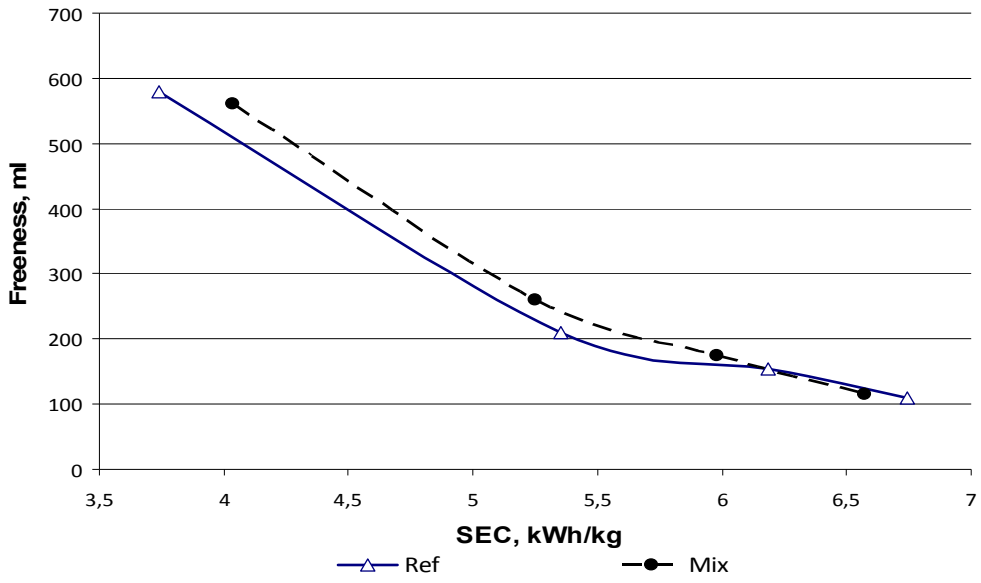

Figure 28. The relationship between specific energy consumption and freeness level (Δ - Ref–untreated chip; ● - Mix–treated with mixture of enzymes (5 kg/t)). The chip was pretreated in small digester and not dried between refining stages.

11.3 Results for fiberized pulp

In this method fiberized pulp, instead of chip, and the small digester for the cooking was used. This is because it was not possible to use the digester with continuous circulation system for the material in pulp form. The pulp:water ratio was 1:4 and 1:10. This is because the contact area is increased and pulp takes water better then unrefined chip. The treated coarse pulp was left for 8 hours at room temperature with purpose to increase the reaction time of enzymes with wood components.

Pulp:water ratio 1:4

The first results were taken with the pulp:water ratio = 1:4. Several enzymes were used in experiments. The enzyme dosage was 5 kg/t, the same in all experiments. On the Figure 29 the differences in energy consumption between untreated and treated with pectinase pulp are showed.

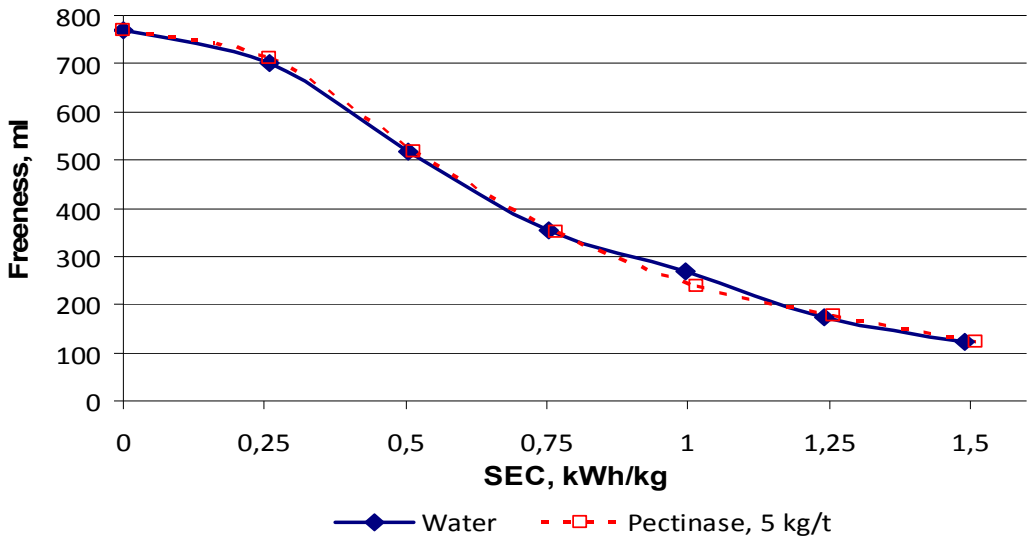

Figure 29. The relationship between specific energy consumption and freeness level for untreated and treated 2 hours by pectinase (5 kg/t) fiberized pulp. (Valley refiner)

As we can see on this figure the pectinase does not have any visible effect on decrease of energy consumption. The effect of mixture of enzymes was also measured by this method. The mixture also does not show the effect on energy consumption. The results of measurements can be seen in Appendix V.

Endoglucanase treatment with dosages 5 kg/t and 10 kg/t had a small decrease in energy consumption in early refining, figures 30 and 31.

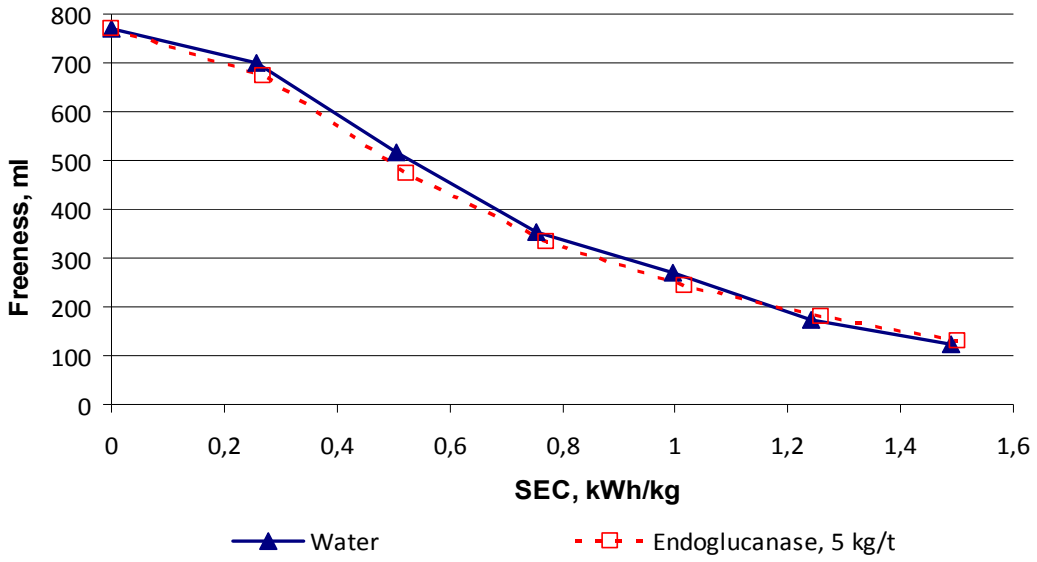

Figure 30. The relationship between specific energy consumption and freeness level for untreated and treated 2 hours by endoglucanse (5 kg/t) fiberized pulp. (Valley refiner)

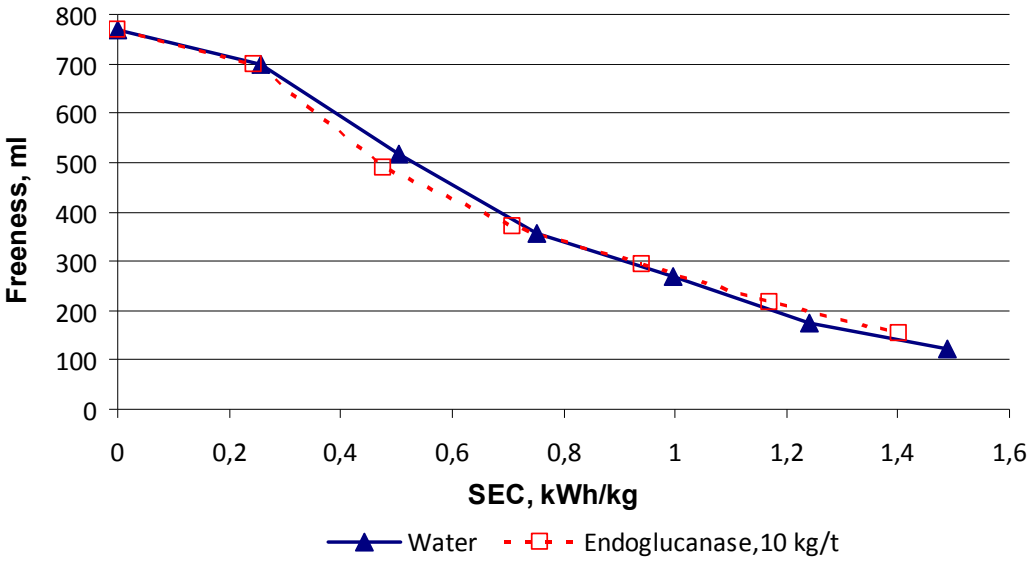

Figure 31. The relationship between specific energy consumption and freeness level for untreated and treated 2 hours by endoglucanase (10 kg/t) fiberized pulp. (Valley refiner)

It can be assumed, that the enzymes cannot penetrate deep in wood and soft reaction takes place at wood surface, thereby in start of refining it requires less energy.

