Wood Sci. Technol. 24:79–101 (1990)

Wood Science and Technology

© Springer-Verlag 1990

Biotechnology in the pulp and paper industry

K.-E. L. Eriksson, Athens, GA, USA

Summary. Biotechnology implies the technical exploitation of biological processes. One of nature's most important biological processes is the degradation of wood and other lignocellulosic materials to carbon dioxide, water, and humic substances. Consequently, there should be possibilities to apply biotechnology to wood conversion. This article summarizes briefly the knowledge relating to the enzymic degradation of cellulose, hemicelluloses, and lignin. However, it is mainly focused upon biotechnological processes in commercial use or in various stages of development for the pulp and paper industry. Areas covered are ethanol and protein production, water purification, development of new bleaching techniques, microbial delignification (biopulping), and development of "biosensors" for analysis of pulp fiber surfaces.

Introduction

Biotechnology implies the technical exploitation of biological processes. This technology has received increasing attention during the past fifteen years because of its commercial potential in many fields. The pulp and paper industry continually tries to improve its processes and products. However, due to economical and technical constraints, new approaches to pulp and paper manufacture are rare. Biotechnology can, however, give rise to new possibilities.

In the forest product industries the raw material is wood. The foundation thus exists for also using biotechnology in these industries since one of nature's most important biological processes is the degradation of lignocellulosic materials such as wood and agricultural wastes to carbon dioxide, water, and humic substances. Consequently, there are many possibilities for applying biotechnology to wood conversion. The virtue of biotechnology lies in its potential to supply more specific reactions, to provide less environmentally deleterious processes, to save energy, and to be used where non-biological chemistry is impractical.

Fungi, rather than bacteria, are the main degraders of lignocellulosic materials, particularly wood. The fungal hyphae penetrate wood very rapidly, up to 1 mm per hour. The fungi attack wood by means of enzymes secreted from their hypha. Research aimed at understanding how wood and wood components are degraded by

* Academy lecture presented on May 18, 1989 at the Fourth International Conference on Biotechnology in the Pulp and Paper Industry. Raleigh, NC, USA

microorganisms and their enzymes has accelerated during the past decade, spurred on by the energy crisis, environmental concern, and very rapid advances in biotechnology in general. As a consequence, considerable knowledge has been accrued from fundamental studies. A brief summary of the knowledge relating to the enzymatic degradation of cellulose, hemicelluloses, and lignin will be presented first as a background to the biotechnical parts of this paper.

Fundamental knowledge

The generally accepted picture of the degradation of cellulose by fungi is that it proceeds by a synergistic action of three types of hydrolytic enzymes: endo-1,4-β-glucanase, exo-1,4-β-glucanase, and 1,4-β-glucosidase. The endo-glucanases cleave the cellulose molecules at random and generate non-reducing ends as substrate for the exo-glucanases (cellobiohydrolases). In contrast to exo-glucanases, endo-glucanases hydrolyze substituted celluloses such as carboxylmethylcellulose (CMC). The mode of action and the reason(s) for the strong interaction exhibited by fungal cellulases is not yet clearly understood (Eriksson, Wood 1985).

The most extensively studied cellulases are those from *Trichoderma reesei.* At least four components of its cellulolytic system have been purified to apparent homogeneity. Amino acid sequences of some of these enzymes are known and, more importantly, full length cDNA copies of all genes from *T. reesei* coding for, respectively, CHB I, CBH II, EG I, and EG III have been isolated (Knowles et al., 1988).

By elucidating the primary structures of these four components of the *T. reesei* cellulase system very obvious homologies between CBH I and EG I (>45%) were observed. Perhaps the most striking feature emerging from the sequence analyses is the presence of short, conserved regions (AB blocks) at either the N-termini (CBH II, EG III) or C-termini (CBH I, EG I), (Teeri et al., 1987) (Fig. 1). Block A (30 amino acids long) is rich in glycine and is stabilized by 2–3 disulfide bridges. Block B (40 amino acids long) is heavily *O*-glycosylated (threonine, serine content >40%) and contains several proline and arginine residues at conserved positions in all the four proteins. This block seems to serve as a hinge region linking Block A to the respective core (active site) enzymes. The core proteins of CBH I and CBH II (50–55 kDa), obtained by partial proteolysis, retain their full catalytic activity against water soluble substrates, but their activity toward microcrystalline cellulose is almost completely (core I) or partially (core 2) lost. Since the loss of the AB Blocks leads to decreased adsorptivity to cellulose, the conclusion is that these blocks are of importance in binding the enzyme to the cellulose substrate.

The tertiary structures of CBH I and CBH II from *T. reesei* have also been deduced from small-angle X-ray scattering studies (Abuja et al. 1988) (Fig. 2). Preliminary evidence exists for similar domain structures in CBH I and CBH II from *Penicillium pinophilum* (Claeyssens 1988) and *Sporotrichum pulverulentum* (CBH I).

In bacteria, unlike fungi, there is a strong evidence for the presence of tightly associated enzyme complexes and the production of multicomponent cellulolytic complexes seems to be a common denominator for cellulose degradation by anaerobic bacteria (Coughlan, Ljungdahl 1988; Lamed, Bayer 1988). Lamed et al. (1983) gave this multicomponent cellulolytic complex produced by *Clostridium thermocellum* the term cellulosome. By SDS treatment of the *C. thermocellum* cellulosome, fourteen

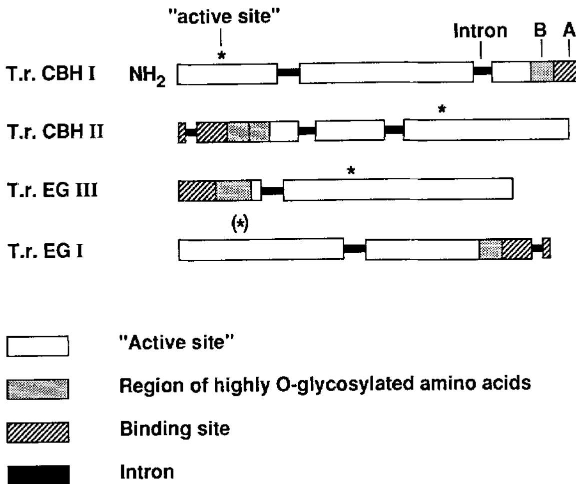

Fig. 1. Structural organization of *Trichoderma reesei* genes coding for cellulolytic enzymes

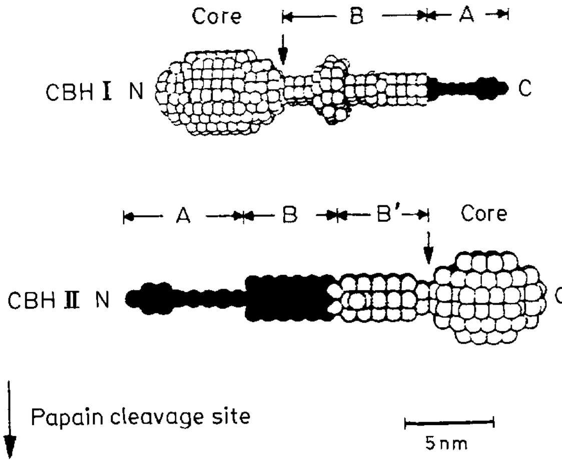

Fig. 2. Tertiary structures of the CBH I and CBH II from *T. reesei* as deduced from small-angle X-ray scattering studies. Abuja et al. (1988)

different polypeptides ranging in M_r from 48,000 to 210,000 can be distinguished by electrophoresis (Lamed, Bayer 1988).

