ENZYMATIC BLEACHING OF WOOD PULP

The dark colour of kraft pulp after the wood chips have been heated at high temperature with NaOH solution is caused by lignin (a complex phenolic polymer) still attached to the cellulose fibres. Traditionally this pulp is treated with chlorine to bleach the pulp to make it suitable for paper making. Unfortunately this leads to the formation of some potentially toxic chlorophenolic compounds of low molecular mass and which can polute the environment.

This article discusses the possible use of enzymes to break down chemical bonds in the phenolic polymers and thus make it easier to remove the lignin from the cellulose. Although "Total Chlorine Free" bleaching has not yet been achieved treatment of the pulp with enzymes after the cooking with NaOH does render the lignin easier to extract in the subsequent bleaching steps, allowing a reduction in use of chlorine. World wide many mills now include enzymatic treatment in their processes.

Research on thermophylic organisms growing in New Zealand hot pools is being undertaken to see if they could improve on those organisms currently being used.

INTRODUCTION

The pulp and paper industry offers a good example of the introduction of biocatalysis to a traditional industry. Like many long-standing industries, pulp and paper manufacture has developed more as an art than as a science and much of the chemistry remains a mystery. The difficulty of interpreting the chemistry underlying the various stages of paper manufacture is exacerbated by the complexity of the starting material - wood. Wood is not a simple, single molecule but is a mixture of complex organic polymers that each have an effect on the final product and each take part in the chemistry of the process. Identification of enzymes which are capable of assisting in the reactions has been a difficult challenge but a worthwhile one primarily because of serious environmental problems caused by the chemical process. At present, global production of chemically manufactured pulp stands at around 100 million tons per year and this figure is likely to increase, despite the trend toward greater utilisation of non-chemical methods and recycled paper. Political pressure from environmental groups and consumers has had a major impact on the industry in many western countries. Ironically, much of the environmental damage is caused by the same consumers having a preference for more attractive, bleached, paper products. The chemical treatments used in chlorine-based bleaching procedures produce harmful chlorinated organic compounds that are sometimes released into the environment. These chlorinated compounds are difficult to recycle and as a result, they are discarded - in some cases directly into nearby lakes and rivers. Additional pressure has fallen on the industry because of fears that some of these compounds (such as dioxin) may escape extraction steps and contaminate paper used for food packaging. The pulp and paper industry is faced with having to change its ways. It is reluctant to do so because the chemical processes are cheap, effective and efficient; but they are also dirty and public tolerance is waning.

CONVENTIONAL METHODS

Bleaching

The complex chemical bleaching procedures used to treat wood pulp are primarily to remove lignin, the coloured component of wood fibre. Lignin gives mechanical load bearing strength to plants and so is present in large amounts in wood with softwoods containing 26-32% and hardwoods 20-28% lignin. Lignin is a complex phenolic polymer which is essentially random in its overall structure and this complexity makes it a difficult target for enzymatic digestion. **Figure 1** is schematic formula of a lignin.