Pulp:water ratio 1:10

The next part of research was based on treatment of the fiberized pulp with pulp:water ratio = 1:10. The coarse pulp was also hold for 8 hours after the 2 hours treatment at 50°C and then was refined in Valley refiner with the same conditions as in the previous set of experiments.

The action of mixture of enzymes with the effect of endoglucanase treatment was compared during the experiments. Different dosages of enzymes (1.5, 2.5, 5, 10 and 20 kg/t) were used.

As we can see from the Figure 32, both enzymes showed the effect in energy consumption decrease, but we can see that treated with endoglucanase pulp was refined faster during all refining process, at the same time when the mixture of enzymes decrease the energy consumption just at the end of refining. So at low CSF level (CSF < 200) both treatments had about the same SEC reduction (about 13%).

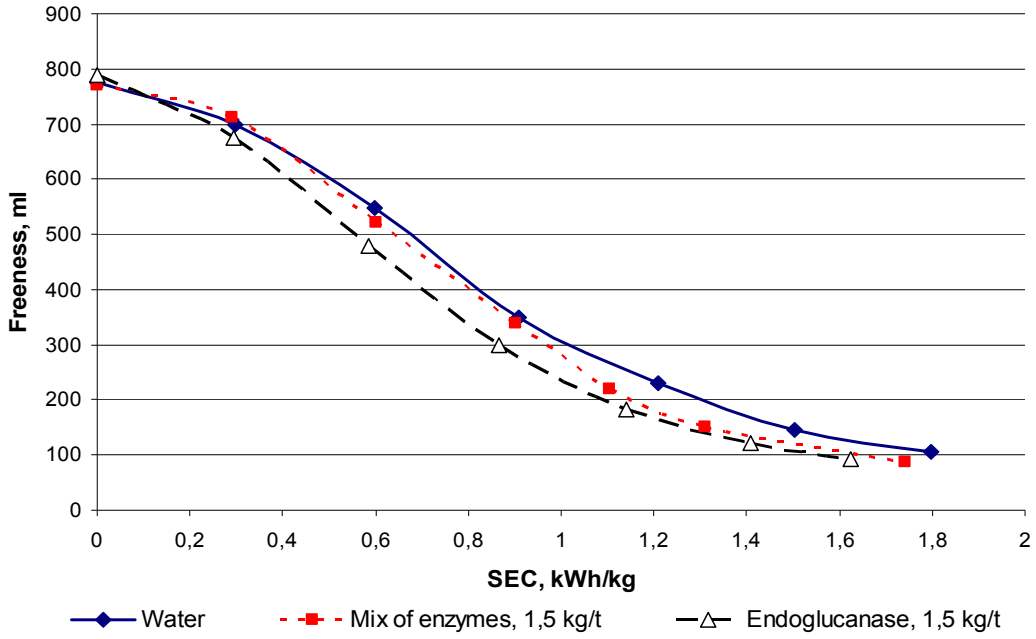

Figure 32. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (1.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

Taking into account that mixture of enzymes started to work when the pulp was already refined till the 350 ml of freeness level, it can be assumed that this enzymes blend works not on the fiber surface, but inside of them.

The further analyze was based on comparison of effect of enzymes dosage on SEC. Figures 33, 34, 35 and 36 show that mixture of enzymes stop to work at high dosages level.

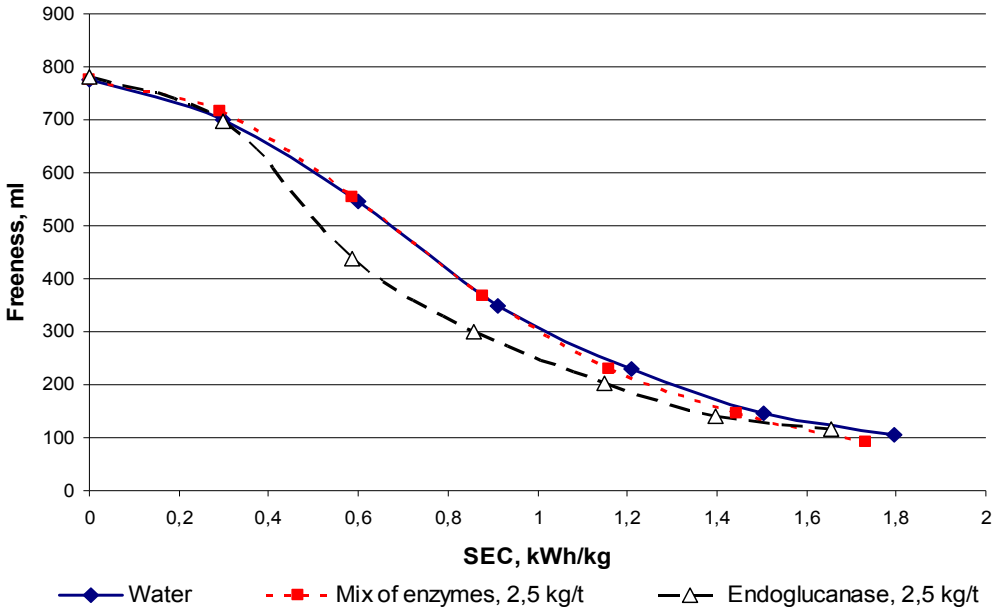

Figure 33. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (2.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

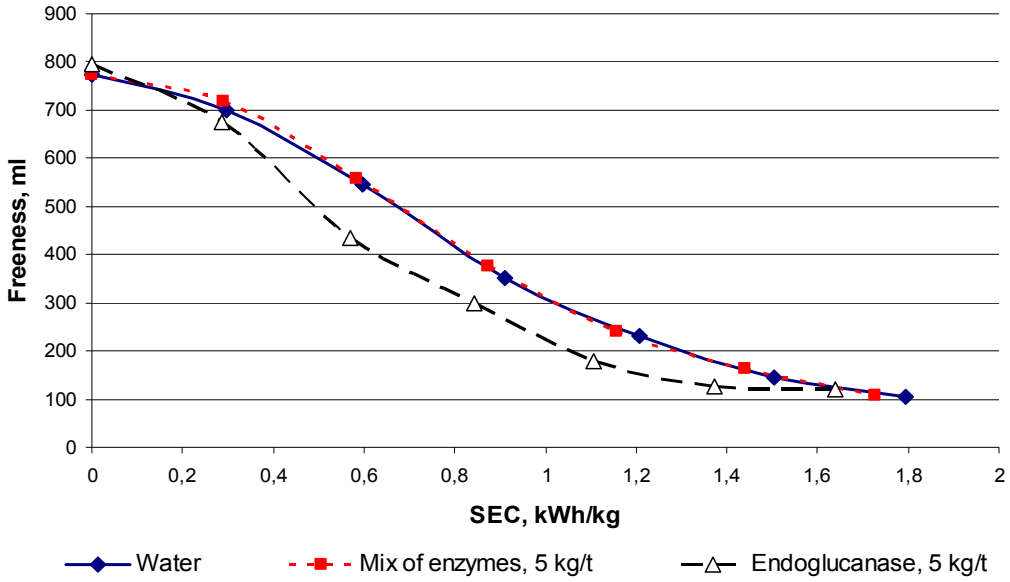

Figure 34. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

The endoglucanase shows on Figure 33 and Figure 34 high effectiveness in SEC reduction. But the treatment with dosages at 10 and 20 kg/t (See the Figure 35, 36) did not show further raise of effect on SEC reduction. Even the treatment with 10

kg/t dosage of endoglucanase showed less effectiveness than treatment with 1.5, 2.5 and 5 kg/t dosages.

