

OXYGEN DELIGNIFICATION AS A PRETREATMENT FOR THE ENZYMATIC  
HYDROLYSIS OF LIGNOCELLULOSIC MATERIAL

by

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## ABSTRACT

With the increasing importance of environmental conservation and the inevitable depletion of the world's fossil fuel supplies, it is necessary to develop renewable and clean sources of energy. Bioconversion, the process of converting plant biomass to fuel grade ethanol, is one process that addresses these concerns. However, despite promising results, the development of a more feasible pretreatment process to be used upstream of enzymatic hydrolysis is required. Oxygen delignification has long been successfully employed in the pulp and paper industry to remove the lignin from pulp samples. Since the general aim of a pretreatment process is to disrupt the close relationship between lignin and cellulose, a study was taken to evaluate the effectiveness of oxygen delignification in this regard.

Softwood Kraft pulp samples were used as a model substrate to determine the potential effectiveness of oxygen delignification. In the initial phase of the study, hemlock pulp samples were taken upstream (kappa number of 34) and downstream (kappa number of 16) of the oxygen delignification unit in a commercial pulp mill. When subjected to enzymatic hydrolysis under standard conditions, the initial rate of sugar saccharification and the final yield of sugars improved by 62 – 82% and 76 – 80%, respectively, for the oxygen delignified sample. These results were sufficiently promising to carry out a more detailed laboratory-scale study.

In an effort to optimize the delignification conditions, lab-scale tests were carried out in a 750-mL reactor over a range of temperature, oxygen partial pressure, retention time and caustic loading based on a factorial experimental design, measuring the kappa number and pulp viscosity as response parameters. The dependence of the kappa number and pulp viscosity on the oxygen delignification conditions were found to follow a linear model:

$$Kappa = 49.73 - 0.19T - 3.09S - 0.05t - 0.05O$$

$$Viscosity (mPa.s) = 55.91 - 0.03t - 0.19T - 4.06S - 0.02O$$

Where  $t$  = retention time (minutes)

$S$  = caustic loading (% NaOH per gram oven-dry weight of pulp)

T = reaction temperature ( $^{\circ}\text{C}$ )

O = oxygen partial pressure (psig)

The laboratory-delignified samples were subsequently hydrolyzed and analyzed for their susceptibility to hydrolysis. Generally, as the kappa number of the pulp decreased, the final yield of sugars and initial rate of sugar saccharification increased. However it was apparent that there is a point when hydrolysis is maximized and as the lignin content is further reduced (below 1.5%), the rate and extent of hydrolysis decrease.

In all cases, the laboratory-delignified pulps were easier to hydrolyze than the original substrate, and based on these positive results, the effectiveness of oxygen delignification was evaluated using a waste lignocellulosic material. Rejects from the post digestion screens in a sulfite mill (22 – 26% lignin) were chosen for this purpose. A portion of the rejects was mechanically refined and both refined and unrefined samples were subjected to oxygen delignification under a standard set of conditions. The particle size appeared to have an effect on the kappa number after the oxygen delignification since a 20% and 25% decrease was recorded for the unrefined and refined samples respectively. The final yield of sugars increased by 21% when the samples were only refined (not oxygen delignified), and increased by 19% and 58% for the unrefined and refined oxygen-delignified samples respectively, further evidence of the importance of particle size to enzymatic hydrolysis.

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## 1. INTRODUCTION

### 1.1 Biomass Energy

Many social, political and environmental issues have sparked the recent interest in the development of technology for converting biomass to transportation-grade fuel. During the early twentieth century, much of the world's energy was supplied by oil from a few countries in the Middle East. In the 1970s, when it was believed that fossil fuel supplies would be quickly diminished, the price of oil rose accordingly. However, investigations performed by OPEC in the early 1980s established that the alleged oil crisis was misleading and the security of the oil supply was brought to the forefront. Regulatory committees have since been established to monitor the price of oil, and the fear of its immediate depletion is no longer an issue. However, these factors initiated the development of new and innovative bioconversion technologies and are the historical basis for our present interest in biomass energy [1].

A more recent concern is the change in the global climate due to the accumulation of carbon dioxide from the combustion of fossil fuels [1,2,6]. It has been predicted that, if the rate of emission of greenhouse gases is maintained, there will be a 2°C increase in the global temperature by 2100, which could lead to increased sea levels and unusual weather patterns [13]. Urban air pollution is another problem that may be remedied by using biomass-derived fuel. Nitrogen oxides, sulfur oxides and ozone are all byproducts of fossil fuel combustion that are currently being investigated for their effects on the respiratory organs. The United States Department of Energy (DOE) has estimated that bioethanol can reduce the net carbon dioxide emissions from vehicles by 90% when a 95% ethanol/gasoline blend is used. Sulfur oxide emissions and the level of reactive hydrocarbons that form ozone would also decrease if a 95% ethanol/gasoline blend were used for transportation [2].

Biomass is the fourth largest source of global energy [4,8], accounting for 33 - 35% of the total energy used in developing countries [3,4,8] and about 13 - 15% of the global energy consumption [4,8,13]. In the US, the use of biomass energy currently stands at about 4% [3,4], while biomass contributes 7 - 8% of the total energy consumption in Canada [3,13,48]. The processes employed to harness the energy contained in biomass are as diverse as the plants from

which the energy can be derived. Biomass can be burnt directly for heat and electricity or converted into gaseous fuels such as methane and hydrogen by gasification. It can also be broken down into its simple sugars and fermented using yeast to produce bioethanol or digested by bacteria to produce biogas [4]. Figure 1.1 summarizes the technologies that have been developed to date to convert biomass into energy.