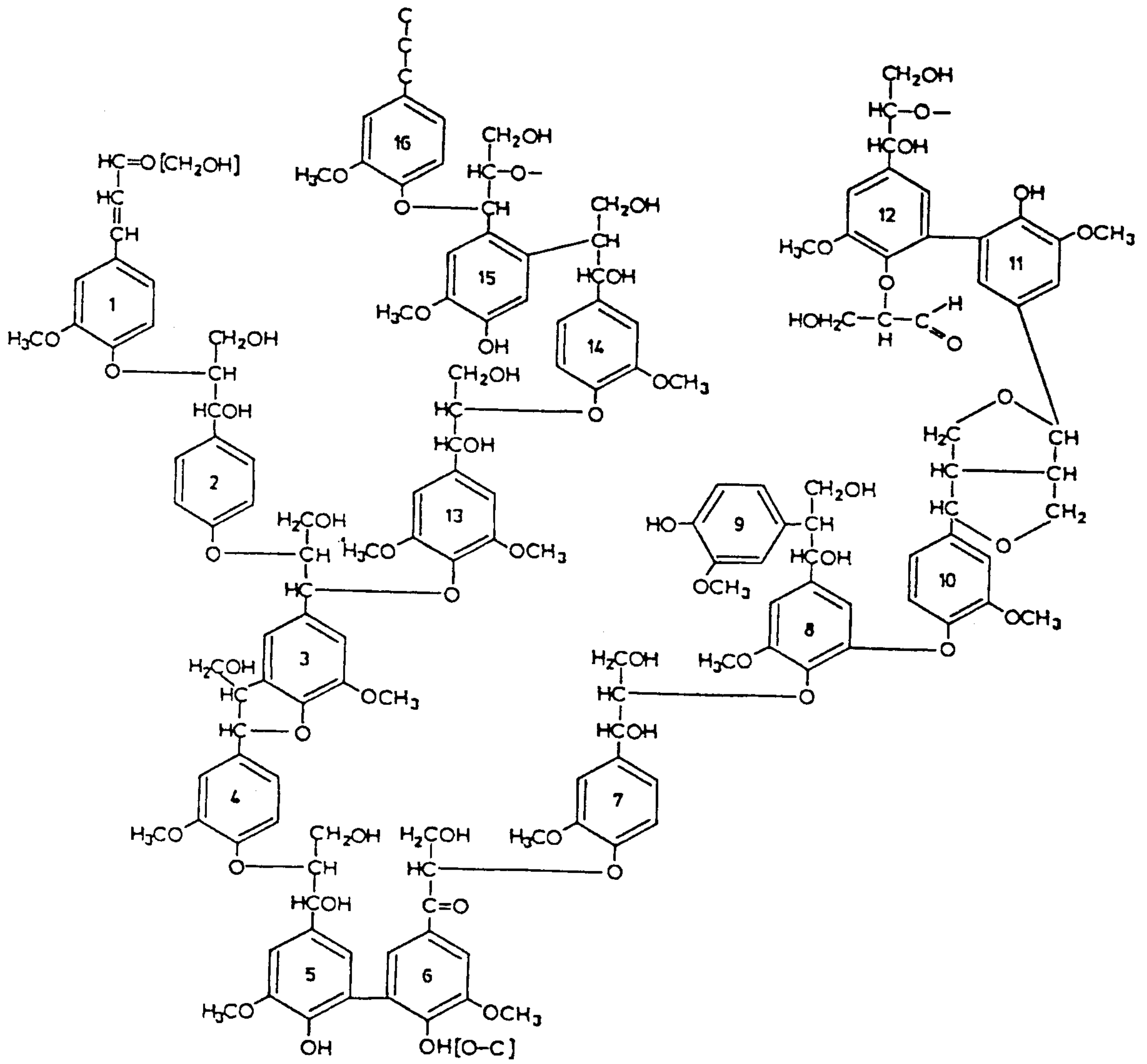

Figure 1. **Schematic formula illustrating the major types of linkages between the phenylpropane units of lignin. The types of linkages between the numbered benzene rings, and their approximate percentage occurrence are: aryl glycerol-β-aryl ether, 48%, 1-2, 2-3, 4-5, 6-7, 7-8; non-cyclic benzyl aryl ether, 6-8%, 15-16; phenylcoumaran, 9-12%, 3-4; biphenyl, 9.5-11%, 5-6, 11-12; 1,2-diarylpropane, 7%, 8-9; diaryl ether, 3.5-4%, 8-10.**

The lignin molecules are formed in plant cells by randomly coupling radical species which arise from the peroxidase-mediated oxidation of the phenylpropane compounds *p*-coumaryl-, coniferyl- and sinapyl-alcohol and cinnamic acid, **Figure 2**. The lignin complex is thought to be covalently linked to cell wall polysaccharides by ether bonds, and possibly phenol-uronic acid ester bonds.