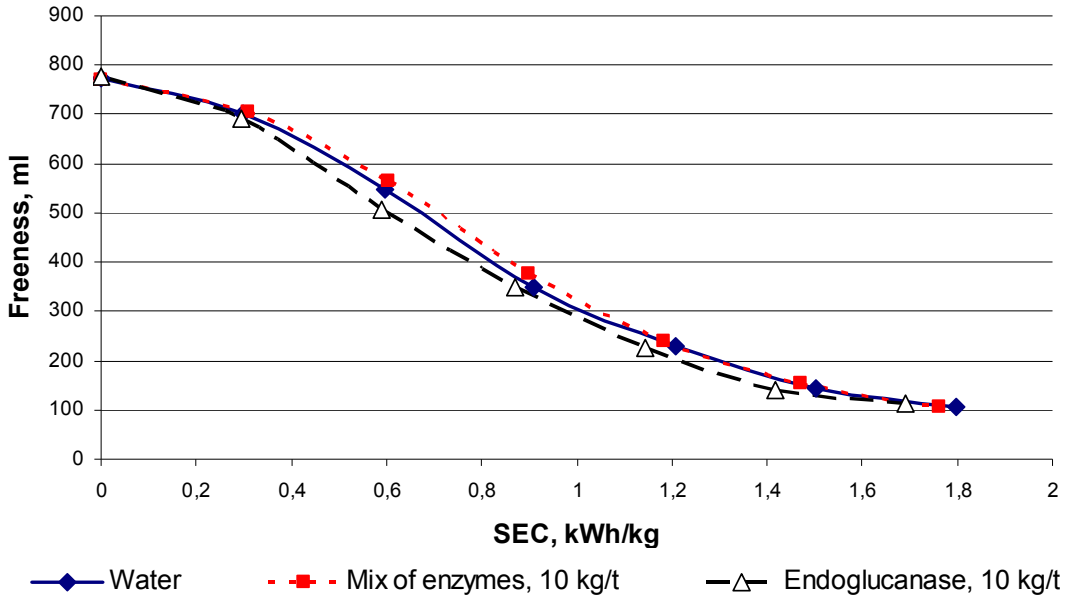

Figure 35. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (10 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

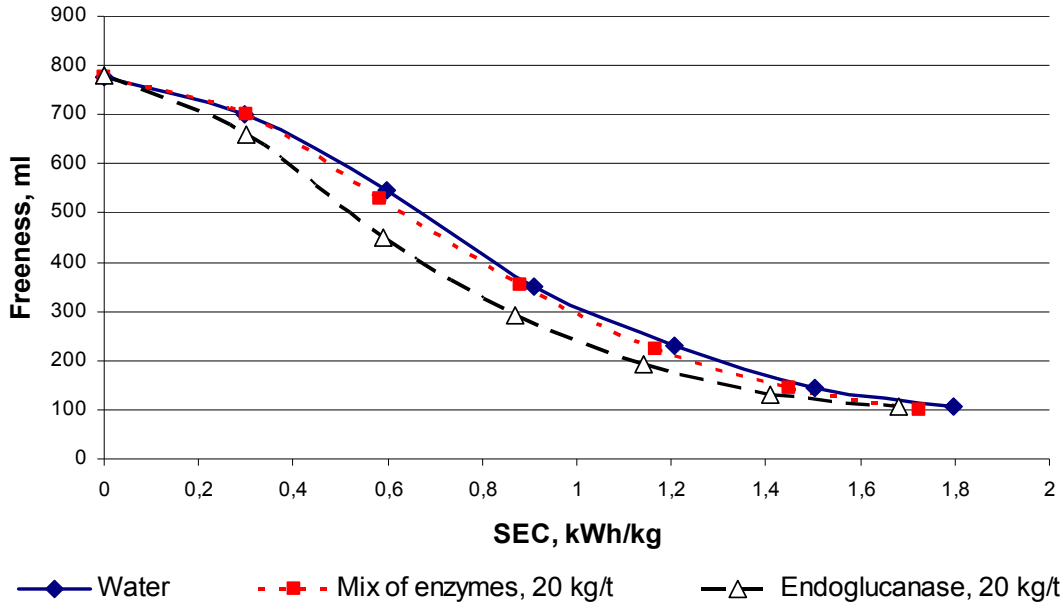

Figure 36. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (20 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

The energy consumption for coarse pulp, which was hold after the treatment long time (66 hours) at room temperature, was measured. The endoglucanase action and mixture of enzymes action were compared. The relationship between SEC and CSF freeness level are presented on Figure 37.

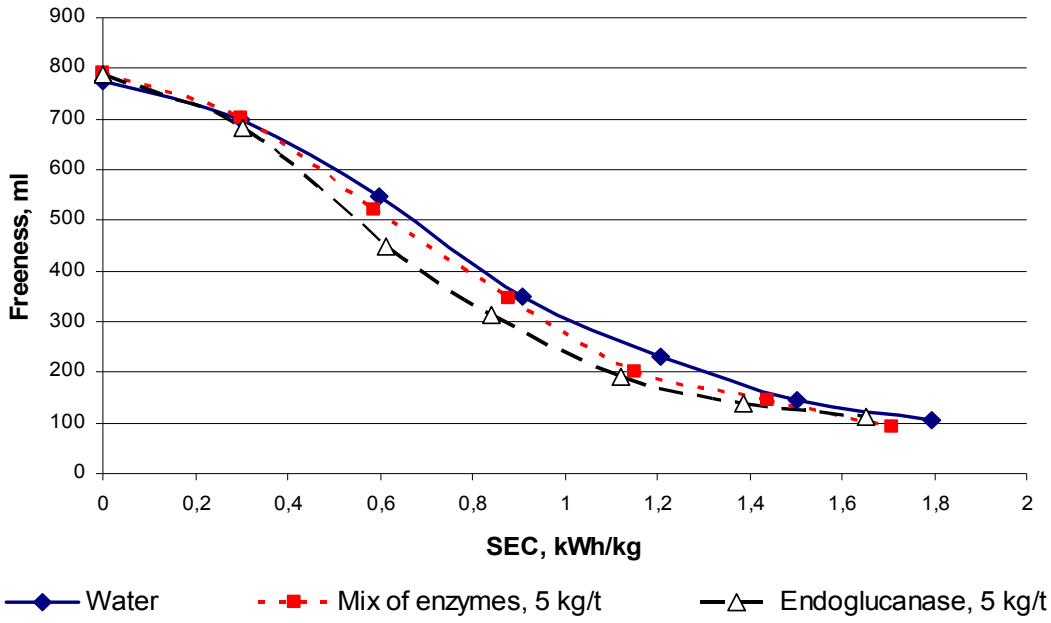

Figure 37. The relationship between specific energy consumption and freeness level for untreated and treated fiberized pulp (5 kg/t enzyme dosage, treatment time 66 hours).

As we can see from the Figure 41 endoglucanase had more significant effect in energy decrease than mixture of enzymes at long time treatment. But the mixture of enzymes showed again the most significant decrease at the end of refining. This phenomenon can prove the assumption that this blend of enzymes works into the inner structure of cell wall. As well known the S2 and S1 layer of wood fibers contains cellulose and hemicelluloses mostly. Therefore in this case can be assumed that blend of enzymes compounds mostly oriented on cellulose and hemicelluloses destruction.

11.4 Morphological analyze of pulp

11.4.1 Relation between fines content and specific energy consumption

The morphological analyze of pulp was oriented on determination of fines content in pulp samples, which were taken after each 15 min during the refining. It was assumed that after the enzymatic treatment the fibers could be softer, weaker and more capability to refining. From enzyme treated fibers the detachment of small fiber parts could be easier than from untreated fibers. Thereby the fine content of pulp can be used as indirect factor in evaluation of enzymatic effect.

On the Figures 38, 39, 40 and 41 the results of measurements of fine content of untreated and treated with different concentration of enzymes pulps are presented.