For hydrolysis of cellulose, only enzymes hydrolyzing 1,4-β-glucosidic linkages are necessary. However, the structures of the hemicelluloses of wood are more variable, involving linear 1,4-β-linked chains of xylose or mannose which, substituted with other sugars, make up the branched heteropolysaccharides, which constitute hemicelluloses. Therefore, a more complex set of enzymes is required for their degradation. In Fig. 3, a hypothetical plant xylan and the sites of attack by microbial hemicellulolytic enzymes are presented. Complete degradation of such a branched, acetylated xylan requires the concerted action of several different hydrolytic enzymes,

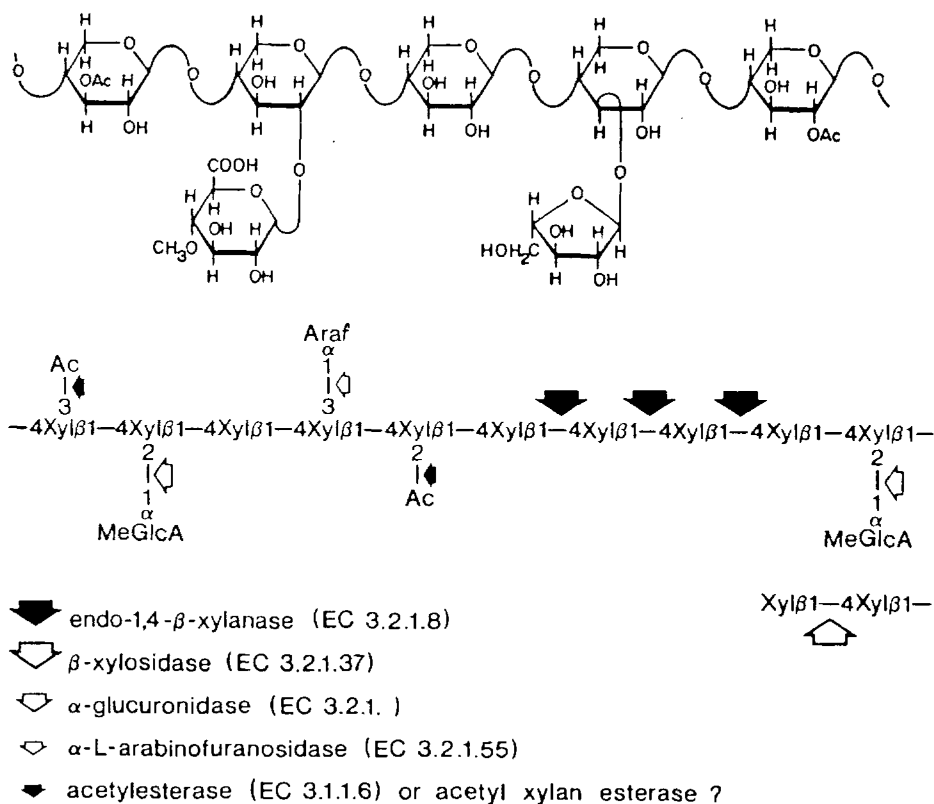

Fig. 3. Sites of attack by microbial hemicellulolytic enzymes on hypothetical plant xylan. Biely (1985)

including endo-1,4-β-xylanase, 1,4-β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase and acetylxylanesterase. Recently, considerable progress has been made in the separation and characterization of these enzymes (Poutanen 1988).

The endoxylanases are the best characterized and most widely studied of the hemicellulolytic enzymes. These enzymes initiate an end-wise attack on the backbone of xylans to produce both substituted and non-substituted short-chain oligomers, xylobiose and xylose. To convert the water-soluble oligomers, dimers, etc. to xylose, β-xylosidases are employed. The enzymes releasing the substituents on the xylan backbone, i.e., α-L-arabinosidase, α-D-glucuronidase, and acetylxylanesterase act in synergism with endoxylanases and β-xylosidases.

The mannan hemicelluloses, galactoglucomannans and glucomannans, are both branched heteropolysaccharides, and the concerted action of several enzymes are again required for their complete hydrolysis. Enzyme preparations suitable for such hydrolyses require the concerted action of several hydrolytic enzymes, namely endo-1,4-β-mannanase, 1,4-β-mannosidase, 1,4-β-glucosidase and α-galactosidase. Enzymatic hydrolysis of mannans has recently been reviewed by Dekker (1985).

The most widely studied and best characterized of the mannan degrading enzymes are the endomannanases. They attack the backbone of the mannans in an end-wise

manner to produce shorter, substituted and non-substituted oligomers, mannobiose, and mannose. β-Mannosidases then convert the water-soluble oligomers and dimers to mannose (Reese, Shibata 1965). The β-glucosidases and α-galactosidases, which release the glucose and galactose substituents on the mannan backbone, act in synergism with the endomannanase and the β-mannosidases.

Lignin, a phenylpropanoid structural polymer of vascular plants, gives the plants rigidity and binds the wood cells together. Lignin also decreases water permeation across cell walls of xylem tissue and makes the wood more resistant to attack by microorganisms (Sarkanen, Ludwig 1971). Although quite resistant to microbial attack, lignin is ultimately degraded to humus, water, and carbon dioxide, following the death of the plant tissues.

Research into lignin biodegradation is important for several reasons. Next to cellulose, lignin is our most important renewable material. While both cellulose and hemicelluloses can be degraded by cellfree enzyme solutions from a large variety of fungi and bacteria, this has not been accomplished with lignin. Lignin degradation occurs aerobically and has so far required the presence of the fungal cell. White rot fungi are the only microorganisms that will degrade lignin to any substantial degree. Studies of the degradative process has led to the picture of the lignin-degrading mechanisms shown in Fig. 4.

It has long been realized that phenoloxidases are necessary for lignin degradation to occur (Ander, Eriksson 1976). With the discovery of ligninases (peroxidases) this fact has been emphatically confirmed (Tien, Kirk 1983; Glenn et al. 1983). However, other enzymes are also necessary since the phenoloxidases produce phenoxyradicals that spontaneously polymerize. These polymerization reactions must be prevented or reversed for actual de-polymerization of the lignin polymer to occur. We do not yet know enough to allow the construction of an enzyme mixture that prevents the polymerization reactions from taking place in a cell-free state. However, it is clear that not only oxidative enzymes, i.e., phenoloxidases, including lignin peroxidase and laccase are involved in the degradation process, but also enzymes reducing the phenoxyradicals or preventing their formation. Such enzymes are cellobiose: quinone oxidoreductase (CBQ) and NAD(P)H: quinone oxidoreductase (Westermark, Eriksson 1974, a, b; Buswell et al. 1979).

What practical use is there for a detailed knowledge of the mechanisms for lignin degradation? In the pulping processes, lignin is degraded at very high temperatures and pressures and at extreme pH-values, using a large amount of energy. It is clear that white rot fungi and their enzymes can do the same thing, delignify wood, at room temperature and at almost neutral pH-values. If we can learn how the enzyme mechanisms function, pulp could eventually be produced and bleached using less energy and under more environmentally favorable conditions. As an example, I can mention, and I will come back to this, that we have succeeded in delignifying and bleaching pulp using hemoglobin in the presence of hydrogen peroxide as a catalyst. Hemoglobin carries the same heme-structure as the lignin peroxidases. The goal, therefore, is probably not to degrade lignin with enzymes but with stable, low-molecular mass organic substances mimicking the reactions catalyzed by lignin degrading enzymes once these are known.

The knowledge of enzymes and enzyme mechanisms in different wood-degrading microorganisms described above is the fundamental knowledge on which the develop-

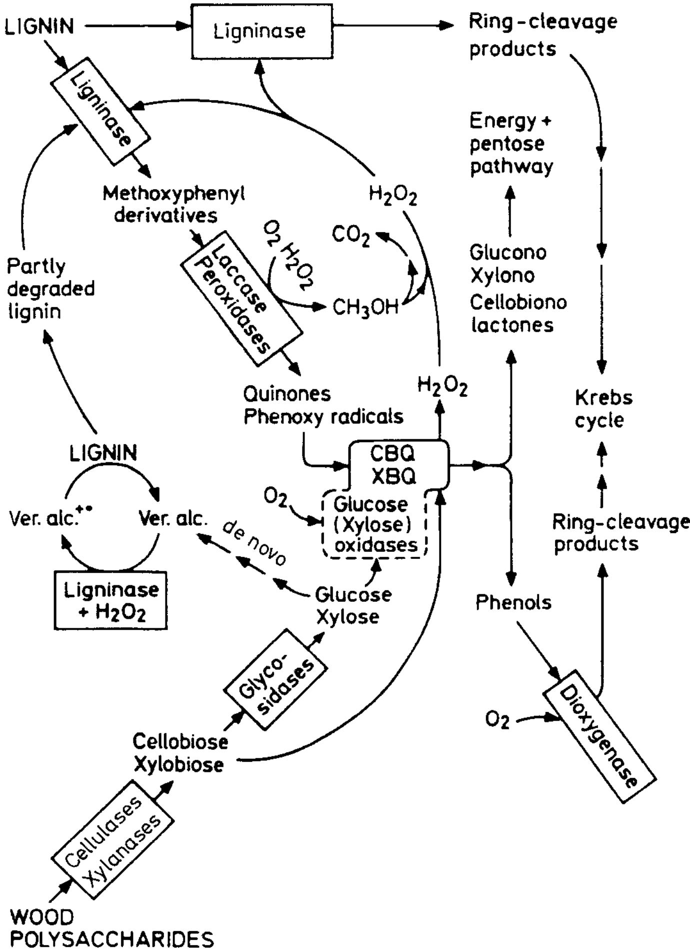

Fig. 4. Metabolic pathways for degradation of components of lignocellulosic materials. Eriksson et al. (1989)

Table 1. Biotechnology for the pulp and paper industry in different stages of development

Processes	Status
Ethanol production on sugars in spent sulfite liquors.	commercial scale
Protein production on sugars in spent sulfite liquor.	commercial scale
Closing of white-water systems in mechanical pulp and paper mills.	pilot plant scale
Microbial delignification (biopulping).	pilot plant scale
Purification of waste bleach waters.	pilot plant scale
New techniques for pulp bleaching.	laboratory scale
"Biosensors" for analysis of pulp fiber surfaces.	laboratory scale

ment of biotechnology for the pulp and paper industries rests. However, as we will see from the following, other microorganisms than those participating in wood degradation can also be of great importance for the processes and products that can be developed by biotechnological means in our industry. A summary of biotechnological processes in different stages of development is given in Table 1.