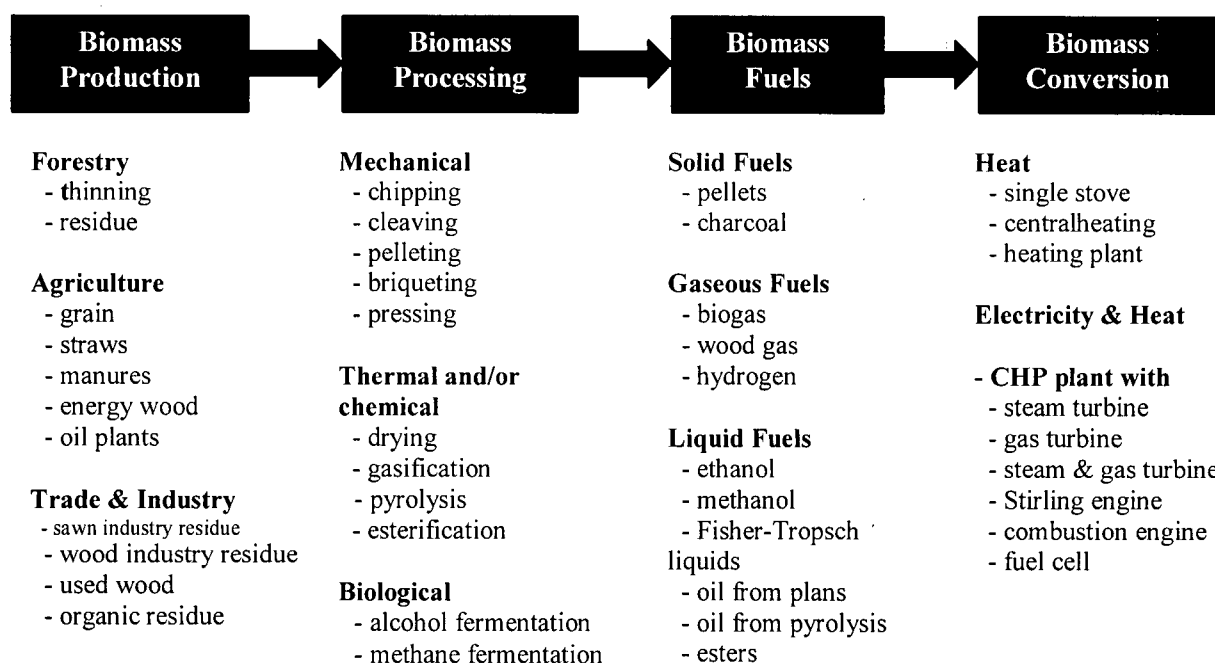


Figure 1.1 Methods for converting biomass to useful fuel [5]

### 1.1.1 Potential sources of biomass for conversion to ethanol

The sources of biomass that can be used for producing energy fall into four main categories: agricultural wastes, forest residues, municipal and industrial wastes and energy crops [3,6].

Most of the agricultural wastes generated in the United States are derived from wheat straw, corn stover (leaves, stalks and cobs) and orchard trimmings. Corn stover alone has a potential harvest of 120 million dry tons [6], while the amount of straw from wheat varies between 1.5 to 4 tonnes per tonne of useful grain [7]. Agricultural wastes are usually plowed back into the ground, but it is possible to claim between one-half to two-thirds for the production of ethanol. The actual grains (wheat, corn, oats, barley, etc.) are also well suited for conversion into ethanol because of their high carbohydrate contents, and although they are generally used as food, it is possible to

increase the current production to accommodate potential conversion processes. Corn is now widely used for the production of fuel ethanol in the US and similar technologies are being developed to convert other grains into useful fuel [7].

Forestry residues, comprised of underutilized wood and logging residues such as bark and sawdust, imperfect commercial trees and trees from thinning of fire-prone forest [6], are also potential feedstocks for bioconversion. In the forest industries, the quantity of wood that ends up as pulp or lumber is considerably less than the quantity of wood that is harvested. Imperfect trees, tops and trimmings, sawdust, shavings, blocks and other sawmill wastes, are either burnt for onsite energy production or landfilled. In Canada, about  $39 \times 10^6$  tonnes of mill and logging residues are available annually for conversion to bioenergy [7,48].

About  $60 \times 10^6$  tonnes of municipal solid waste and  $90 \times 10^6$  tonnes of industrial waste are generated globally each year [8]. The large volumes have caused the use of landfills to become quite expensive and in some cases, landfills have been closed due of their inability to meet the stringent regulations required for their operation [6]. Thirty seven percent of municipal solid waste is paper, which could potentially be converted into bioethanol by fermentation and another 30% is organic matter such as food, which could be converted to biogas or methane [7].

Energy crops are grown specifically for fuels and include shrubs and grasses, as well as many fast-growing trees such as poplar, willow and eucalyptus [6,7]. They can be grown on spare acreage such as surplus agricultural lands, riverbanks and in natural forests and wetlands and, while trees can only be harvested every ten years, shrubs and grasses require a shorter time period [6]. With proper crop rotation and harvesting, it is possible to run a biomass conversion facility most of the year.