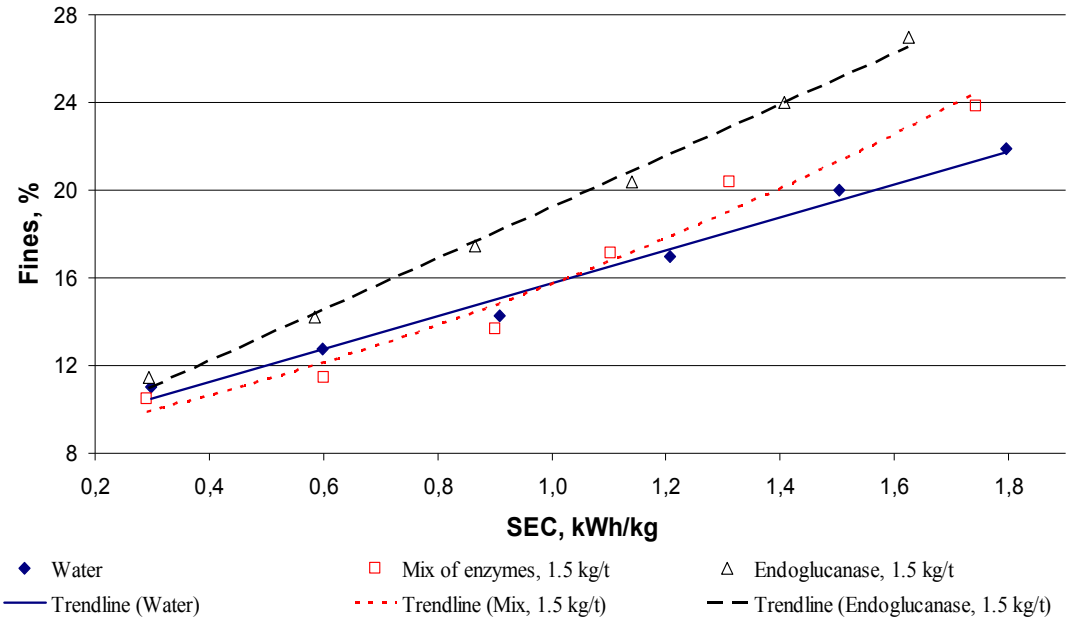

Figure 38. The relationship between fine content and SEC for refining of untreated and treated fiberized pulp (1.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

The fine content of pulps correlates with the specific energy consumption. The pulps after endoglucanase treatment have higher fines content than the untreated pulps. The highest dosage of endoglucanse (5 kg/t) also showed the highest fine content (See the Figure 44).

Figure 42 shows that the mixture of enzymes had small influence on fine content of pulp with the treatment at low dosage (1.5 kg/t). Especially the raise in fine content with the mixture of enzymes was noticed after 1.1 kWh/kg SEC level.

Thereby it can prove the assumption enzymes blend activity not on the surface, but inside of fibers.

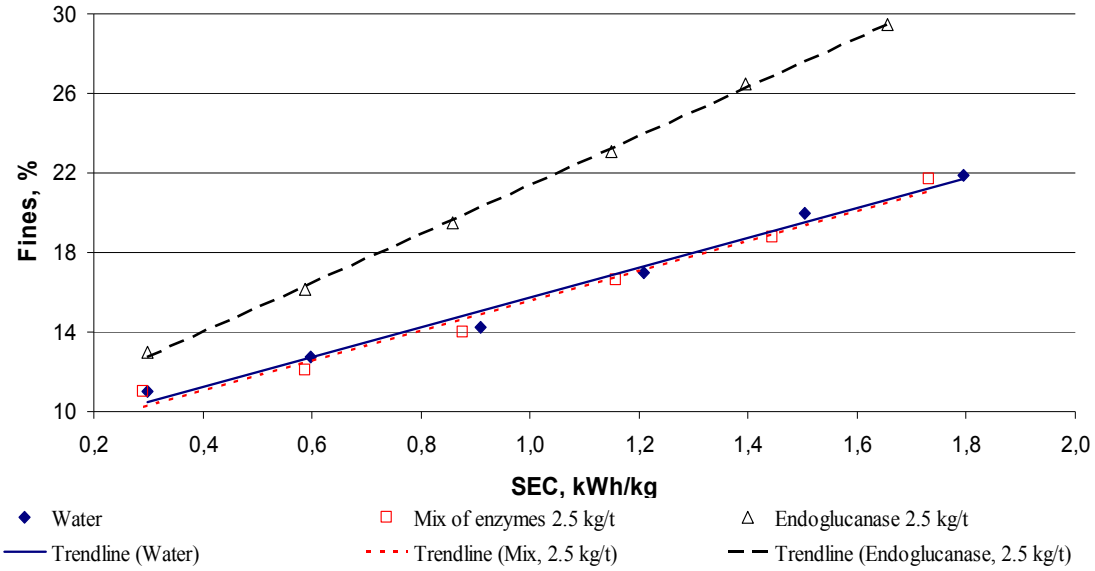

Figure 39. The relationship between fine content and SEC for refining of untreated and treated fiberized pulp (2.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

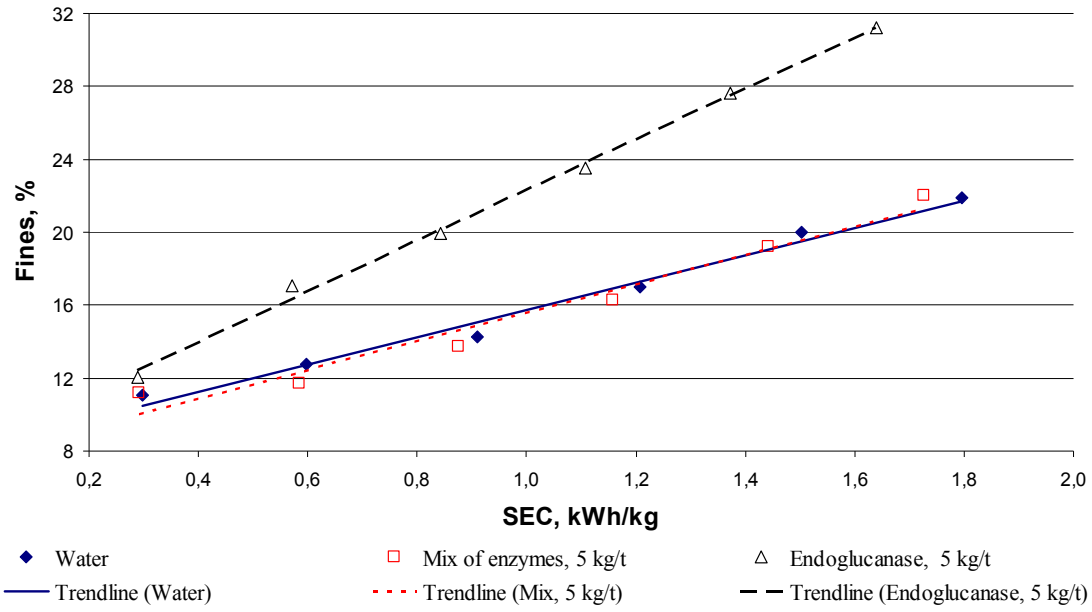

Figure 40. The relationship between fine content and SEC for refining of untreated and treated fiberized pulp (5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

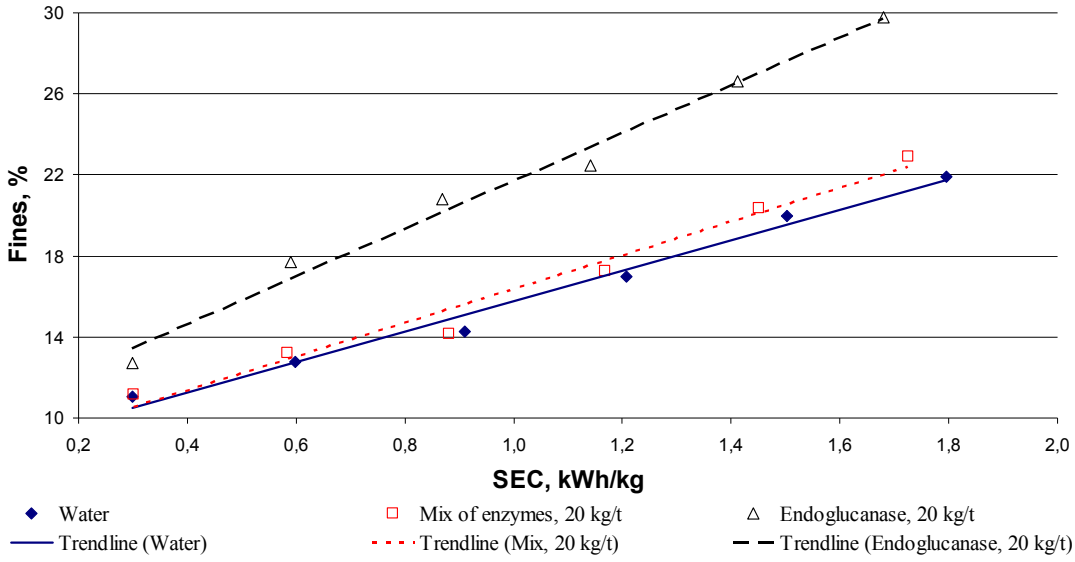

Figure 41. The relationship between fine content and SEC for refining of untreated and treated fiberized pulp (20 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

On the Figure 42 the results of refining the pulp, which was treated with enzymatic solution and hold after for 66 hours at room temperature, are presented. It can be seen that endoglucanase has strong effect on fine generation during the refining. The treatment by mixture of enzymes with dosage 5 kg/t does not show so significant effect on fine generation. But the same raise of fine content after 1.1 kWh/kg SEC level can be also noticed.