Ethanol production

The first biotechnological process to be developed in the pulping industry was ethanol production from sugars available in spent sulfite liquors. Ethanol produced in this way was once an important byproduct of pulping. Now, ethanol production from spent sulfite liquors has declined due to the closure of most mills of this type. Most of the potable alcohol is now produced by fermentation of starch from potatoes or grain.

The oil crisis induced much research into the production of ethanol from lignocellulosic materials. Since this crisis no longer appears acute, some of these activities have ceased. However, it still seems likely that ethanol, sooner or later, will find large-scale usage as a motor fuel. Production from renewable resources will then be necessary. Appropriate stimuli could include a new oil crisis, a desire to reduce the greenhouse effect caused by production of gases from non-renewable resources, or for other environmental reasons.

Ethanol production from lignocellulosic materials is technically not as simple as ethanol production from starch or sugars. Lignocellulosic materials must be pretreated, at least if enzymic saccharification is to be used in sugar production (Vallander, Eriksson, to be published). Much progress has recently been made in enzymic saccharification of lignocellulosics, including a more efficient reutilization of the cellulolytic enzymes (Vallander, Eriksson 1987). However, much of the sugars from wood is xylose and other pentoses for which there is no commercial fermentation technology. Development of such technology is recognized as essential if ethanol production from lignocellulosic materials is going to be economically feasible. Progress toward this goal is being made, but lack of funding and interest has slowed down research. Right now, ethanol from lignocellulosic materials remains a distant goal and will probably not be realized until a major crisis in oil supply or in environmental concern develops.

Protein production

The second biotechnological operation run by forest industries is the production of microbial protein from spent sulfite liquors. Two different processes have been used for this purpose, namely, the so-called Candida and Pekilo processes, based on the yeast *Candida utilis* and on the fungus *Paecilomyces varioti*, respectively. In both cases, both 5- and 6-carbon sugars are utilized. The Candida process for protein production originated at the beginning of this century while the Pekilo process is a more recent development in Finland (Forss et al. 1974). In addition to monomeric sugars, acetic acid is also a substrate for both organisms, while disaccharides and higher oligosaccharides are utilized only to a limited extent. Industrial fermentations

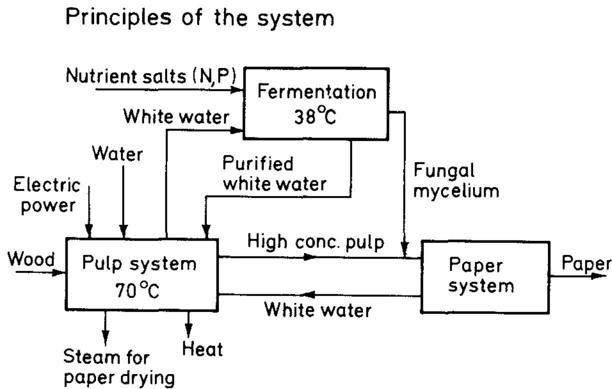

Fig. 5. Flow sheet of a closed white-water system of a newsprint pulp and paper system. The fermenter with a white rot fungus acts as a "kidney" and depletes the circulating water of the solid organic materials. Eriksson (1985)

for microbial protein production based on sulfuric acid hydrolyzates of wood operate in the Soviet Union and probably also in other eastern European countries. However, in western economies, such processes cannot compete economically with soybean protein.

Solid lignocellulosic wastes can also be converted to protein by fungi producing enzymes degrading cellulose and hemicelluloses. Two such processes have been developed based on the white rot fungus *Sporotrichum pulverulentum* (*Phanerochaete chrysosporium*) and the mold *Chaetomium cellulolyticum* (Ek, Eriksson 1980; Moo-Young et al. 1978). These processes have been developed at the Swedish Pulp and Paper Research Institute (STFI) and University of Waterloo, Canada, respectively. On evaluation, the STFI process at least was found not to be economically feasible, since the protein produced again could not compete on a price basis with soybean protein unless the substrate used in the fermentation had a negative value. It seems likely that this applies to all microbial protein production processes in western countries which are based on lignocellulosic substrates.

Closing of white water systems in mechanical pulp and paper mills

Production of mechanical pulps means that organic materials in the form of sugars, low-molecular weight lignins, extractives, etc., are released from the wood and appear in the circulating white waters. This is particularly true for the thermomechanical pulping (TMP) and chemithermomechanical pulping (CTMP) processes. The dissolved sugars in the waste waters are a mixture of monomeric and oligomeric water soluble substances, excellent substrates for fungi producing extracellular enzymes for hydrolysis of these polymers. Thus, released organics from mechanical pulping are an example of a negative-value substrate on which microbial protein could be produced and other benefits also obtained. At STFI, we have developed a water purification process where a fermenter with the white rot fungus *S. pulverulentum* was inserted as a kidney in the circulating water (Ek, Eriksson 1980). The principles of this process are presented in Fig. 5.

The process was tested on a pilot plant scale in a newsprint mill with a residence time in the fermenter of 17 hours. No build up of organic materials took place in the waste water system. We expect that recirculation in the fermenter of part of the mycelium produced in the continuous process would reduce the residence time and decrease the need for a large fermenter volume. The resulting fungal mycelium, appearing in the form of pellets of 0.2 to 0.4 mm diameter, are easily separated from waste water by filtration (Ek, Eriksson 1980). The process was also operated on a 25 m^3 scale to produce enough fungal mycelium for feeding trials. In this case, the waste water from a board factory was used as substrate (Ek, Eriksson 1980). Feeding trials with the resulting protein were carried out at the Swedish University of Agricultural Sciences in Uppsala, Sweden. The results, particularly with ruminants, were essentially positive (Thomke et al. 1980).

Instead of usage as cattle feed, the mycelium can be incorporated into the paper without influencing the paper quality. The fungal mycelium will, in a closed process, correspond to approximately 1.5% of the paper weight. The advantages of the process are as follows: (1) external effluent treatment unnecessary, (2) water savings due to recirculation, (3) increased paper (board) production by incorporation of the fungus in the product, (4) possible energy savings.

Based on all these virtues, the process should have excellent commercial possibilities. Research and Development particularly of recirculation of mycelium, is now being initiated in cooperation with the Austrian company Voest-Alpine with the application of this process on a commercial scale the goal. There is a good chance that this will be one of the new biotechnological processes developed for the pulp and paper industry.

Biopulping

The use of fungi for the pretreatment of wood chips, bagasse or straw prior to pulping is often referred to as "biopulping". It is based on the ability of white-rot fungi to colonize such lignocellulosic materials rapidly and thereby degrade the lignin (Eriksson 1987). The normal effect on wood would be a simultaneous attack on both the polysaccharides and the lignin. A totally specific attack on the lignin component probably does not occur. However, if the capacity of white rot fungi to degrade lignin was made more specific, it should be possible to obtain energy savings, mainly in mechanical pulping. Research on the development of cellulase-less mutants of white rot fungi and their use in microbial delignification was pioneered at the Swedish Pulp and Paper Research Institute in Stockholm (STFI). Since this is now a fairly old concept, there is no need to go into any detail, but simply summarize where we now stand.

In spite of the fact that very potent lignin degraders were developed by selection, mutation, and intercrossing of hemokaryotic strains of *Phaneorchaete chrysosporium* (Johnsrud, Eriksson 1985), no very striking energy savings have been obtained during the mechanical pulping of such fungal pretreated wood chips. However, biopulping of sugarcane bagasse has been more successful. In a cooperative venture between STFI and the Cuban laboratory ICIDCA, energy savings of approximately 40% have been accomplished in a process using the STFI-developed cellulase-less mutants from

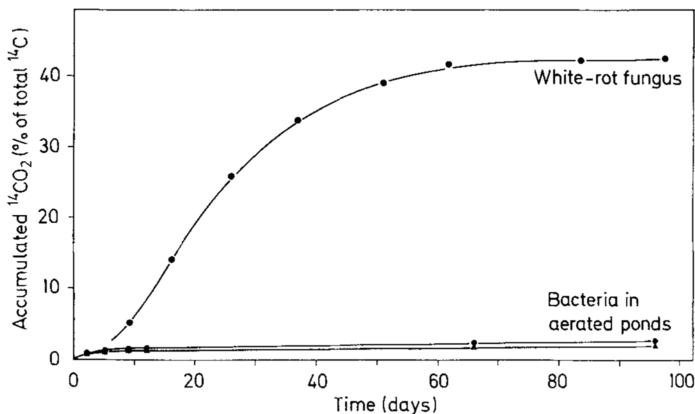

Fig. 6. Degradation of high-molecular mass ^{14}C-chlorolignins by the white rot fungus *Phanerochaete chrysosporium* and two different mixed bacterial populations. Eriksson, Kolar (1985)

P. chrysosporium followed by cold soda/thermomechanical pulping (Johnsrud et al. 1987). The results obtained indicate that grassy materials, such as wheat straw and bagasse, are better suited for fungal pretreatment than wood chips. A Biopulping Consortium in Madison, Wisconsin, is investigating the concept of biopulping using wild-type fungi rather than Cel^- mutants. The Consortium involves 17 pulp and paper companies working together with the U.S. Forest Products Laboratory and various other laboratories. Lignin removal has ranged from 3 to 37% in 4-week fungal pretreatments. Energy requirements for the refining are claimed to decrease up to approximately 50% (Leatham et al. 1989). However, the fungal pretreatment time is still too long to be economically feasible, and current work is therefore focused on reducing this time. It is too early yet to make any forecasts for the future of biopulping, but it is still a viable area of research, as indicated by patents on biopulping recently taken out in Japan.