## **1.2 Biomass composition and chemistry**

Lignocellulosic material is made up of cellulose, lignin, hemicelluloses and extractives. Each component has a specific function in the plant and their relative quantities depend on the source of the biomass. Both softwoods and hardwoods generally have similar cellulose contents (about 40 - 45%) but softwoods usually contain less lignin and more hemicelluloses than hardwoods

(Figure 1.2). Other compounds (mainly extractives) make up less than 5% of the dry mass in both types of wood [11].

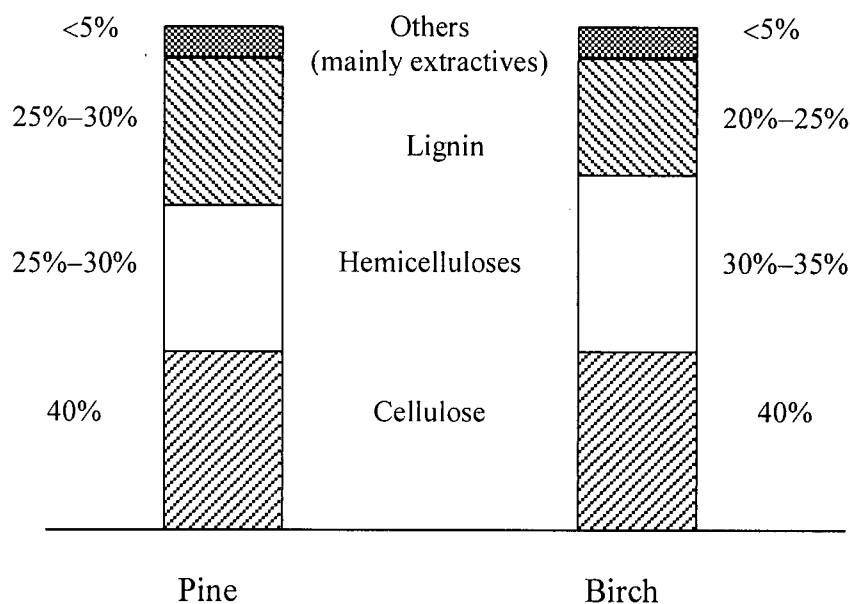


Figure 1.2 Comparison of the composition of softwoods and hardwoods [11]

### 1.2.1 Cellulose

Cellulose is a linear polymeric molecule made up of repeating units of cellobiose, a dimer of glucose, joined via  $\beta$ -1,4-glycosidic linkages. The cellulose molecules native to wood generally have degrees of polymerization (DP) between 8 000 and 10 000 (expressed in terms of glucose) [7,10,11], but it is possible to find cellulose molecules with DP as high as 10 000 to 15 000 in young cotton bolls, the purest form of naturally occurring cellulose [7,11].