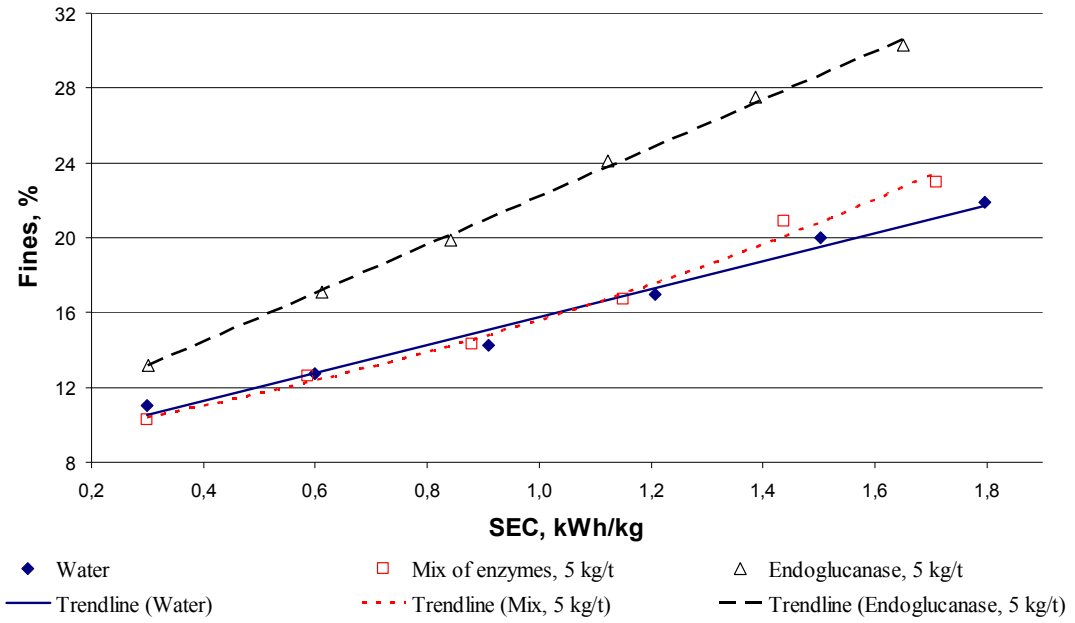

Figure 42. The relationship between fine content and SEC for refining of untreated and treated fiberized pulp (5 kg/t enzyme dosage, treatment time 66 hours).

11.4.2 Relation between fiber length and specific energy consumption

The fiber length of each pulp samples was measured during the experiments. The relationship between fiber length and SEC are presented on Figures 43, 44, and 45. The relationships between fiber length and SEC for other dosages treatment are presented in Appendix VII.

All figures show that endoglucanase treatments have influence on fiber length, and treatment with higher dosage more decreases the fiber length. The treatment by mixture of enzymes did not show strong affect on fiber length. Only at 1.1 kWh/kg of SEC level some small decrease can be noticed. But the higher dosage of mixture of enzymes does not have any effect on fiber length.

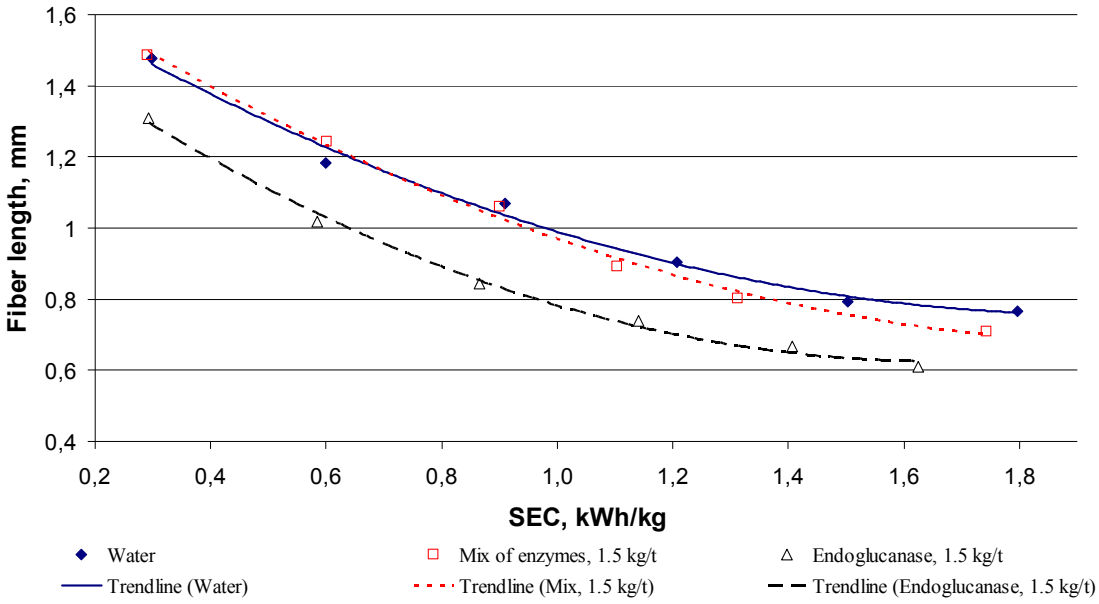

Figure 43. The relationship between fiber length and SEC for refining of untreated and treated fiberized pulp (1.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

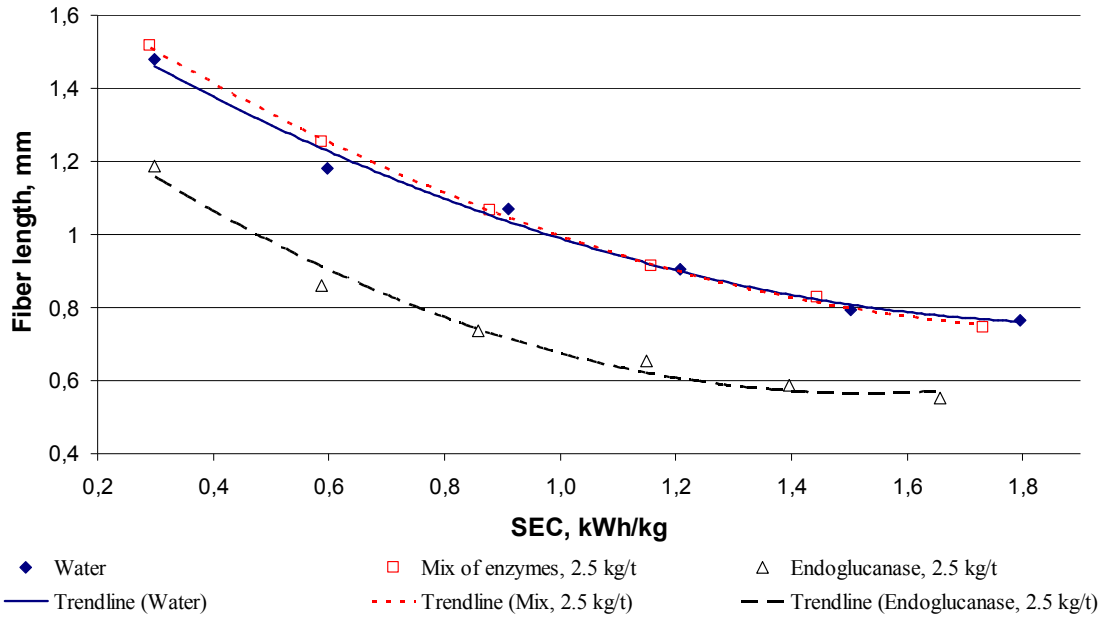

Figure 44. The relationship between fiber length and SEC for refining of untreated and treated fiberized pulp (2.5 kg/t enzyme dosage, treatment time 2+8 hours). (Valley refiner)

Results of long time endoglucanase treatment (Figure 45) show that time of treatment does not have effect on fiber length and it is the same for short time treatment. Long time treatment by mixture of enzymes has the same effect as 8 hours treatment at 1.5 kg/t dosage.