Purification of waste bleach waters

The most important environmental problem created by the pulp and paper industry is the release of waste bleach waters from conventional bleaching of chemical pulps into receiving waters. The techniques presently used for treatment of these waste waters are aerated lagoons and activated sludge plants. The acute toxicity is normally removed in the aerated lagoons but the reduction of chlorinated compounds is very low. In a cold climate, the efficiency of aerated lagoons also decrease substantially (by at least 25% of the summer effect) during the winter. A common feature of the biological treatments used is that high-molecular mass chlorinated lignins are not removed. Eriksson and Kolar (1985), using high-molecular mass ^{14}C-labelled chlorinated lignins, showed that this material cannot be degraded by bacterial consortia isolated from aerated lagoons (Fig. 6). However, it can be seen in the same figure that the white-rot fungus *S. pulverulentum* (*P. chrysosporium*) is able to convert 40–50% of the high-molecular mass ^{14}C-labelled chlorinated lignins to ^{14}C-labelled CO_2 in 40–50 days.

Furthermore, it has been shown (Eriksson et al. 1985) that the high-molecular mass chlorinated material is not as stable as was thought earlier. It is slowly transformed into, among other substances, chlorinated catechols and guaiacols. The chlorinated aromatics are, to some extent, converted by microorganisms to the corresponding, more persistent and more hydrophobic, chlorinated veratrols (Neilson et al. 1983; Eriksson et al. 1985).

The observations presented above make it clear that an effective purification of bleach plant effluents must involve elimination of both low-and high-molecular mass chlorinated compounds. Removal of the latter compounds can be achieved in a number of different ways. One possibility would be to use ultrafiltration (UF), which is presently used to remove high-molecular mass chlorolignins in Japan. Another possibility would be to use white rot fungi, which would also degrade industrially modified lignins, such as kraft lignin (Hiroi, Eriksson 1976; Lundquist et al. 1977) and chlorinated high-molecular mass lignins from pulp bleaching (Eriksson, Kolar 1985) (Fig. 6). However, there is no microorganism known that can grow on lignin alone, and lignin degradation by white rot fungi seems to be an energy requiring process. White rot fungi have so far found no technical usage in the degradation of chlorinated aromatic compounds in bleach plant effluents. The reason for this is their complicated physiological demands when degrading lignin, which are not so easy to satisfy on a technical scale. In spite of this, a process for the degradation of chlorinated organic compounds and for reducing the amount of color in bleach waste waters is being developed at North Carolina State University in Raleigh. This so-called "Mycor-process" utilizes a fixed-film reactor described as a "rotating biological contactor" (Chang et al. 1986). The fungus is immobilized on the surfaces of the rotating disks of the reactor. As already mentioned, white rot fungi need an extra, more easily degradable carbon source than lignin to provide energy and hydrogen peroxide for lignin degradation. This is also true for lignin degradation in the Mycor-process. Therefore, it is difficult to accept the economic feasibility of adding the amounts of an extra carbon source necessary for the purification of spent bleach liquors by white rot fungi.

A modification of the Mycor method, termed Mycopor, is presently being studied in Austria (Messner et al. 1989). The *P. chrysosporium* fungus is used in this process immobilized on foam squares which serve as a trickling filter. Promising decolorization and AOX reductions have been obtained with this process.

For reasons given above, showing how difficult it is to use white rot fungi for purification of waste bleach waters on a large-scale, the use of white rot fungi alone to achieve this goal was abandoned at STFI. The obvious alternative to finding biological processes that degrade or dechlorinate high-molecular mass chlorinated materials is to adopt other means of eliminating such compounds. Currently, ultrafiltration (UF) of the alkaline stage effluent seems to produce the best effect and can also be carried out at a reasonable cost. Ultrafiltration of the entire bleach plant effluent is not yet economically realistic because of the large volumes involved. Ek and Eriksson (1987) investigated a combination of ultrafiltration and biological treatment. Such treatments are gradually being introduced into the pulp and paper industry (Boman et al. 1988). Anaerobic treatment is mainly used for warm and concentrated waste waters, such as recycling paper effluents and semichemical pulp effluents (Velasco et al. 1986) and for evaporation of condensates (Särner 1986). However,

waste bleach effluents are an entirely different matter, since they are currently more dilute than is desirable for anaerobic treatments. Therefore, to avoid an excessively long hydraulic retention time in the system, very good solids retention must be achieved. Secondly, bleach plant effluents vary considerably from time to time with respect to toxicity. This means that the microflora needs protection from too heavy exposure to toxicity shocks. Both these considerations favor the use of immobilized anaerobic bacteria for treatment of these effluents.

Another factor that must be considered concerning biological treatment of bleach plant effluents is chlorate formation when chlorine is replaced by chlorine dioxide in the bleaching process. Experiments have shown that chlorate reduction can be achieved under anaerobic conditions. When an anaerobic treatment of bleach plant effluents was being considered, we thought it useful to combine it with an aerobic treatment since anaerobic treatments result in the formation of substances such as hydrogen sulfide and organic acids, which give rise to odor problems. A combination of a closed anaerobic system with a small aerobic step, however, would be an effective remedy for such problems.

Based on the reasons given above, it was decided to construct a pilot plant in Sweden which was a combination of UF with anaerobic/aerobic treatment. After several months of operation, during which time the anaerobic purification stage in particular had to be stabilized, we now believe that the results from the biological treatment presented in Table 2 can be accomplished in between 10–15 hours of anaerobic residence time (Ek 1989). The calculated values in the table are based on earlier experiences with UF and assume that the effects are completely additive. As can also be seen in Table 2, the acute toxicity is completely eliminated after the combined UF and biological treatments. A comparison is also made in the table of the reduction of essential parameters obtained with the new technique and in aerated lagoons, respectively.

The treatment of waste bleach waters by ultrafiltration plus the anaerobic/aerobic process is now scaled up to larger pilot plant scale in Sweden, and hopefully this will also be done in the U.S. These pilot plants must be built in conjunction with a pulp mill since large volumes of waste bleach water will be involved that cannot be transported to other locations. The possibility of developing a commercial process based on the design described above looks promising and it seems highly probable

Table 2. Purification of waste bleach waters obtained with the technique under development (UF plus anaerobic/aerobic treatment) and the now used technique (aerated lagoon)

Parameters	UF plus anaerobic/ aerobic treatment; predicted reductions, %	Aerated lagoon estimated reductions, %
BOD	95	40–80
COD	70–85	15–30
AOX	70–85	20–30
Color	~50	0
Toxicity	100	variable
Chlorinated Phenols	~80	0–60
Chlorate	>99	variable

that this will be the first modern biotechnological process to be developed all the way from basic research into commercial use by the pulp and paper industry.

Development of new bleaching techniques

From what has been said earlier it is clear that the waste products from pulp bleaching should be reduced or eliminated to avoid harmful effects on the environment. The best way to eliminate environmentally hazardous compounds would be to develop new techniques for bleaching where such compounds are not produced or by developing efficient waste water purification systems such as that described above. At STFI a new waste water purification technique has been developed in parallel with a new biotechnical process for bleaching.

It was suggested by Dordick et al. (1986) that different peroxidases, unable to degrade lignins in aqueous solutions, could do so if the same enzymes were used in dioxane containing 5% of aqueous buffer. However, enzymes will bind to the pulp fibers and thus be difficult to recycle. Enzyme treatments of solid substrates will therefore be expensive. In spite of this, Farrell (1986) recently tried to use ligninases for pulp bleaching. In our approach to the development of a new bleaching technique, the bleaching effects obtained with enzymes, both in water and dioxane environments, have been compared with the effects obtained with porphyrin structure substances, mimicking the peroxidase enzyme reactions (Pettersson et al. 1988 a).