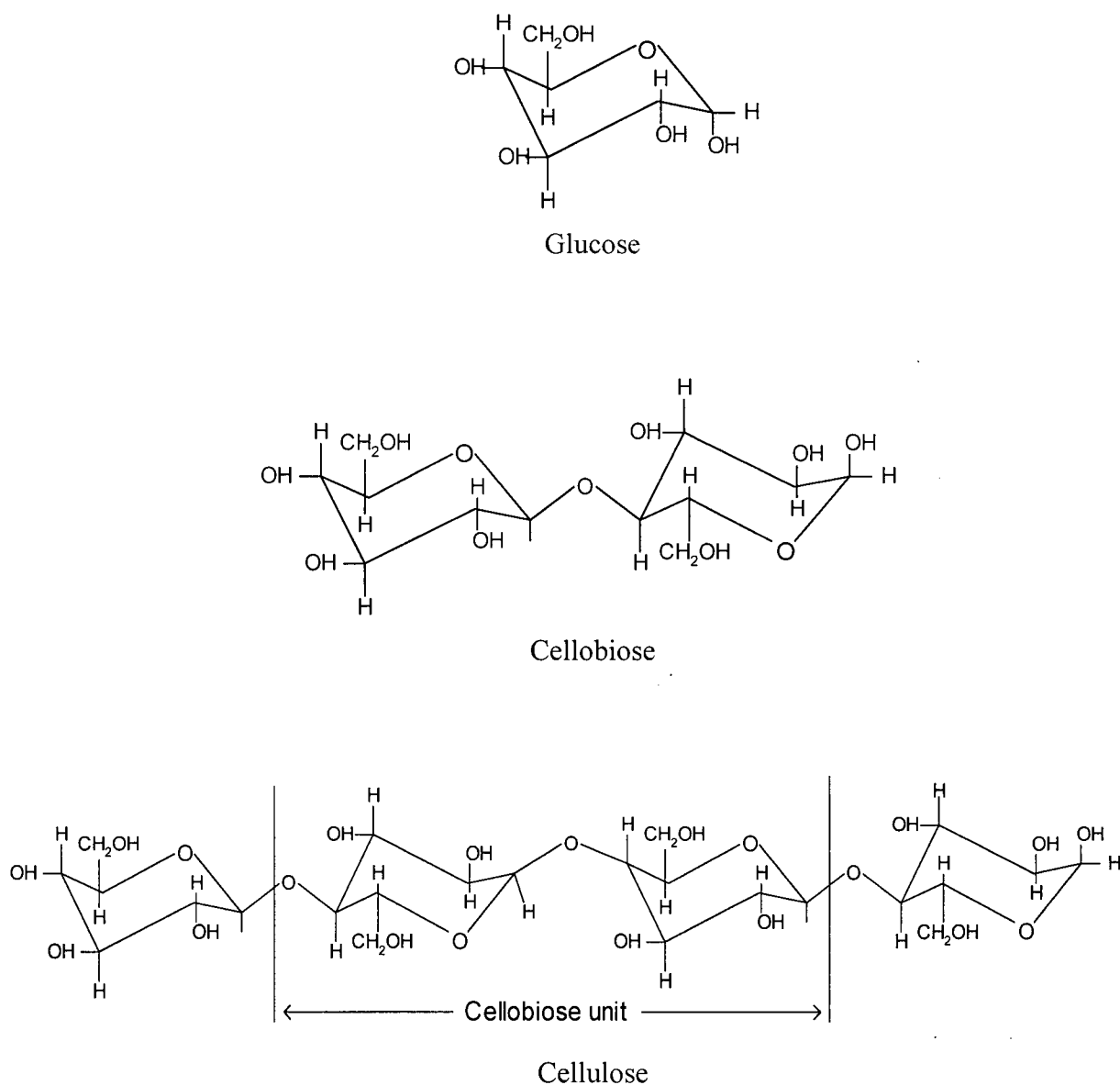


Figure 1.3 The chemical structures of glucose, cellobiose and cellulose [7]

Cellulose molecules have a strong tendency to form hydrogen bonds between functional groups within and between glucose molecules and, as a result, cellulose molecules are generally bundled into small groups called microfibrils [11,12]. The orientation of cellulose molecules in these microfibrils can be highly ordered, giving rise to a crystalline region, or it can be a more random orientation, giving rise to amorphous regions. The crystalline regions exhibit a higher degree of hydrogen bonding and generally constitute about 60 to 75% of the total cellulose in wood, which causes cellulose to be highly unreactive [7,11,12]. It is insoluble in water and will only dissolve

in a few alkaline solvents such as cupriethylenediamine (CED) and cadmiumethylenediamine (Cadoxen) [7,11,12]. Microfibrils form the building blocks of larger bundles of cellulose called fibrils, which in turn give rise to cellulose fibres [12], secured in a matrix of lignin and hemicellulose molecules.

It is possible to degrade cellulose into the individual glucose molecules using strong acids, alkaline copper solutions or some naturally occurring proteins (called enzymes) [7]. During this process, the  $\beta$ -1,4 glycosidic bond between the molecules is broken and a water molecule is taken up, hence the term 'hydrolysis'.

### 1.2.2 Hemicelluloses

The other polysaccharide molecules found in wood are collectively termed hemicelluloses. Due to their lack of crystallinity, hemicelluloses are less chemically and thermally stable than cellulose, and consequently are easily hydrolyzed to their monomers [11]: L-arabinose, D-galactose, D-glucose, D-xylose and D-mannose as well as small amounts of L-rhamnose [7,11,12].

Softwood hemicelluloses are made up primarily of galactoglucomannans, accounting for about 20% of the dry weight of many softwood species. Galactoglucomannan is essentially a linear chain of  $\beta$ -D-glucopyranose and  $\beta$ -D-mannopyranose units with some 1,6 – linked  $\beta$ -D-galactopyranose side units [11,12].

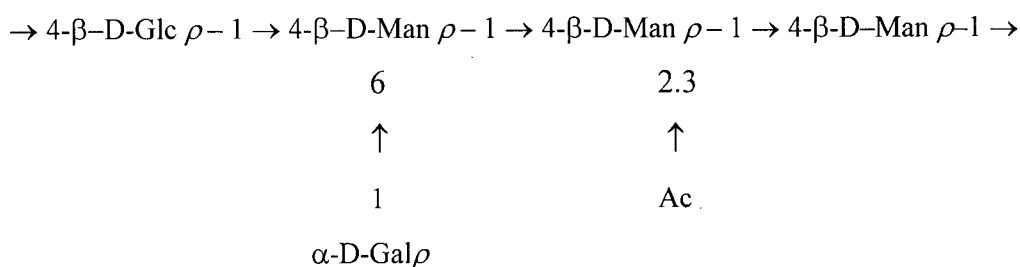


Figure 1.4 Schematic representation of a section of galactoglucomannan [11]

Arabinoglucuronoxylans (xylans) make up about 5 – 10% of the dry mass of softwoods. The main chain is composed of 1,4-linked  $\beta$ -D-xylopyranose with 4-O-methy- $\alpha$ -D-glucuronic acid and  $\alpha$ -L-arabinofuranose side groups [11,12].

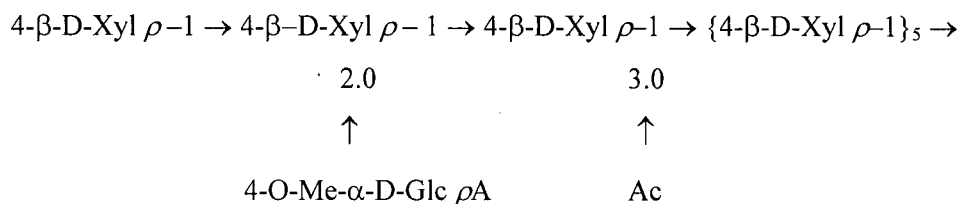


Figure 1.5 Schematic representation of a section of arabinoglucuronoxylan [11]

There are a number of other polysaccharides that occur in softwoods in minor quantities. They include arabinogalactans, starches (amylose and amylopectin) and pectic substances such as rhamnogalacturonans, arabinans, galactans and galacturonans [11,12].