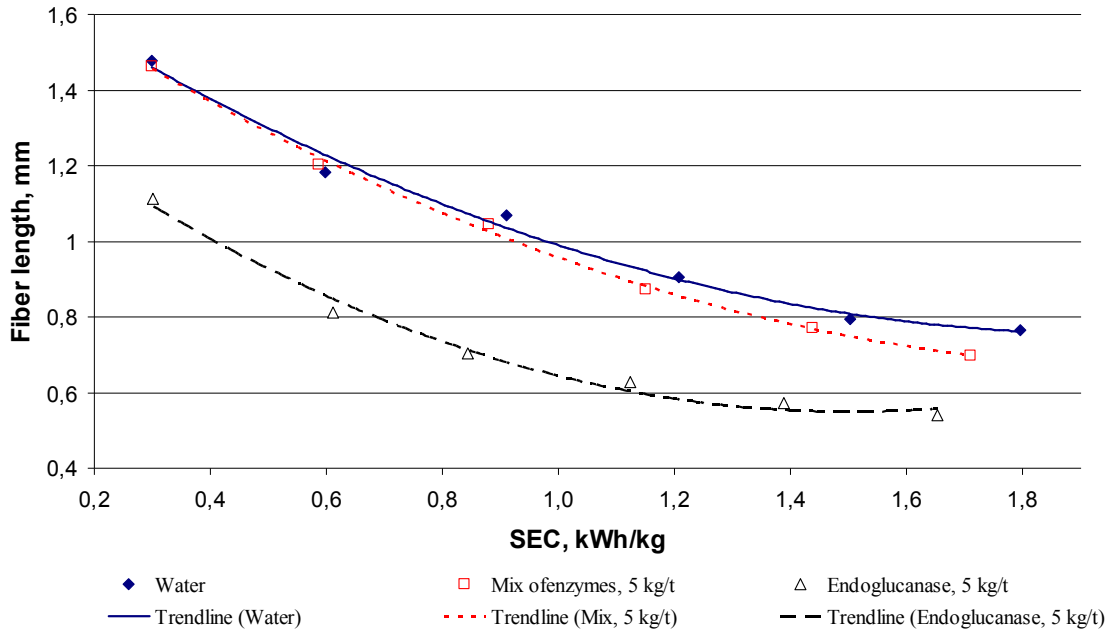

Figure 45. The relationship between fiber length and SEC for refining of untreated and treated fiberized pulp (5 kg/t enzyme dosage, treatment time 66 hours).

11.5 Results of knowledge discovery

The knowledge discovery in databases was done with target to find out possible mixtures of enzymes or some additives to enzymes, which can improve the enzymatic action and thereby increase the energy saving in TMP process. The interactive software "Arrowsmith" was used. The discovery process in this software is based on search in articles in biomedical literature and different databases (e. g. "Medline", "Science Direct", "Springer Link" and so on). In the Table 9 can be seen several examples of the combinations of key words, which were studied.

Table 9. Examples of used combinations of key-words.

List A	List C
Enzyme	Wood softening
Enzyme	Lignin degradation
Enzyme	Thermomechanical pulp
Wood softening	Cellulase
Wood softening	Pectinase
Wood softening	Laccase
Cellulase	Pectinase
Cellulase	Xylanase
Cellulase	Laccase

The B-list, made by "Arrowsmith" software, contains title words and phrases (terms) that appeared in both the A and the C literature. The articles related to found words were analyzed. After that it was possible to make several assumptions concerning the enzymatic effect on energy consumption.

Pectinase + Xylanase + Cellulase

Based on literature articles it was assumed that pectinase, xylanase and cellulase mixture should show the good results in energy savings. It was noticed that pectinase with cellulase works together and show high activity in hydrolyzing of wood polysaccharides./69/ The blend of cellulase and xylanase also showed enhanced action on wood. /71/ Thereby the coactions of these enzymes can be effective.

Laccase +Xylanase + Cellulase

Laccase can work in tandem with xylanase and also possible to add cellulase to achieve good results in wood softening. Laccase and xylanase have oriented on

lignin and hemicelluloses respectively, but both of them showed good results in complexes with cellulase. /87, 88/

Polygalacturonase

In previous study was reported about significant effect of bisulfite-stable polygalacturonase on cherry wood softening by fungi. /89/ It was assumed, that would be interesting to check the effect of polygalacturonase addition on spruce wood softening.

Surfactants

The surfactants showed the enhancement of enzymatic activity on wood components. /61, 63, 90/ Therefore it was concluded, that addition of surfactants during the enzymatic treatment of wood chip, can also have positive effect on SEC decreasing of chip refining.

11.6 Reliability of the results

The bulk density measurements of one chip sample were done with 3-4 repeat and these results were not reliable. The results were depended on chip sample and chip dimensions, which cannot be the same in all experiments.

The refining experiments with chip in Bauer refiner and with fiberized pulp in Valley Hollander were done once for each dosage of enzymes. The experiment was repeated when the results were completely incomparable or experimental technique has been violated. Tests for the reference points were 2-3 times done. Therefore the results of SEC measurements from refining experiments can be taken as relatively reliable results.

The results of fiber length and fines content measurements were done 3-4 times and average values with standard errors of the mean were calculated. Thereby these results can be considered as reliable.

12 Summary

In this study, we have examined the possibility of using pectinase, endoglucanase and mixture of enzymes to decrease SEC of refining. The main objective was to find out reliable method for measuring the effect of enzymatic treatment on SEC of wood refining. Our assumption of enzymes' effect on wood

properties was supported by the study of previous researches and by results of previous studies.

In this thesis, we reviewed the composition and main properties of wood components. The enzymes of lignocelluloses complex were reviewed and their action on wood structure and wood components was presented. The information about enzymes application in mechanical process from previous research was estimated. The other conditions such as temperature, pH value and additives, have been described with purpose to find out suitable conditions for high enzymes activity.

During the experiments the bulk density and dry mater content of normal size chip and crushed chip were measured before and after treatment. The results of the measurements showed that steaming and cooking of chip decrease the dry mater content and increase the bulk density of the chip.

The measurements of SEC during the refining at "Bauer" laboratory disc refiner showed controversial results. The energy consumption for cooked and steamed crushed chip refining was higher than for refining of steamed crushed chip. Also the SEC of chip refining treated by mixture of enzymes was mainly higher than SEC for refining of untreated chip. At 5 kg/t dosage of mixture of enzymes the SEC of refining in "Bauer" refiner was, however, less than SEC for untreated chip refining, but the results were not repeatable and were different at different sets.

The method of Kraft pulp refining, which was used during the refining of fiberized pulp in Valley refiner showed more stable and repeatable results of experiments. At pulp water:ratio = 1:4 the effect of enzymes at different dosages on SEC was poorly visible. Just high dosage (10 kg/t of endoglucanase) treatment has showed small effect on SEC decrease. The pulp:water ratio = 1:10 was more efficient for energy saving. At this ratio endoglucanase treatment showed on laboratory scale about 20% decrease of SEC at 2.5-5 kg/t dosage. The mixture of enzymes treatment at dosage 1.5 kg/t was most effective and showed about 15% of SEC decrease.

The morphological analysis of pulp during the refining in Valley refiner showed that fines content and fiber length depends on the effectiveness of enzymatic treatment. Thereby at highest SEC decrease after 5 kg/t endoglucanase treatment a significant increase of fines content and decrease of average fiber length were

noticed. Also the connection between SEC decrease after mixture of enzymes treatment and fiber length and fines content of pulp was visible.

The knowledge discovery system with target to find new mixtures of enzymes or other additives to enhance enzymes' activity was described in the literature part and was tested as part of the experimental part. The results of knowledge discovery were evaluated and discussed in the experimental part too.

13 Conclusions

The main target of this study was to develop a laboratory method for evaluation of enzymatic treatment's effect on SEC. The reliability of the method was checked by comparison of the results of the previous studies.

After the experiments it was concluded that accessibility of fiberized pulp to enzymatic treatment and liquid penetration without special impregnating equipment is higher than for normal size chip and for crushed chip. This is because the fiberized pulp has larger wood surface area and thereby the contact area between the enzymatic solution and wood is also larger.

The results of refining in “Bauer” refiner can be evaluated as not so reliable. The most probable reason for this was that this kind of refiner has many variables during the experiments. The most important were different refining time and refining consistency, which were dependent on the feeding capacity and it was difficult to control it well enough. The refining in Valley refiner was more stable and results of measurements were more reliable. Thereby it can be concluded that using of fiberized pulp and Valley refiner for evaluation of enzymes' effectiveness in SEC decrease is more suitable than normal size chip and crushed chip with “Bauer” refiner.