The new approach to use so called biomimetic catalysts such as porphyrins instead of enzymes has recently received increasing attention since such catalysts would be more economical and ideal for use on an industrial scale (Cui et al. 1989). Hemoglobin has been our catalyst of choice and bleaching experiments with hemoglobin have been carried out under many different conditions. Hemoglobin is a tetrameric molecule (M_r 64,000) built up of two types of peptide chains, each carrying its own prosthetic group, the heme group, which consists of an organic moiety protoporphyrin IX and an iron atom. Hemoglobin is a well known oxygen carrier in blood cells of animals and man. In Table 3 are presented the results from the bleaching of unbleached kraft pulp, both pine and birch, and O_2-bleached kraft pulp (pine) with hemoglobin in 90% dioxane – 10% water solvent. A considerable decrease in kappa number, from 30 to 21.4, was obtained in 30 hours for bleached kraft pulp (pine) while the viscosity only decreased from 1180 to 1041 dm^3/kg and the brightness increased from 23.5 to 35.4%. For unbleached kraft pulp (birch), the brightness was raised to 53.8% (ISO) during the same treatment. Similar values were obtained in other studies, i.e. a considerable decrease in kappa number, a moderate decrease in viscosity, and approximately 10 (ISO) points increase in brightness.

It could also be demonstrated that catalysts other than hemoglobin, such as cytochrome C, peroxidase, and vitamin B_{12}, also caused a decrease in kappa number. With some of these catalysts an increase in brightness is not obtained, since they are inherently strongly colored.

In addition to hemoglobin, the bleaching mixture used also contains palmitoyl chloride and hydrogen peroxide. Palmitoyl chloride seems to be an important and necessary ingredient in the bleaching solution. Its effect is most likely to increase the specificity of attack on the lignin. This increased specificity seems to have a positive influence upon pulp viscosity.

Table 3. Bleaching of unbleached and O_2-bleached kraft pulp (pine) and unbleached kraft pulp (birch) with hemoglobin in 90% dioxane solvent (Pettersson et al. 1988a)

Pulp	Property	Bleaching time, h									
		Ref	1	2	4	6	22	24	26	28	30
Unbleached kraft pulp (pine)	Kappa no.	30	29.0	27.3	26.2	25.4	23.1	–	22.0	–	21.4
	Viscosity (dm^3/kg)	1180	1170	1151	1162	1117	1062	–	1051	–	1041
	Brightness	23.5	33.7	34.3	34.6	34.7	35.8	–	35.6	–	35.4
O_2-bleached kraft pulp (pine)	Kappa no.	22.5	20.6	19.5	18.2	16.4	15.6	14.3	13.5	–	–
	Viscosity (dm^3/kg)	1050	988	946	932	886	881	878	876	–	–
	Brightness	30.5	41.4	43.0	41.6	43.2	42.5	40.5	4.7	–	–
Unbleached kraft pulp (birch)	Kappa no.	18	16.5	14.4	13.9	13.2	–	12.9	12.3	11.9	11.6
	Viscosity (dm^3/kg)	1250	1214	1204	1187	1147	–	1123	1083	1091	1061
	Brightness	40.0	50.1	51.0	51.1	51.6	–	52.2	52.3	53.3	53.8

Table 4. A comparison of results obtained with oxygen and hemoglobin bleaching respectively (Pettersson et al. 1988a)

Pulp	Bleaching method	Kappa number		Viscosity, dm^3/kg		Brightness (ISO)	
		Before bleaching	After bleaching	Before bleaching	After bleaching	Before bleaching	After bleaching
Unbleached kraft pulp (pine)	Oxygen bleaching (SCA Pulp AB)	30	22.5	1180	1050	23.5	35.9
	Hemoglobin bleaching (26 h)	30	22	1180	1051	23.5	35.6
Oxygen bleached pulp (pine)	Hemoglobin bleaching (26 h)	22.5	13.5	1050	876	30.5	40.7
Unbleached kraft pulp (birch)	Oxygen bleaching (SCA Pulp AB)	18	14	1250	1140	40.0	44.8
	Hemoglobin bleaching (26 h)	18	12.3	1250	1083	40.0	52.3

Since pre-bleaching of pulp with oxygen is environmentally favorable, this technique has been developed considerably in recent years. In Table 4 the results obtained with O_2-bleaching and hemoglobin bleaching are compared. It is clear from this table that very similar bleaching levels are obtained with each of the two separate techniques. However, it can be observed that additional bleaching of O_2-bleached pulp is achieved with hemoglobin. Although it is not imposssible to bleach pulp in a dioxane water mixture also under technical conditions, it would be more favorable if the bleaching could be carried out in water only. Bleaching of unbleached kraft pulp (pine) in water and in the presence of H_2O_2 at 40 °C at three different palmitoyl chloride (PC)-modified hemoglobin levels 0.5, 0.75, and 1.0% calculated on the basis of pulp dry weight was therefore carried out. It was found that 1% PC-hemoglobin causes a dramatic reduction in pulp viscosity at a very early stage. Thus, after only 30 minutes the viscosity decreased from 1,100 dm^3/kg to 930 dm^3/kg. However, at a level of 0.5% PC-hemoglobin the viscosity remained relatively stable. A PC-hemoglobin dosage of 0.75% was found to be optimal and caused only a moderate reduction in viscosity from 1,100 dm^3/kg to 980 dm^3/kg after 90 minutes of bleaching. Temperature studies reveal that 60 °C was optimal for bleaching and kept the viscosity at a higher level than obtained at 40 °C or 50 °C.

The results show that pulp bleaching with PC-hemoglobin in an aqueous medium in the presence of hydrogen peroxide is very effective for lignin removal. The attack on the lignin also seems to be quite selective and pulp viscosity is kept rather stable if the amount of added hemoglobin is no higher than 0.75%. The PC-hemoglobin solution is prepared by pre-treating hemoglobin with palmitoyl chloride, which probably reacts with the peptide chains of hemoglobin to form a more hydrophobic complex. Since lignin is more hydrophobic than polysaccharides, the PC-hemoglobin binds to it more strongly, which facilitates the reaction between the lignin and the PC hemoglobin complex.

The so-called biomimetic bleaching technique presented here is by no means optimized. Our work will continue so that the problem can be attacked from different angles. A better knowledge of the enzyme mechanisms involved in lignin degradation would also most certainly facilitate a positive development.

Analysis and modification of pulp fiber surfaces

The structural and chemical composition of pulp fiber surfaces are of importance for the paper strength (Franzeń, 1986; Mohlin 1987) since those properties strongly influence the bonds between fibers and fiber fragments in a paper sheet. Refiner mechanical pulp (RMP), thermomechanical pulp (TMP) and chemi-thermomechanical pulp (CTMP) pulp types have been developed during the last 40 years. Due to the high yields obtained with these pulps compared to chemical pulps, production has attracted a growing interest. Improved strength and other important quality properties of these new mechanical pulps compared to the older types allow the production of newsprint without the addition of chemical pulps. Also, these better quality pulps make them very viable economically as furnish components in many papers (Franzeń 1986). A better understanding of the chemical composition of pulp fiber surfaces might serve to optimize mechanical and chemimechanical production processes. With

these goals in mind we started at STFI to develop bioassay techniques that would allow us to gain this necessary knowledge.

Pulp fibers are usually characterized using physical methods, including measurements of mechanical strength and hydrodynamic pulp properties. Forgacs (1963) and Garceau et al. (1975) studied different Bauer McNett fractions of thermomechanical pulp in relation to their bonding ability and printing properties. Chemical analysis and UV-microscopy were assessed as possible methods for investigating the chemical composition of mechanical pulps and the morphological elements of fibers by Chang et al. (1979), Douek et al. (1976) and Boutelje and Eriksson (1982). However, as early as 1971, Boutelje et al. (1971) used purified xylanase enzyme to investigate the composition of spruce holocellulose.

The mechanical pulps used in our studies were all produced at Sunds Defibrator AB, Research and Development Laboratory, Sundsvall, Sweden. Norway spruce (*Picea abies*) was selected as the raw material. Fractionation according to SCAN-M 6:69 was carried out using Bauer McNett fractionation. Four fractions, >16 mesh, 16–30, 30–50, and 50–100 mesh sizes were collected. The smaller fractions passing through the 100 mesh screen, estimated as amounting to about 35% of the total pulp material, was not collected due to the large volume in which it was suspended (200 liters).