### 1.2.3 Lignin

Lignin is an amorphous polymer composed of phenylpropane units with no seemingly systematic order. Native lignin is mostly made up of three precursors or building blocks: coniferyl alcohol, sinapyl alcohol and coumaryl alcohol, and the relative amounts of each precursor depends on the origin of the lignin (Figure 1.6). For example, guaiacyl lignins make up close to 95% of the lignin found in softwoods and are derived from *trans*-coniferyl alcohol with the remainder coming primarily from *trans*-coumaryl alcohol. The lignins in hardwoods (guaiacyl-syringyl lignin) however, is 50% *trans*-coniferyl and 50% *trans*-sinapyl alcohol [11,12].

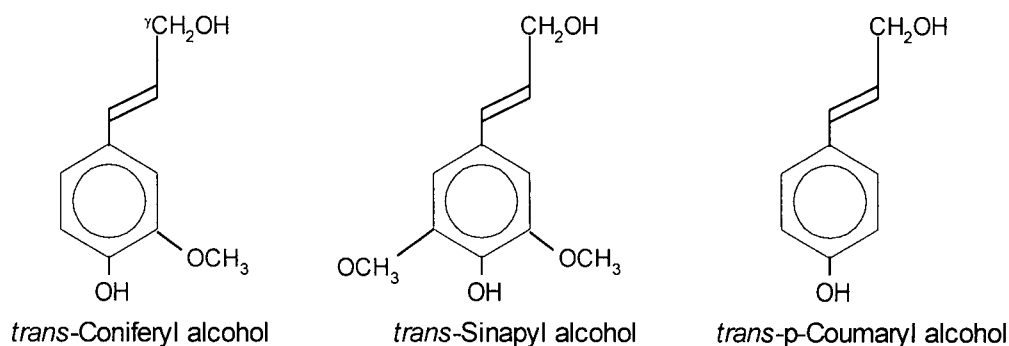


Figure 1.6 The three precursors of lignin [11]

The biosynthesis of lignin comprises a number of complex reactions catalyzed by enzymes, which begins with the generation of the lignin precursors from glucose molecules. The precursors are bound to each other by ether linkages (C-O-C) or carbon-carbon (C-C) bonds, with the ether linkages accounting for over two thirds of these bonds [12]. Figure 1.7 illustrates typical spruce lignin, showing the many ether and carbon-carbon bonds between lignin precursors.