The endoglucanase treatment and mixture of enzymes treatment show different effects at different dosages. Also both of them did not work properly at very high dosages. This dependence between dosage and effectiveness can be related to the molecular weight of enzymes. Thereby it can be assumed that endoglucanase has lower MW than mixture of enzymes; and higher amount of short polypeptide chains of proteins can easier penetrate into the wood structure. It can be also confirmed by higher effectiveness of mixture of enzymes at low dosages. Also the more significant effect of mixture of enzymes treatment at the end of refining was noticed. It means

that refining of inner areas of wood was easier than refining of surface areas. Based on this results it can be assumed that mixture of enzymes contains such enzymes which work into the inner structure of cell wall. There are S1 and S2 layers, which contain cellulose and hemicelluloses mostly. Thereby it can be assumed that blend of enzymes can contain cellulases and hemicellulases.

Pectinase treatment did not show effect on SEC of refining. Probably, not efficient impregnation of enzymatic solution into the wood structure and impossibility to achieve pectin substrate was the main reason for this.

The knowledge discovery in databases with special interactive device "Arrowsmith" was used during the work. The most important assumptions were to use the surfactants for enzymes' activity enhancement and to try mixture of cellulase, pectinase and xylanase as possible effective blend of enzymes. These assumptions should be tested in future.

14 Recommendations for the future

The developed laboratory scale method for evaluation of enzymatic effect on specific energy consumption during the refining process can be used in future to find out effective enzymes and mixtures of enzymes. During the experiments the one of the most important problem was enzymes penetration into the wood structure. Thereby it can be recommended to use special impregnators for normal size and crushed chip to increase the enzymatic liquid penetration.

During the experimental part was not possible to check the effect of all enzymes on specific energy consumption. It can be recommended to continue the research in this area and to check other enzymes.

The knowledge discovery system can be also recommended as effective approach to find new ideas and solutions for enzymes operation and application.

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LIST OF APPENDICES

.

APPENDIX I (1/3)

Control data from Bauer refiner with chip drying between refining stages

Table I. Refining parameters and characteristics for pre steamed normal size chip.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	1000	43	25	370	4,7778	3,3367	344	29,1	4,7778
2	940	6	10	275	0,6698	0,508	48	31,2	0,7125
3	1090	5,5	3	170	0,5671	0,4286	35	29,4	0,5203
4	1030	7	1	92	0,7873	0,5583	20	25,8	0,7644

Table II. Refining parameters and characteristics for pre steamed crushed chip.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	1000	38,5	35	470	3,9564	2,8086	385	27,4	3,9564
2	1000	8	10	220	1,098	0,7658	48	28,6	1,098
3	1000	9,25	3	110	1,1911	0,8296	57	23,4	1,1911
4	1000	5,58	1	85	0,8559	0,61132	31	22	0,8559

Table III. Refining parameters and characteristics for pre steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	836	26,75	35	470	3,2812	2,2496	260	35,2	3,9249
2	733	9,67	10	255	1,0927	0,8101	48	26,8	1,4907
3	758	5,17	3	160	0,7593	0,5428	25	25,4	1,0017
4	807	6,42	1	80	0,8732	0,605	32	26,7	1,0820

APPENDIX I (2/3)

Table IV. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	1076	36,83	35	450	4,3154	2,9371	368	36,4	4,0106
2	994	8,5	10	230	1,0547	0,7346	48	30,4	1,0611
3	953	9,58	3	135	1,2672	0,8602	48	23	1,3297
4	915	8,42	1	80	1,1774	0,8198	42	23,1	1,2868

Table V. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	1154	38,33	35	450	4,3203	3,0586	380	35,7	3,7438
2	1051	12,83	10	230	1,4724	1,0566	64	28,9	1,401
3	883	7,17	3	140	0,9726	0,6594	35	24,5	1,1015
4	814	6,33	1	75	1,2181	0,8238	40	23,8	1,4964

Table VI. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by mixture of enzymes (dosage 2.5 kg/t).

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Energy consumption free running, kWh	Water, l	Dry Matter Content, %	SEC, kWh/kg
1	1121	44,33	35	550	4,1167	3,0964	390	37,5	3,6723
2	1005	16,67	10	250	1,7156	1,2559	83	31,2	1,7071
3	881	9,83	3	140	1,2672	0,866	48	24,7	1,4384
4	799	5,5	1	90	0,7609	0,5275	37	22,3	0,9523

APPENDIX II (1/2)

Control data from Bauer refiner without chip drying between refining stages

Table I. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1000	39,83	35	520	4,7202	380	4,7202
2	888	4,25	10	370	0,6784	21	0,763964
3	776	3,46	3	190	0,5145	17	0,663015
4	664	3,33	1	125	0,4129	16,5	0,621837

Table II. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1000	35	35	590	4,1359	350	4,1359
2	895	8,62	10	280	1,0369	43,1	1,158547
3	790	3,52	3	160	0,7319	17	0,926456
4	685	2,7	1	100	0,4127	13,5	0,602482

Table III. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1051	42,83	35	500	4,994	428	4,751665
2	946	5,57	10	270	0,972	28	1,027484
3	841	3,92	3	160	0,6719	20	0,79893
4	736	2,33	1	130	0,3666	12	0,498098

APPENDIX II (2/2)

Table IV. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1000	34,33	35	640	3,3389	342	3,3389
2	899	12,5	10	290	1,4956	63	1,663626
3	798	4,51	3	165	0,8263	23	1,035464
4	698	2	1	110	0,313	10	0,448424

APPENDIX III (1/1)

Control data from Bauer refiner without chip drying between refining stages. Chip was treated in rotating digester.

Table I. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by water.

Refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1000	35,4	35	580	3,7383	354	3,7383
2	887	9,4	10	210	1,4297	47	1,611838
3	774	3,73	3	155	0,6448	18,65	0,833075
4	661	2,42	1	110	0,3709	12,1	0,56112

Table II. Refining parameters and characteristics for two times steamed crushed chip, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Number of refining stage	Weight, g	Ref. time, min	Gap distance, mm	CSF level, ml	Energy consumption, active power, kWh	Water, l	SEC, kWh/kg
1	1000	34,92	35	560	4,0332	349,2	4,0332
2	889	5,92	10	260	1,0795	29,6	1,214286
3	778	4,37	3	175	0,5694	21,85	0,731877
4	668	2,53	1	115	0,3974	12,65	0,59491

APPENDIX IV (1/2)

Control data from Valley refiner with coarse pulp:water ratio during the treatment = 1:4

Table I. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by water.

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,575	0
15	700	0,148	0,569	0,257
30	518	0,1404	0,563	0,247
45	355	0,1397	0,557	0,248
60	270	0,1363	0,551	0,245
75	175	0,1347	0,545	0,244
90	124	0,1349	0,539	0,248

Table II. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Pectinase (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,575	0
15	713	0,1496	0,569	0,260
30	519	0,1448	0,563	0,254
45	350	0,1417	0,557	0,252
60	240	0,1387	0,551	0,249
75	178	0,1343	0,545	0,244
90	124	0,1376	0,539	0,252

Table III. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,575	0
15	710	0,1488	0,569	0,259
30	505	0,1481	0,563	0,260
45	355	0,1511	0,557	0,268
60	253	0,1374	0,551	0,247
75	168	0,1334	0,545	0,242
90	115	0,1399	0,539	0,257

Table IV. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,575	0
15	675	0,1543	0,569	0,268
30	472	0,1443	0,563	0,254
45	335	0,1406	0,557	0,250
60	242	0,1373	0,551	0,246
75	180	0,1334	0,545	0,242
90	130	0,1312	0,539	0,241

Table V. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Cellulase (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	788	0	0,569	0
15	715	0,1477	0,563	0,260
30	535	0,1353	0,557	0,240
45	400	0,1392	0,551	0,250
60	280	0,1293	0,545	0,235
75	195	0,1333	0,539	0,245
90	143	0,1265	0,533	0,235

Table VI. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 10 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,572	0
15	700	0,1401	0,566	0,245
30	490	0,1311	0,56	0,232
45	370	0,1302	0,554	0,2325
60	295	0,1276	0,548	0,230
75	215	0,1269	0,542	0,232
90	155	0,1248	0,536	0,230

Control data from Valley refiner with coarse pulp:water ratio during the treatment = 1:10

Table I. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by water.