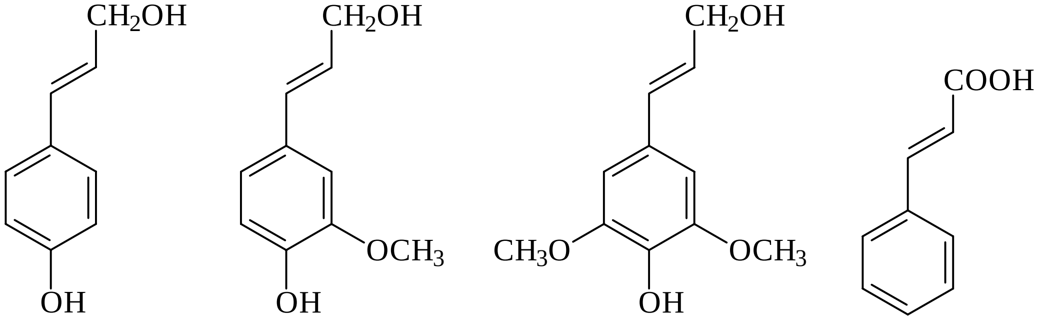

Figure 2 - The major phenylpropane subunits found in plant lignin polymers

Harmful organic chlorides result almost entirely from reactions between lignin and the chlorine (Cl_2) used to remove it. Most of the chlorine incorporated in the early steps of the bleaching process is displaced by OH^- ions in subsequent alkaline extractions but some remains as both polymeric chlorolignin (75%), and in low molecular weight chlorophenolic compounds. Conventional pulp bleaching of softwoods produces about 5 kg of organically bound chlorine per tonne of pulp, with almost all of this discharged as effluent. Some governments are now imposing limits on the allowable discharge from pulp processing plants. Limits are usually expressed in terms of ‘adsorbable organic halide’ quantities that are released as waste. (Adsorbable Organic Halides are referred to in the trade as ‘AOX’). Typical goals are 1.5 to 2.5 kg AOX per tonne of pulp produced, but the Swedish National Environmental Protection Board is proposing further reductions to 0.5 kg per tonne by the year 2004 and to 0.1 kg per tonne by the year 2010. Several measures have already been taken to reduce the amount of AOX released into the environment. For example, the incorporation of chlorine dioxide (ClO_2), ozone (O_3) and hydrogen peroxide (H_2O_2) into pulp bleaching sequences has significantly reduced the problem. For the same amount of Cl_2, ClO_2 produces only one-fifth the amount of AOX and more efficient pulping methods prior to bleaching helps to reduce the amount of lignin which reaches the bleaching stages.

Pulping

The most commonly used pulping method is known as the Kraft Process. It has evolved over a period of 100 years to become highly refined and currently, about 70% of the world's annual pulp output is produced by this process. Despite its shortcomings, it is the most cost effective, versatile and efficient wood delignification method available and because of the large amount of capital already invested in kraft pulping, it is unlikely that the process will be replaced in the near future.

The first stage of the kraft process involves cooking wood chips in a solution of NaOH at high temperature (*ca*170 C) and pressure. During a kraft cook of wood chips, a large amount of the lignin in the wood fibres is fragmented and rendered soluble by the cleavage of the ether linkages between the phenyl propane units. The reaction rarely reaches completion, and generally 10-15% of the ether linkages are still present after the cooking process. What remains of the lignin is heavily modified. The dark colour of the pulp results from various compounds including: quinones, complexed catechols, chalcones and stilbenes, all of which absorb visible light to make the pulp brown, - the colour of brown paper and cardboard which is manufactured from pulp taken as far as this stage. Almost half of the world's kraft pulp is subsequently bleached to remove the residual lignin and give white paper. Residual lignin is degraded and dissolved by treatment with various sequences of bleaching and extraction steps which are listed in **Table 1**. The most reactive bleaching chemicals are chlorine, ozone and peroxy-acids, which react with the aromatic structures in lignin. Chlorine dioxide and oxygen react with the lignin subunits that have a free phenolic hydroxyl group, whilst sodium hypochlorite and hydrogen peroxide react with functional groups such as double bonds. Effective delignification is achieved by sequential treatment with different types of bleaching chemicals, each active on different sites in the lignin molecule.

Table 1. The chemicals used in pulp-bleaching. The symbols are used by the paper manufacturing industry to describe consecutive pulp bleaching stages. For example, CEHH describes a Cl_2 treatment (C), followed by an alkali extraction (E), and two hypochlorite treatments (HH).

Agent	Chemicals	Symbols
Elemental chlorine	Cl_2	C
Alkali Extraction	NaOH	E
Hypochlorite	NaOCl + NaOH	H
Chlorine dioxide	ClO_2	D
Peroxide	Na_2O_2 + NaOH or H_2O_2 + NaOH	P P/E
Oxygen	O_2 + NaOH	O
Ozone	O_3	Z

ENZYMATIC METHODS OF LIGNIN REMOVAL

"Biobleaching" has been examined recently in an effort to eliminate chlorine altogether. Countless micro-organisms take advantage of the worlds largest source of stored energy, plant biomass, and these organisms produce a broad spectrum of enzymes that catalyse the hydrolysis of the various components that are present in plant material. By identifying those enzymes which target specific components of wood fibre, it should be possible to find an enzyme (or enzymes) which assist in the removal of lignin. At present, enzymes are being considered as only partial replacements for chemicals, but there is hope that as the mechanisms of enzymatic treatment are elucidated, refinements in the process may allow enzyme assisted "Total Chlorine Free" (TCF) bleaching sequences to become an effective and economic method of pulp treatment.