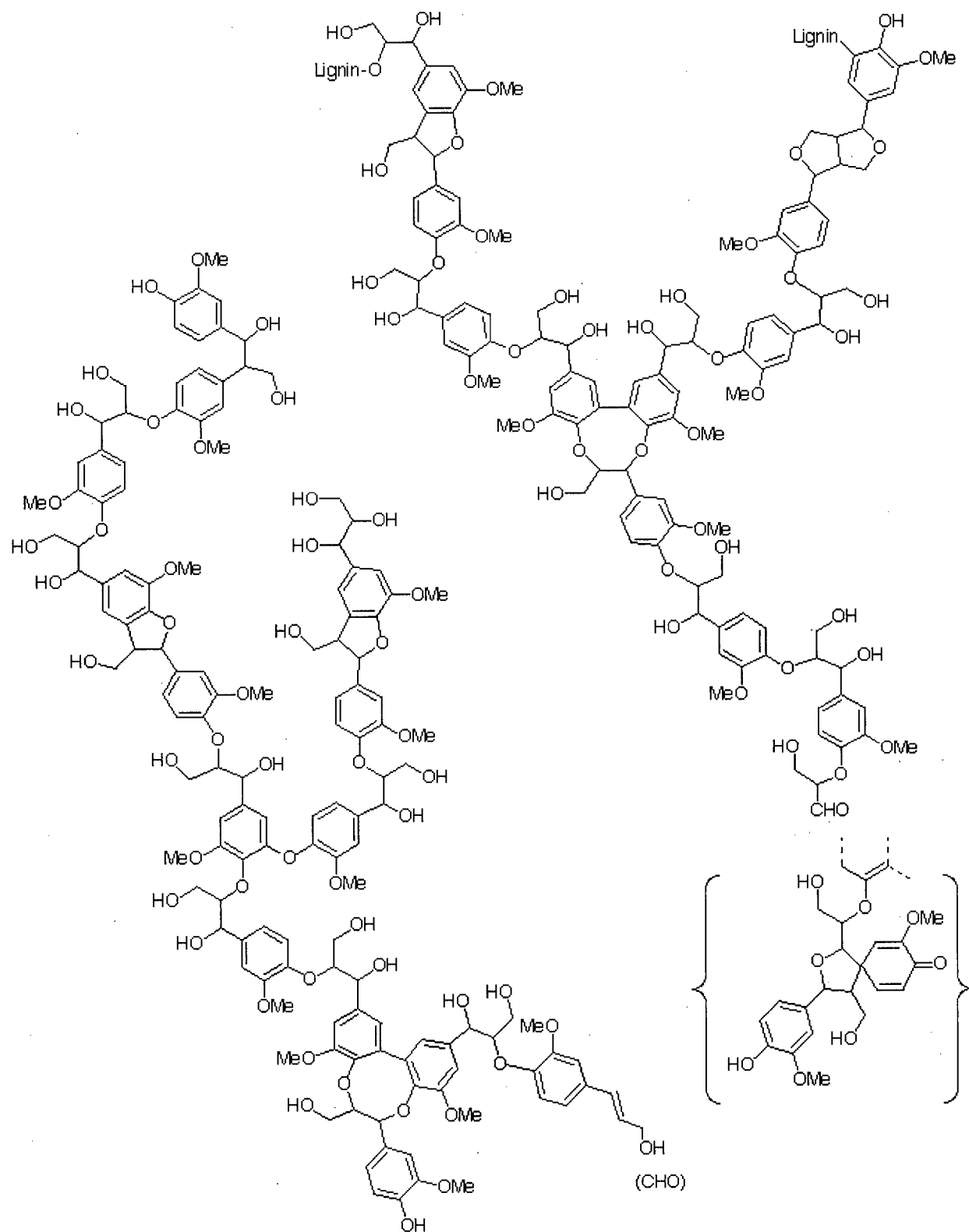


Figure 1.7 Chemical structure of a section of softwood lignin [49]

Reactions with lignin are difficult to initiate and although it is possible to separate lignin from the rest of the biomass components, this is usually accompanied by some degradation. For this reason, the actual molecular weight of lignin is still unknown although many methods have estimated the molecular weight of softwood lignin to be in the range of 15 000 to 20 000 Daltons [12].

#### **1.2.4 Extractives**

Biomass also contains a small amount of low molecular weight compounds that are very easily removed with water, alcohol, ether or benzene. Thus, extractives may be either hydrophilic (able to dissolve in water) or hydrophobic (able to dissolve in common organic solvents) [11,12]. The hydrophobic compounds, excluding phenolics, are collectively termed “resins” and they impart the odour, flavour and some of the colour that we associate with wood, as well as provide protection against attack from insects and microbiological organisms. The water-soluble extractives, called gums or industrial gums, are colorless, odourless, tasteless and non-toxic polysaccharides that are subject to microbiological attack [11].

The extractives content and composition is highly species specific. It usually accounts for less than 10% of the dry weight of lignocellulosic material, but can vary from traces in some species to close to 40% in others [12].

### **1.3 Enzymatic hydrolysis**

#### **1.3.1 The importance of ethanol**

The transportation sector consumes more than 97% of the total fossil fuel consumed in the United States and contributes close to 30%, 67%, 41%, 51% and 23% of total emissions of carbon dioxide, carbon monoxide, nitrogen oxides, reactive hydrocarbons and particulates, respectively [2]. It is evident from these statistics that the development of bioethanol facilities is of particular importance to the transportation industry. This technology could not only reduce the dependence on foreign oil (and hence increase national security), but also yield a considerable reduction in toxic emissions which is a benefit to all residents of urban areas. It has been suggested that ethanol could be used instead of the highly toxic tetra ethyl lead to improve the octane rating of unleaded gasoline [8,26] and can be substituted for methyl tertiary-butyl ether (MTBE) to enhance fuel combustion and reduce emissions [8,46].

In addition to being a source of renewable fuel, ethanol is also the feedstock for many other useful chemicals (Figure 1.8).

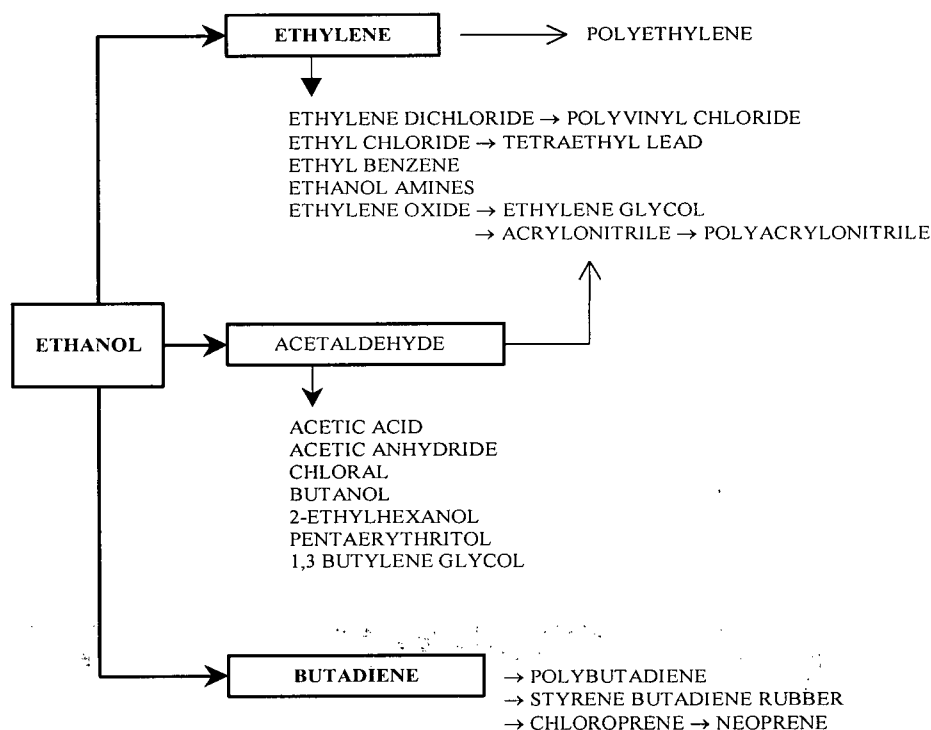


Figure 1.8 Useful chemicals that are produced from the ethanol [12]