In our first study, endo and exo-1,4-β-glucanases from a culture filtrate of the fungus *Trichoderma reesei* QM 9414 were used separately and in combination to treat the fractionated RMP, TMP, CTMP pulp fibers under identical experimental conditions (Yang et al. 1988a). Using the same fiber fraction, the greatest amount of reducing sugars was released from RMP and the lowest levels from the TMP sample. The order of sugar released, i.e. RMP>CTMP>TMP did not change no matter which fiber fraction was used. However, the amounts increased with decreasing fiber fraction sizes, i.e., the shorter the pulp fiber the greater the quantity of reducing sugars liberated from the fiber surfaces. In Fig. 7 the release of reducing sugars from the 30–50 mesh fractions of RMP, TMP and CTMP fibers is presented. The individual endo- and exo-glucanase components can hydrolyze only amorphous cellulose. To degrade crystalline cellulose a combination of both enzyme types is necessary. Thus, a synergistic action exists between these two enzymes. Therefore, the amount of reducing sugars released by the combined endo- and exo-glucanases is considerably higher than the sum of reducing sugars released by the separate endo- and exo-glucanase components.

HPLC analyses of the released sugars as a means of examining the specificity of the cellulolytic enzymes gave the following results: About 95% of the reducing sugars released were cellobiose and glucose. The amount of xylose was less than 1%, while the levels of galactose and arabinose plus mannose accounted for between 1.0 to 2.5% of the total sugars. The conclusion is that the endo- and exo-glucanases used here are specific enough for the purpose we had in mind. The results from our sugar release studies strongly suggest that cellulose is exposed on the surface of the different mechanical pulps in the same order as sugar is released, i.e. RMP>CTMP>TMP. This conclusion is strongly supported by the breakdown mechanisms of the wood matrix in mechanical pulping proposed by Franzeń (1986).

To further investigate the composition of the mechanical pulp fiber surfaces, lignin specific peroxidase (Yang et al. 1988b), as well as lignin specific antibodies (Pettersson

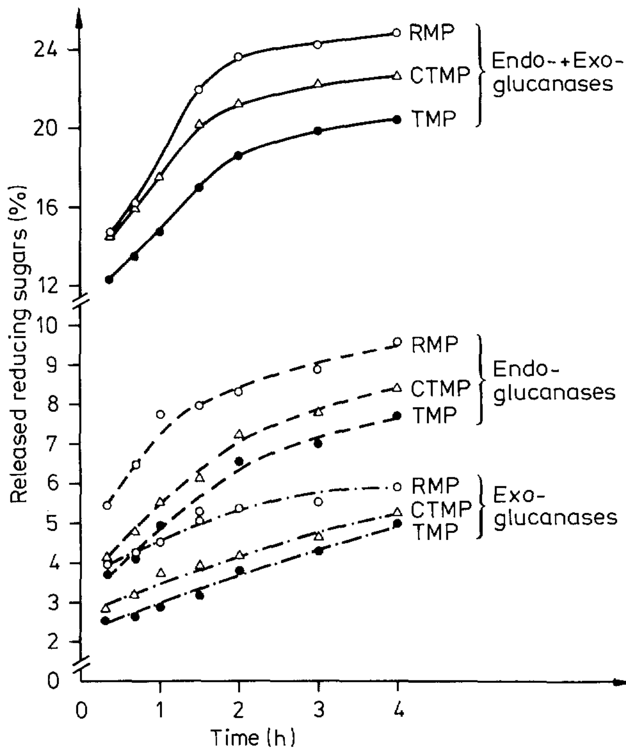

Fig. 7. Release of reducing sugars (in percent of fiber dry weight) from the surfaces of Bauer McNett/RMP, TMP, CTMP fibers, 30–50 mesh. Yang et al. (1988a)

et al. 1988b), were used to assay for exposed lignin on the fiber surfaces of the same Bauer McNett fractions of RMP, TMP and CTMP used in our previous study (Yang et al. 1988a). Peroxidases are enzymes with a high affinity for the phenolic type compounds which serve as substrates (Ander, Eriksson 1978; Crawford 1981; Kirk, Shimada 1985). These types of structures are abundant in lignin, indicating that peroxidases might exhibit a higher affinity and specific binding capacity for the lignin component of the mechanical pulp fiber surfaces compared to other major wood components. Our results strongly support this contention. In Fig. 8, the relative amounts of peroxidase adsorbed on the surfaces of the 30–50 mesh fractions of RMP, TMP and CTMP are presented. It is clear from this figure that the amount of peroxidase bound to the different types of pulps decreases in the order TMP > CTMP > RMP. The same order applies to all the Bauer McNett fractions studied. It should be observed that the peroxidase binding order is precisely the reverse to that in which cellulose was presumed to be exposed on these pulp fiber surfaces. It should also be observed in Fig. 8 that practically no peroxidase was adsorbed to bleached sulfate pulp.

Adsorption of horseradish peroxidase on the mechanical pulp fiber surfaces was determined by assaying for peroxidase activity after carefully washing the fibers following incubation with the enzyme. The fibers were then suspended in a solution of phenol 1.2-phenoldiaminedihydrochloride solution serving as a substrate for the

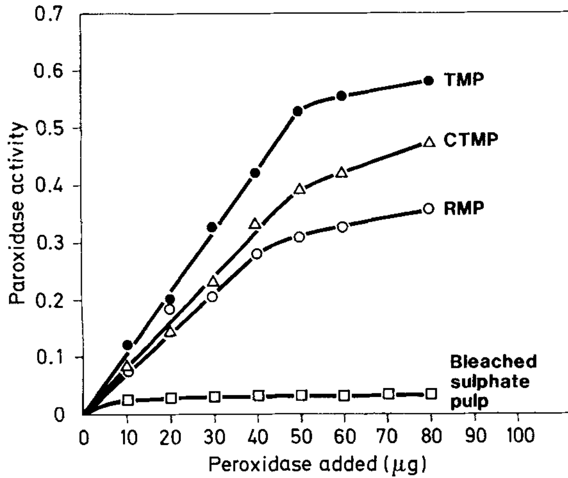

Fig. 8. Relative amounts of peroxidase adsorbed on the surfaces of Bauer McNett/RMP, TMP, and CTMP fibers, 30–50 mesh. Yang et al. (1988b)

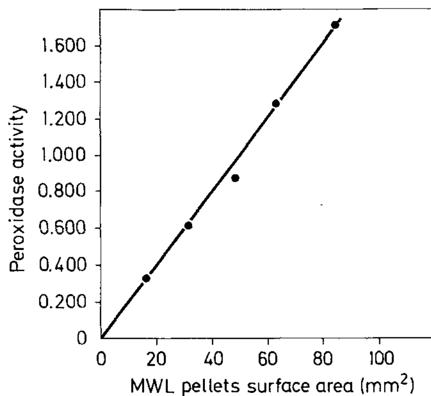

Fig. 9. Relative amounts of peroxidase adsorbed per mm^2 of milled wood lignin (MWL) pellets. Yang et al. (1988b)

peroxidase. After 60 seconds, the reaction was stopped with one molar H_2SO_4 and the mixture centrifuged. Quinone formation, resulting from peroxidase activity, was determined by measuring the adsorption at 492 nm.

To estimate the maximum amount of horseradish peroxidase adsorbed per unit area of lignin, pellets of milled wood lignin (MWL) with known diameters were prepared. The thicknesses of these pellets were then determined by scanning electron microscopy (SEM) and the surface area calculated from these dimensions. Horseradish peroxidase was added to a number of test tubes containing increasing quantities of these MWL pellets and maintained under the same experimental conditions used during incubation of the pulp fiber fractions with the enzyme. After washing the MWL pellets until no trace of peroxidase could be detected in the washing buffer, the amount of peroxidase adsorbed was determined. It is seen in Fig. 9 that a linear relationship exists between the total amount of horseradish peroxidase adsorbed per surface area of the pellets. Using Fig. 9 as a standard curve, the area of lignin exposed

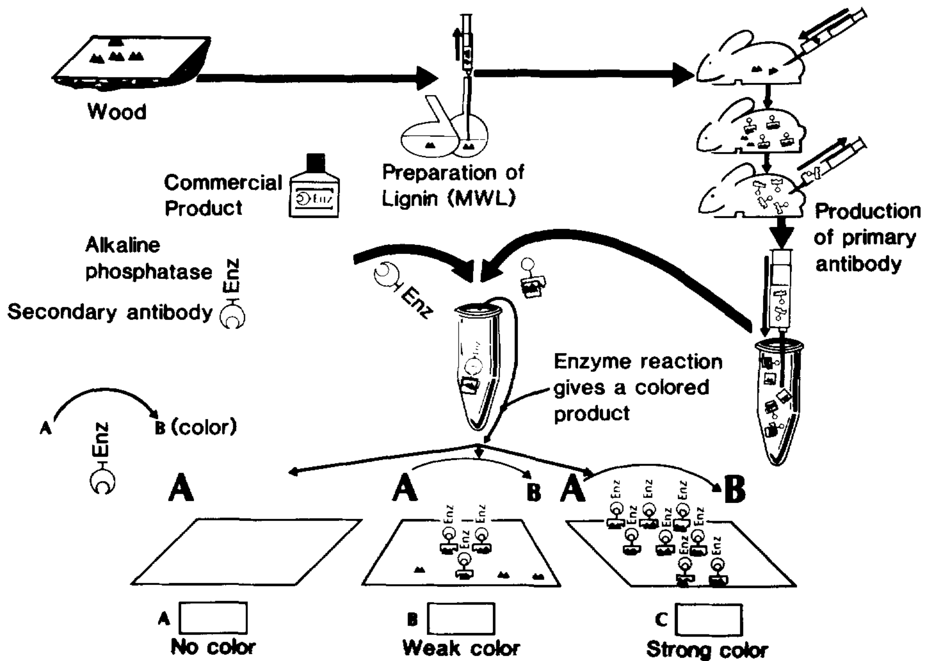

Fig. 10. Principles for the production of lignin-specific antibodies and analyses of antibody binding to the surface of mechanic pulp fibers. Pettersson et al. (1988b)

on the surfaces of the different pulp fibers can readily be calculated. For the 30–50 mesh RMP fibers used in Fig 8, the surface area occupied by lignin is calculated to be approximately 1.3 mm^2/mg of fibers (Yang et al. 1988b).