Several different biocatalysis approaches have been trialed. The most successful method is known as "biobleaching". Biobleaching involves the treatment of cooked pulp with enzymes isolated from naturally occurring bacteria and fungi, or with enzymes produced by genetically engineered bacteria and fungi. The most obvious line of attack would be to find enzymes which degrade the lignin itself. Lignin is metabolised by several fungi and bacteria in processes that are thought to be a combination of oxidative and reductive reactions involving aromatic ring cleavage, cleavage of propyl side chains and reduction in methoxyl content. The most extensively studied enzymes are: lignin peroxidases, manganese-dependent peroxidases, laccases and enzymes that produce hydrogen peroxide. The chemistry, however, is inherently complex. It involves oxidative and reductive enzymes, cofactors and chelators, and also depends on several enzymes working other in biochemical pathways. This complexity has limited the success of most trials using a cell-free, enzymatic lignin-degradation system.

A far more effective strategy is to attack hemicelluloses, a group of heteropolymers implicated in reducing the solubility of lignin. The most thoroughly researched hemicelluose degradation system involves xylan, a polymer of the sugar xylose (**Figure 3**) which is degraded by endo-1,4-ß-xylanases (although also of importance is a second polymer mannan, a polymer of mannose, which is present in many of the softwoods used in the Southern Hemisphere as the raw material for paper).

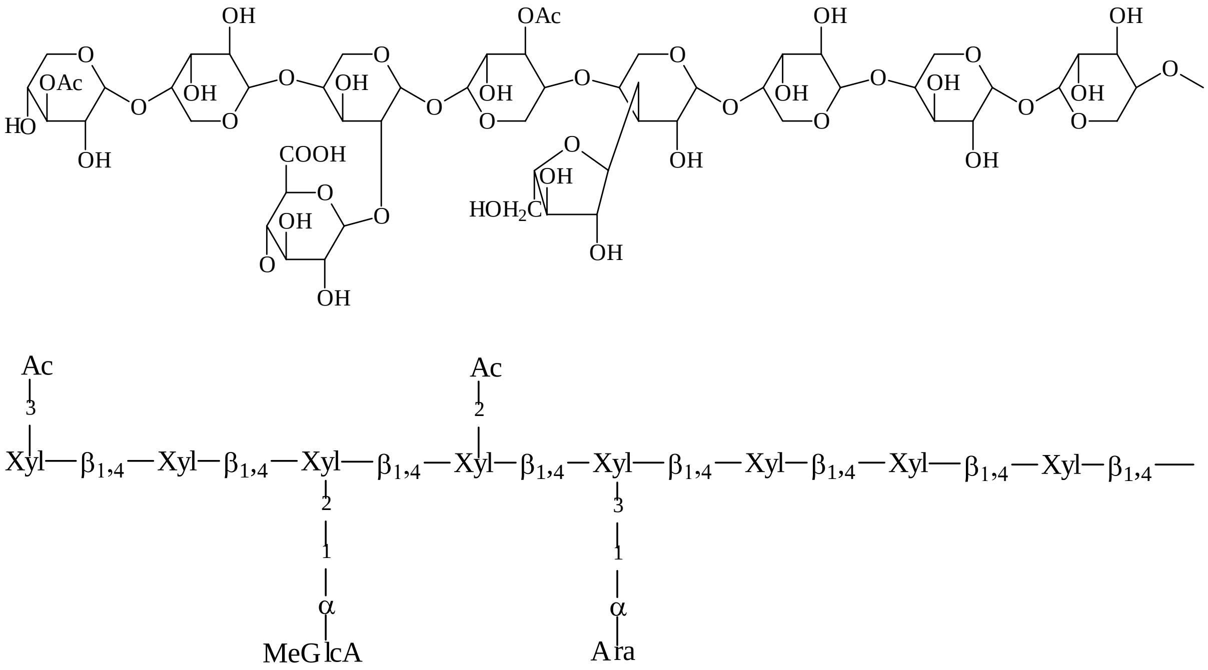

Figure 3. **The structure of xylan, a hemicellulose polymer of xylose sugar. Molecules of xylan are substituted to a varying degree with side-groups of acetyl (Ac) , arabinose sugars (Ara) and α-methylglucuronic acid (MeGlcA). Endo-1,4-ß-xylanase cleave the backbone internally at alternate ß1,4 glycosidic bonds to produce after complete hydrolysis mainly xylobiose dimers.**