Since it is an alcohol, the process for producing fuel-grade ethanol is similar to that employed in the production of beers and wines. During hydrolysis, the carbohydrates in the biomass feedstock are first broken down into their monomeric sugars after which, these sugars are fermented using yeast. The hydrolysis reaction can be effected using strong acids, or enzymes but it is advantageous to employ the enzymatic hydrolysis process because:

- (1) enzymatic hydrolysis has the potential to give higher glucose yields [15,47],
- (2) enzymatic hydrolysis can be performed at temperatures only slightly higher than room temperature ( $50 \pm 5^\circ\text{C}$ ) [3,15,47],

- (3) acid hydrolysis requires more expensive materials to prevent corrosion of the equipment [3,15,47],
- (4) there is no need to neutralize the hydrolysate from enzymatic hydrolysis before fermentation [47].

### 1.3.2 Cellulases

The enzymes that degrade cellulose into glucose (collectively called cellulases) are divided into three groups, based upon their mode of attack and the nature of the substrates on which they act. The simplest model that has been proposed to describe the action of cellulases on lignocellulosic material during enzymatic hydrolysis assumes that endoglucanases randomly attack the cellulose structure [7,15,17], cleaving  $\beta$ -1,4 glycosidic bonds in the amorphous regions [15] and giving rise to reactive chain ends [17] and cellodextrins [7,14]. Exoglucanases cleave cellobiose molecules from the reactive chain ends [15] and convert cellodextrins to cellobiose [7,15,17]. Finally,  $\beta$ -glucosidase (cellobiase), degrades cellobiose, and in some cases, other small cello-oligomers, into glucose [2,7,14,15]. In commercial enzyme preparations such as Celluclast and Novozym 188 from Novozym, North America, it is not uncommon to find enzymes that will also degrade hemicelluloses.

Endo/exoglucanase activity is usually measured in filter paper units (FPU) and is based on the ability of the enzymes to hydrolyze filter paper, a very pure form of cellulose (see Section 4.4).  $\beta$ -glucosidase activity is measured in cellobiose units (CBU), based on the ability of the enzyme to liberate p-Nitrophenol from a p-Nitrophenyl- $\beta$ -D-glucoside stock [45]. Since the inhibitory effects of glucose on cellobiase is minor compared to the inhibitory effect of cellobiose on endo/exoglucanases [14,15,26], it is usual to use at least a 1:2 FPU to CBU ratio during hydrolysis to minimize the overall inhibition of the enzymes.

### 1.3.3 Factors affecting enzymatic hydrolysis

Enzymatic hydrolysis is a heterogeneous reaction - there is an insoluble substrate (the lignocellulosic material) and a soluble catalyst (the enzymes), and the rate and extent of the hydrolysis reaction depend on the physical characteristics of the substrate as well as the specific activity of the enzyme [9].



The hydrolysis reaction has two distinct phases: a rapid initial phase, when the rate of glucose saccharification is greatest, followed by a slower final phase (Figure 1.9).

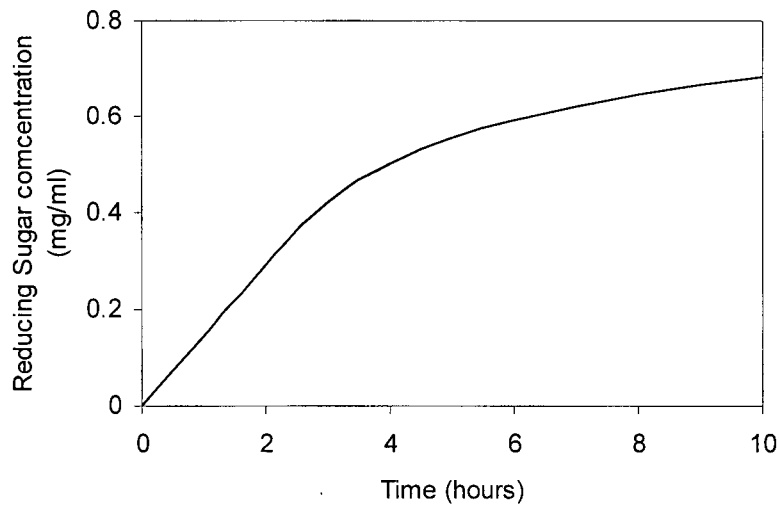


Figure 1.9 Characteristic time course of the enzymatic hydrolysis reaction

The reduction in the rate of hydrolysis as the duration of the reaction increases has been attributed to:

1. depletion of the more reactive, amorphous region of the cellulose early in the reaction leaving behind a substrate that is harder to hydrolyze [17,19,29],
2. enzyme desorption from the surface of the substrate [17,29],
3. enzyme deactivation [15,17,29],
4. lack of residual active sites on the substrate as they are consumed faster than they can be generated [17].

All enzymes have a set of conditions at which their activity is optimized, which depends on the type of substrate and the source of the enzyme. The general operating parameters for many cellulases include a pH between 3.3 and 5.5 at a temperature of  $50 \pm 5$  °C [14,15,16,27]. The temperature is high enough to prevent the growth of most microorganisms, which eliminates possible carbon losses due to contaminant metabolism [15].

The complex nature of cellulose and the lignin-carbohydrate relationship suggests that there are a number of physical parameters that affect enzymatic hydrolysis. Untreated biomass is very difficult to hydrolyze using enzymes and therefore a number of pretreatment processes have been developed to alter the material and hence, make it more digestible [9]. There are two main effects of pretreatment processes on cellulose – they increase the accessibility of the substrate to the enzyme and they increase the reactivity of the substrate [17].