To further confirm the results obtained in the studies of mechanical pulp fiber surfaces using cellulolytic enzymes and peroxidase, antibodies towards MWL lignin were produced. This was accomplished by injection of lignin into rabbits. The lignin specific antibodies were isolated from blood serum of the animal. In addition to the isolated primary antibodies we have also used commercially available anti-immunoglobins to rabbits as secondary antibodies. An enzyme, in our case alkaline phosphatase, is bound to the secondary antibodies and the total antibody complex has been used as a probe to characterize mechanical pulp fiber surfaces. The principles for the production of lignin-specific antibodies and analyses of antibodies binding to the fiber surfaces are given in Fig. 10.

Results for the 30–50 mesh fiber fractions are presented in Fig. 11. They clearly show that the amount of lignin specific antibodies binding to the different fiber types rank in the order TMP>RMP>CTMP. This means that the immunoassay ranks RMP and CTMP in the reverse order compared to the lignin specific peroxidase (Yang et al. 1988b). The reason for this discrepancy is not known. However, the most likely explanation is that sulfonation of the lignin in the CTMP pulp may have changed the structure of the lignin so that the antibodies do not "recognize" the sulfonated lignin as antigen in the same way as they "recognize" native lignin.

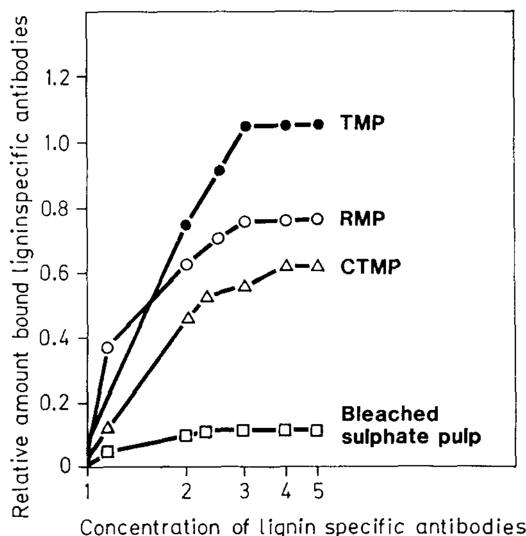

Fig. 11. Binding of lignin-specific antibodies to the surface of three different types of mechanical pulp fibers and to bleach kraft pulp fibers (30–50 mesh). Pettersson et al. (1988b)

Conclusions

In this review some examples of approaches for the creation of biotechnological processes for the pulp and paper industry have been given. While biotechnological developments in other industries, mainly pharmaceutical and medical, have focused on high value processes and products, the applications discussed here for the forest industries fall in the category of lower value processes and products. This is a much more difficult field to develop economically feasible new processes, particularly if one considers the large volumes that generally have to be processed in the forest industries.

It may be that certain applied developments of the areas described above have started too early, i.e. before enough basic knowledge has been collected. Premature approaches may sometimes curb interest when results do not appear fast enough. However, the overall spending on biotechnological research and development in the forest industries is small. A more substantial input of research and development would make it possible to evaluate more quickly just how promising such biotechnological approaches really are. Another prerequisite for the development of biotechnology in the pulp and paper industries is that the forest industries employ people with a more biologically oriented educational background than has been the case in the past. A better communication between biotechnologists and industry technical staff is necessary. A better trained staff that will speak the language of the biotechnologists is therefore highly desirable. Until this has become effective, we researchers should continue to explore the potentials that biotechnology has in the pulp and paper industry.

References

Abuja, P.; Pilz, I.; Claeyssens, M.; Tomme, P. 1988: Domain structure of cellobiohydrolase II as studied by small angle X-ray scattering: Close resemblance to cellobiohydrolase I. Biochem. Biophys. Res. Commun. 156: 180–185

Ander, P.; Eriksson, K.-E. 1975: Influence of carbohydrates on lignin degradation by the white-rot fungus *Sporotrichum pulverulentum*. Svensk Papperstidn. 78: 643–652
Ander, P.; Eriksson, K.-E. 1978: Lignin degradation and utilization by microorganisms. Progress in Industr. Microbiol. Vol. 14: 1–58. Ed. M. J. Bull. Elsevier, Amsterdam
Biely, P. 1985: Microbial xylanolytic systems. Trends Biotechnol. 3: 286–290
Boman, B.; Frostell, B.; Ek, M.; Eriksson, K. E. 1988: Some aspects on biological treatment of bleaching pulp effluents. Nordic Pulp and Paper Res. J. 3: 13–18
Boutelje, J. B.; Eriksson, I. 1982: An UV-microscopy study of lignin in middle lamella fragments from fibers of mechanical pulp of spruce. Svensk Papperstidn. 85: R39–42
Boutelje, J.; Eriksson, K.-E.; Hollmark, B. H. 1971: Specific enzyme hydrolysis of the xylan in a spruce holocellulose. Svensk Papperstidn. 74: 32–37
Buswell, J. A.; Hamp, S.; Eriksson, K.-E. 1979: Intracellular quinone reduction in *Sporotrichum pulverulentum* by a NAD(P)H: Quinone oxidoreductase: Possible role in vanillic acid catabolism. Febs Lett. 108: 229–232
Chang, H-m.; Sinkey, J. D.; Yan, J. F. 1979: Chemical analysis of refiner pulps. Tappi 63: 9, 103–106
Chang, H.-m.; Joyce, T. W.; Matsumoto, Y.; Yin, C.; Vasudevan, B.; Bocchat, C.-A. 1986: Decolorization and dechlorination of bleach plant effluents by a white-rot fungus. Proc. 3rd Inter. Conf. Biotechnol. in the Pulp and Paper Ind., Stockholm, 120–123
Claeyssens, M. 1988: The use of chromophoric substrates and specific assays in the study of structure – activity relationships of cellulolytic enzymes. FEMS Symp. No. 43. Biochemistry and Genetics of Cellulose Degradation (J.-P. Aubert, P. Beguin and J. Millet, Eds.). Academic Press, London, 393–397
Couglan, M. P.; Ljungdahl, L. G. 1988: Comparative biochemistry of fungal and bacterial cellulolytic enzyme systems. In: Aubert, J.-P., Beguin, P., Millet, J. (eds.), Biochemistry and genetics of cellulose degradation, FEMS symp. no. 43. Academic Press, London, 11–30
Crawford, R. L. 1981: Lignin biodegradation and transformation. New York: John Wiley & Sons
Cui, F.; Dolphin, D.; Wijesekera, T.; Farrell, R.; Skerker, P. 1989: Biomimetic studies of lignin degradation and bleaching. In Biotechnology in Pulp and Paper Manufacture. Butterworths, Stoneham, MA. In press
Dekker, R. F. H. 1985: Biodegradation of the hemicelluloses. In: Higuchi, T. (Ed), Biosynthesis and biodegradation of wood components. Tokyo: Academic Press
Dordick, J. S.; Marletta, N. A.; Klibanov, A. M. 1986: Peroxidases depolymerize lignin in organic media but not in water. Proc. Natl. Acad. Sci. U.S.A. 83
Douek, M.; Heitner, C.; Lamandé, L.; Goring, D. A. I. 1976: The measurement of visible absorption of morphological elements in wood. Trans. Tech. Sect. Can. Pulp Pap. Assoc. 2: 3, 78–82
Ek, M. 1989: Reduction of AOX in bleach plant effluents by ultrafiltration and biological methods. In Biotechnology in Pulp and Paper Manufacture. Butterworths, Stoneham, MA. In press
Ek, M.; Eriksson, K.-E. 1980: Utilization of the white-rot fungus *Sporotrichum pulverulentum* for water purification and protein production on mixed lignocellulosic waste waters. Biotechnol. Bioeng. 22: 2273–2284
Ek, M.; Eriksson, K.-E. 1987: External treatment of bleach plant effluent. 4th Int. Symp. on Wood and Pulping Chemistry, Paris
Eriksson, K.E. 1985: Swedish developments in biotechnology related to the pulp and paper industry, Tappi 68: 46–55
Eriksson, K.-E. 1987: Microbial delignification – basics, potentials and applications. Proceedings FEMS Symposium, Biochemistry and Genetics of Cellulose Degradation, 285–302, Paris, September 1987. London: Academic Press
Eriksson, K.-E.; Kolar, M. C. 1985: Microbial degradation of chlorolignins. Environ. Sci. Technol. 19: 1086–1089
Eriksson, K.-E.; Blanchette, R. A.; Ander, P. 1989: Microbial and enzymatic degradation of wood and wood components. Heidelberg: Springer, In press
Eriksson, K.-E.; Wood, T. 1985: Biodegradation of cellulose. In Higuchi, T (Ed): Biosynthesis and biodegradation of wood components. London: Academic Press