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	775	0	0,46	0
15	700	0,1373	0,454	0,299
30	547	0,1361	0,448	0,300
45	350	0,1395	0,442	0,311
60	230	0,1318	0,436	0,298
75	145	0,1287	0,43	0,295
90	105	0,1259	0,424	0,293

Table II. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 10 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,46	0
15	705	0,142	0,454	0,307
30	565	0,134	0,448	0,295
45	375	0,1319	0,442	0,294
60	240	0,1264	0,436	0,286
75	155	0,1242	0,43	0,285
90	105	0,1254	0,424	0,292

Table III. Refining parameters and characteristics fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 10 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	778	0	0,46	0
15	690	0,136	0,454	0,296
30	505	0,1337	0,448	0,295
45	348	0,1253	0,442	0,280
60	227	0,1208	0,436	0,273
75	142	0,1193	0,43	0,274
90	112	0,1186	0,424	0,276

Table IV. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 20 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	780	0	0,46	0
15	660	0,1375	0,454	0,299
30	450	0,1318	0,448	0,290
45	293	0,1253	0,442	0,280
60	192	0,1207	0,436	0,273
75	132	0,1173	0,43	0,269
90	105	0,1158	0,424	0,269

Table V. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 20 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	775	0	0,46	0
15	700	0,1385	0,454	0,301
30	530	0,1288	0,448	0,284
45	355	0,1332	0,442	0,297
60	225	0,1265	0,436	0,286
75	145	0,1228	0,43	0,282
90	100	0,1181	0,424	0,275

Table VI. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 30 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	780	0	0,46	0
15	706	0,1347	0,454	0,293
30	466	0,1297	0,448	0,286
45	310	0,1224	0,442	0,273
60	185	0,1207	0,436	0,273
75	143	0,1134	0,43	0,260
90	118	0,1164	0,424	0,271

Table VII. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 2.5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	775	0	0,46	0
15	715	0,1337	0,454	0,291
30	553	0,1346	0,448	0,296
45	368	0,1301	0,442	0,290
60	231	0,1239	0,436	0,280
75	147	0,1249	0,43	0,286
90	92	0,1238	0,424	0,288

Table VIII. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 2.5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	780	0	0,46	0
15	697	0,1371	0,454	0,298
30	437	0,1313	0,448	0,289
45	301	0,121	0,442	0,270
60	202	0,1293	0,436	0,292
75	141	0,1072	0,43	0,246
90	115	0,1121	0,424	0,261

Table IX. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	773	0	0,46	0
15	717	0,1337	0,454	0,291
30	558	0,133	0,448	0,293
45	377	0,1302	0,442	0,291
60	241	0,1254	0,436	0,284
75	163	0,1235	0,43	0,283
90	108	0,1227	0,424	0,285

Table X. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	796	0	0,46	0
15	675	0,1325	0,454	0,288
30	435	0,1285	0,448	0,283
45	298	0,1217	0,442	0,272
60	179	0,1172	0,436	0,265
75	126	0,1155	0,43	0,265
90	120	0,1151	0,424	0,268

Table XI. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by mixture of enzymes (dosage 1.5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	770	0	0,46	0
15	712	0,1334	0,454	0,29
30	521	0,1411	0,448	0,311
45	338	0,1343	0,442	0,300
60	220	0,1301	0,436	0,294
75	150	0,0617	0,43	0,142
90	88	0,1852	0,424	0,430

Table XII. Refining parameters and characteristics for fiberized pulp, which were treated 2 hours by Endoglucanase (dosage 1.5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	790	0	0,46	0
15	675	0,1349	0,454	0,293
30	478	0,1324	0,448	0,292
45	300	0,1259	0,442	0,281
60	182	0,121	0,436	0,274
75	122	0,1165	0,43	0,267
90	93	0,0935	0,424	0,217

Control data from Valley refiner with coarse pulp:water ratio during the treatment = 1:10. The chip was hold for 66 hours after treatment.

Table I. Refining parameters and characteristics for fiberized pulp, which were treated 66 hours by mixture of enzymes (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	790	0	0,46	0
15	702	0,1376	0,454	0,299
30	520	0,1307	0,448	0,288
45	347	0,1312	0,442	0,293
60	200	0,1197	0,436	0,271
75	145	0,1253	0,43	0,287
90	92	0,1167	0,424	0,271

Table II. Refining parameters and characteristics for fiberized pulp, which were treated 66 hours by Endoglucanase (dosage 5 kg/t).

Time,min	Freeness,ml	Energy consumption, kWh	Pulp amount, g	SEC, kWh/kg
0	788	0	0,46	0
15	684	0,1386	0,454	0,301
30	450	0,1411	0,448	0,311
45	313	0,1033	0,442	0,231
60	190	0,1239	0,436	0,280
75	138	0,1153	0,43	0,264
90	111	0,1136	0,424	0,264

APPENDIX VII (1/3)

Fines content and fiber length of pulp samples which were taken during the refining in Valley refiner

Table I. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by water.

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	11,035±0,09	1,478±0,0035	0,298
30	12,756±0,14	1,182±0,0104	0,598
45	14,249±0,02	1,069±0,0056	0,910
60	16,981±0,11	0,905±0,09	1,208
75	19,998±0,03	0,794±0,0851	1,503
90	21,896±0,07	0,765±0,0377	1,796

Table II. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by mixture of enzymes (dosage 1.5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	10,5±0,001	1,4865±0,0038	0,290
30	11,45±0,13	1,243±0,053	0,601
45	13,7±0,231	1,06±0,0044	0,901
60	17,15±0,064	0,893±0,0546	1,103
75	20,4±0,019	0,8035±0,072	1,312
90	23,85±0,112	0,7105±0,0032	1,742

Table III. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by Endoglucanase (dosage 1.5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	11,45±0,14	1,3095±0,0075	0,293
30	14,2±0,032	1,018±0,0413	0,585
45	17,45±0,211	0,8445±0,009	0,866
60	20,4±0,724	0,738±0,0101	1,140
75	24±1,003	0,6665±0,0059	1,407
90	27±0,938	0,6105±0,0267	1,624

Table IV. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by mixture of enzymes (dosage 2.5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	11±0,0078	1,516±0,104	0,291
30	12,1±0,008	1,255±0,0331	0,587
45	14±0,958	1,066±0,0735	0,878
60	16,6±0,164	0,915±0,0648	1,158
75	18,8±0,526	0,829±0,0224	1,444
90	21,7±1,102	0,746±0,007	1,732

Table V. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by Endoglucanase (dosage 2.5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	13±0,329	1,188±0,312	0,298
30	16,15±0,662	0,86±0,009	0,587
45	19,5±0,583	0,738±0,1	0,857
60	23,1±0,172	0,653±0,0028	1,150
75	26,5±0,487	0,5885±0,0477	1,396
90	29,45±1,008	0,553±0,0153	1,656

Table VI. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by mixture of enzymes (dosage 5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	11,2±0,982	1,443±0,0736	0,291
30	11,7±0,322	1,228±0,0012	0,58
45	13,75±0,998	1,037±0,0883	0,874
60	16,3±1,005	0,904±0,0516	1,158
75	19,25±0,989	0,7955±0,0044	1,441
90	22±1,079	0,7165±0,0018	1,727

Table VII. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by Endoglucanase (dosage 5 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	12,05±0,673	1,1795±0,0743	0,288
30	17,05±0,992	0,845±0,0962	0,571
45	19,95±0,709	0,706±0,0581	0,843
60	23,55±0,534	0,6395±0,0656	1,108
75	27,6±1,351	0,5725±0,0367	1,373
90	31,25±2,003	0,529±0,0279	1,640

Table VIII. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by mixture of enzymes (dosage 20 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	11,167±0,256	1,450±0,0911	0,301
30	13,200±0,831	1,155±0,0638	0,585
45	14,167±0,448	1,020±0,0325	0,882
60	17,267±1,336	0,874±0,0645	1,168
75	20,367±0,012	0,775±0,0107	1,450
90	22,933±0,516	0,713±0,0332	1,725

Table IX. Fiber length and fines content of fiberized pulp samples, which were treated 2 hours by Endoglucanase (dosage 20 kg/t).

Time, min	Fines content, %	Average fiber length, mm	SEC, kWh/kg
15	12,7±0,998	1,13±0,0035	0,299
30	17,69±0,796	0,81±0,00708	0,589
45	20,83±0,945	0,701±0,016	0,869
60	22,46±0,567	0,635±0,0671	1,142
75	26,6±1,036	0,573±0,001	1,411
90	29,8±0,056	0,53±0,0835	1,680