The ability of xylanases to facilitate the bleaching of kraft pulp was first reported in 1986 by the Finnish group led by Dr Lisa Viikari. Crude xylanase preparations from *Aspergillus awamori* and *Streptomyces olivochromes* were shown to enhance the peroxide delignification of Birch and (to a lesser extent) Pine kraft pulp. The actual mechanism is still pretty much a mystery. Treating unbleached kraft pulp with xylanase does not, in itself, release significant amounts of lignin. However, the enzyme appears to render the lignin easier to extract in the subsequent bleaching steps and now a number of mills include enzymes in their bleaching sequences. There are two mechanisms that have been proposed to account for action of xylanases:

1. Under alkaline conditions, much of the xylan in wood fibre is dissolved into the liquor. As cooking proceeds and the alkali concentration decreases, the dissolved xylan precipitates back onto the crystalline cellulose fibres. It is thought that a loss of the side-groups from the xylan chains allows the xylan to re-precipitate in a crystalline form which is less soluble than native xylan and it is this re-precipitated, insoluble xylan that hinders the solubilisation of residual lignin (**Figure 4**).

2. Hemicellulases have also been proposed to cleave hemicellulose bonds near points of attachment between lignin and hemicellulose and it is possible that this leads to the improved solubilisation of lignin.

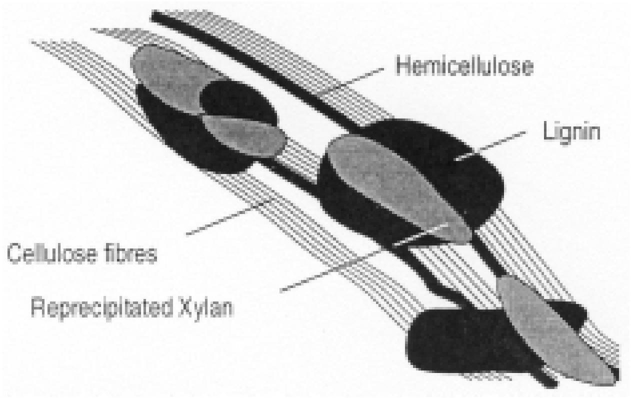

Figure 4. A hypothetical structure of Kraft pulp fibres. Xylanases are thought to promote pulp bleaching *via* the hydrolysis of the re-precipitated xylan.

The addition of a xylanase stage in the bleaching process allows a 30% reduction in chlorine consumption without reducing the brightness or strength of the final paper. In 1992 more 10 mills were reported to use xylanases continuously for improved 'bleachability' of kraft pulps and almost 100 mill trials have been carried out.

The Future of Enzymes in Pulp and Paper Manufacture.
The enzymes currently in use by the pulp and paper industry are less than perfect. Kraft pulping requires high temperatures and high a pH and as most of the currently available xylanases are from mesophilic organisms, they rapidly lose activity at temperatures above 50 C and at pHs above 7. To include the commercially available enzymes in the bleaching stages requires cooling and neutralisation. The result is a costly change to the plant, an increased energy consumption and increased process time. Understandably, mill owners are reluctant to embrace this technology. The race is on for finding new enzymes which alleviate at least some of these problems. Many research groups have turned to xylanases and mannases produced by thermophilic organisms and already enzymes are under trial which have excellent activity at a pH of 9 and in boiling water. These conditions don’t quite reach the high expectations of the industry but they are much closer than anything presently available. Recent research with enzymes isolated from thermophilic organisms growing in hot pools around New Zealand,

suggest that these could replace currently available commercial contenders. With some 50 million metric tonnes of bleached pulp being made worldwide each year, it has been predicted that the market for pulp bleaching enzymes could reach US$200 million by the turn of the century. The technology may be considered esoteric by the more traditional industrialists, but a potential reward as high as this has proven a good incentive for the enzyme industry to invest in research. In parallel, the benefits to the environment have convinced many governments to play their part in the funding of Research and Development.

Written by Dr David J. Saul (School of Biological Sciences, University of Auckland, Private Bag 90219, Auckland, New Zealand), Dr Moreland D. Gibbs and Professor Peter L. Bergquist (both of the School of Biological Sciences, University of Macquarie, Sydney, New South Wales 2109, Australia).