Eriksson, K.-E.; Kolar, M. C.; Ljungquist, P.; Kringstad, K. P. 1985: Studies on microbial and chemical conversion of chlorolignins. Environ. Sci. Technol. 19: 1219–1224

Farrell, R. L. 1986: Kraft pulp bleaching with ligninolytic enzymes. In Biotechnology in the Pulp and Paper Industry, Stockholm, pp. 61–63

Forgacs, G. L. 1963: The characterization of mechanic pulps. Pulp and Paper Mag. Can. 84: T 89–118

Forss, K. G.; Gadd, G. O.; Lundell, R. O.; Williamson, H. W: Process for manufacture of protein containing substances for fodder, foodstuff and technical application. U.S. Patent 3.809.614, patented May 7, 1974

Franzén, R. 1986: General and selective upgrading of mechanical pulps. Nordic Pulp Paper Res. J. 1:3, 4–13

Garceau, J. J.; Lavallee, H. C.; Law, K. W. 1975: Beyond "L" and "S". Pulp Paper Mag. Can. 76: 45–51

Glenn, J. K.; Gold, M. H. 1983: Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 45: 1741–1747

Hiroi, T.; Eriksson, K.-E. 1976: Microbial degradation of lignin. Part 1. Influence of cellulose of the degratation of lignins by the white-rot fungus *Pleurotus ostreatus*. Svensk Papperstidn. 79: 157–161

Johnsrud, S. C.; Eriksson, K.-E. 1985: Cross-breeding of selected and mutated homokaryotic strains of *Phanerochaete chrysosporium* K-3: New cellulase deficient strains with increased ability to degrade lignin. Appl. Microbiol. Biotechnol. 21: 320–327

Johnsrud, S. C.; Fernandez, N.; Lopez, P.; Gutierrez, I.; Saez, A.; Eriksson, K.-E. 1987, August: Properties of fungal pretreated high yield bagasse pulps. Nordic Pulp Pap. Res. J. Special Issue Börje Steenberg 75, p. 47–52

Kirk, T. K.; Shimada, M. 1985: Lignin biodegradation: The microorganisms involved and the physiology and biochemistry of degradation by white rot fungi. In Higuchi (ed.) Biosynthesis and biodegradation of wood components, 579–605. New York: Academic Press

Knowles, J.; Teeri, T.; Lehtovaara, P.; Pentila, M.; Saloheimo, M. 1988: The use of gene technology to investigate fungal cellulolytic enzymes. FEMS Symposium No. 43. Biochemistry and Genetics of Cellulose Degradation (Aubert, J.-P–Beguin, P. and Millet, J.; Eds.), 153–169. London: Academic Press

Lamed, R.; Bayer, E. A. 1988: The cellulosome concept: Exocellular/extracellular enzyme reactor centers for efficient binding and cellulolysis. In: Aubert, J.-P., Beguin, P., Millet, G. (eds.), Biochemistry and genetics of cellulose degradation, FEMS symp. no. 43. Academic Press, London, 101–116

Lamed, R.; Setter, E.; Kenig, R.; Bayer, E. A. 1983: The cellulosome – A discrete cell surface organelle of *Clostridium thermocellum* which exhibits separate antigenic, cellulose binding and various cellulolytic activities. Biotechnol. Bioeng. Symp. 13: 163–181

Leatham, G. F.; Myers, G. C.; Wegner, T. H.; Blanchette, R. A. 1989: Energy savings in biomechanical pulping. In Biotechnology in Pulp and Paper Manufacture. Stoneham: Butterworths MA

Lundquist, K.; Kirk, T. K.; Connors, W. J. 1977: Fungal degradation of kraft lignin and lignin sulfonates prepared from synthetic ^{14}C-lignins. Arch. Microbiol. 112: 291–296

Messner, K.; Ertler, G.; Jaklin-Farcher, S. 1989: The treatment of bleach plant effluents by the MYCOPOR system. In: Biotechnology in Pulp and Paper Manufacture. Stoneham: Butterworths

Mohlin, U.-B. 1987: Massans kvalitet bestämmer papperets funktion. Svensk Papperstidn. 90: 11, 14–20

Moo-Young, M.; Chahal, D. S.; Vlach, D. 1987: Single cell protein from various chemically pretreated wood substrates using *Chaetomium cellulolyticum*. Biotechnol Bioengin 20: 107–118

Neilson, A. H.; Allard, A.-S.; Hynning, P.-A.; Remberger, M.; Landner, L. 1983: Bacterial methylation of chlorinated phenols and quaiacols: Formation of veratroles from guaiacols and high-molecular-weight chlorinated lignin. Appl. Environ. Microbiol. 45: 774–783

Pettersson, B.; Yang, J.-L.; Eriksson, K.-E. 1988a: Biotechnical approaches to pulp bleaching. Nordic Pulp Pap Res. J. 3: 198–202

Pettersson, B.; Yang, J.-L.; Eriksson, K.-E. 1988b: Characterization of pulp fiber surfaces by lignin specific antibodies. Nordic Pulp Pap Res. J. 3: 152–155

Poutanen, K. 1988: Characterization of xylanolytic enzymes for potential applications. Diss. Techn. Res. Centre, Finland. Publications 47

Reese, E. T.; Shibata, Y. 1965: β-Mannanases of fungi. Can. J. Microbiol. 11: 167–183

Sarkanen, K. V.; Ludwig, C. H. 1971: Definition and nomenclature. In: Sarkanen, K. V.; Ludwig, C. H. (eds.), Lignin. Ocurrence, formation, structure, and reactions. New York: Wiley-Interscience

Särner, E. 1986: Pilot and full-scale anaerobic-aerobic treatment of mixed wastewater from a sulphite pulp mill and a cellulose derivate manufacturing company. In Biotechnology in the Pulp and Paper Industry, Stockholm

Teeri, T. T.; Lehtovaara, P.; Kauppinen, S.; Salovuori, I. and Knowles, J. 1987: Homologous domains in *Trichoderma reesei* cellulolytic enzymes: Gene sequence and expression of cellobiohydroase II. Gene 51: 43–52

Thomke S.; Rundgren, M.; Eriksson, S. 1980: Nutritional evaluation of the white-rot fungus *Sporotrichum pulverulentum* as a feedstuff to rats, pigs, and sheep. Biotechnology and Bioengineering 22: 2285–2303

Tien, M.; Kirk, T. K. 1983: Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science 221: 661–663

Vallander, L.; Eriksson, K.-E. 1987: Enzyme recirculation in saccharification of lignocellulosic materials. Enzyme Microb. Technol. 9: 714–720

Velasco, A. A.; Greene, M.; Frostell, B. 1985: Full scale anaerobic-acrobic biological treatment of a semichemical pulping wastewater. In Proceedings, 40th Ind. Waste Conf., Purdue University, 297–304, Ann Arbor Science

Westermark, U.; Eriksson, K-E. 1974a: Carbohydrate-dependent enzymic quinone reduction during lignin degradation. Acta Chem. Scand. B28: 204–208

Westermark, U.; Eriksson, K-E. 1974b: Cellobiose: Quinone oxidoreductase, a new wood-degrading enzyme from white-rot fungi. Acta Chem. Scand. B28: 209–214

Yang, J.-L.; Pettersson, B.; Eriksson, K.-E. 1988a: Development of bioassays for the characterization of pulp fiber surfaces. I. Characterization of various mechanical pulp fiber surfaces by specific cellulolytic enzymes. Nordic Pulp and Paper Res. J. 3: 19–25

Yang, J.-L.; Pettersson, B.; Eriksson, K.-E. 1988b: Development of bioassays for the characterization of pulp fiber surfaces. II. Characterization of various mechanical pulp fiber surfaces by a lignin specific peroxidase. Holzforschung 42: 319–322

(Received June 2, 1989)

Prof. Karl-Erik L. Eriksson
The University of Georgia
Department of Biochemistry
Athens, GA 30602
USA