The main obstacle to enzyme penetration is lignin. Lignin forms a “mutually interpenetrating system” [9] with native cellulose and is covalently bonded to hemicelluloses in some areas [11,12]. Hence, the presence of lignin in the substrate inhibits the diffusion of the enzyme to the cellulose fibre and generally, the more lignin in the substrate, the lower the extent and rate of hydrolysis. Many pretreatment processes aim to disrupt the carbohydrate-lignin relationship and hence make the cellulose more accessible by either dissolving the lignin (as in the organosolv pretreatment process [30]) or by separating the lignin from the cellulose (as is the case in steam explosion [26,29]). It is not necessary to remove all the lignin to obtain a significant increase in digestibility, 20 – 65% removal can be sufficient depending on the source of the cellulose [9].

Accessibility can also be improved by increasing the surface area, which can be effected in a number of ways:

1. By reducing the size of the particles as is the case with many physical pretreatment processes. Lee and Fan [21] and Pieterse *et al.* [18] have both reported an increase in the rate and extent of enzymatic hydrolysis as the surface area of the substrate was increased, while Mooney *et al.* [19] found that smaller particles were hydrolyzed more efficiently than larger ones.
2. By fibre swelling. It is possible to increase the surface area of a dry cellulose fibre 100-fold if it is allowed to swell in water [9].
3. By increasing the pore volume Mooney *et al.* [20] reported a significant improvement in substrate digestibility when the pore volume was increased.

The reactivity of cellulose also depends on the chemical characteristics of the fibrils, such as the crystallinity and the number of active sites (cellulose chain ends) on the substrate. Experiments

performed by Sinitsyn *et al.* [22] have shown that the rate and extent of enzymatic hydrolysis decreased linearly as the crystallinity index of the substrate was increased. Bertran and Dale [24] also found that a lower initial crystallinity equated to a higher extent of hydrolysis.

Increasing the initial number of active sites on the substrate will generally cause an increase in the rate and extent of the reaction. In the presence of an adequate supply of reactive chain ends, increasing the enzyme concentration will also improve hydrolysis. However, when the enzyme occupies a large percentage of the surface of the substrate, the activity diminishes so that the rate of hydrolysis is not proportional to the enzyme loading, likely due to steric hindrance or competitive adsorption between enzyme molecules [17,23].

#### **1.4 Enzymatic hydrolysis pretreatment processes**

There is a number of mechanical, chemical and biological processes that have been developed to improve enzymatic hydrolysis. For commercial purposes, the chemical processes are the most practical since they generally require the least reaction time to give an adequately modified substrate.

##### **1.4.1 Physical pretreatment methods**

The shearing forces of the milling processes are very effective at breaking down lignocellulosic material, thus increasing the surface area, reducing the degree of crystallinity and decreasing the degree of polymerization. However, in order to obtain sufficiently high levels of hydrolysis, the milling process must be prolonged for days and is therefore not commercially feasible [9,26].

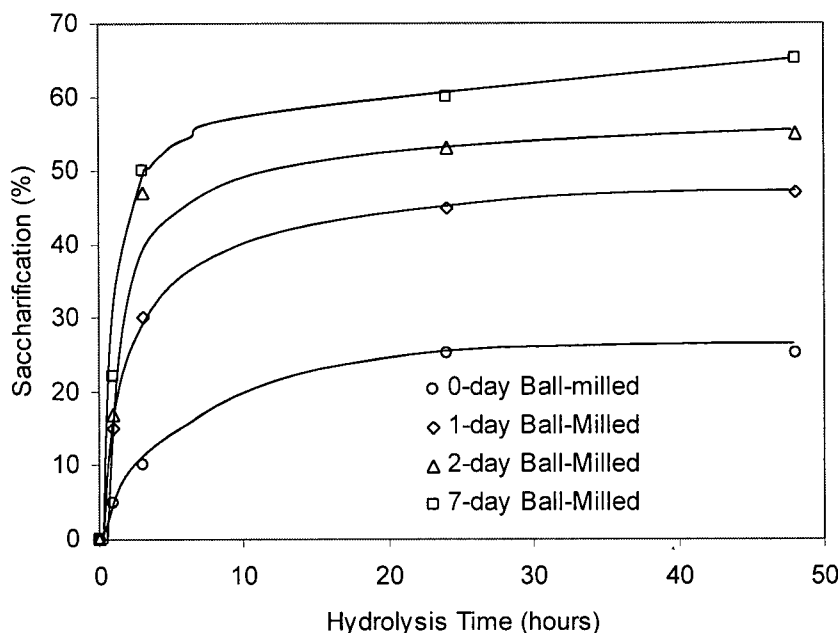


Figure 1.10 Dependence of enzymatic hydrolysis of ball-milled newspaper on milling time [9]

#### 1.4.2 Biological pretreatment methods

White-rot fungi have been used for the biological pretreatment of lignocellulosic material and was found to effectively degrade lignin by the action of three enzymes: laccase, manganese peroxidase and lignin peroxidase [9,25,43]. Like the physical pretreatment, biological pretreatments using the actual fungi take several days to weeks to produce significant delignification although the process can be sped up if the purified enzymes are used directly [25].

Lignin peroxidase was the first of the three enzymes to be isolated from the white-rot fungi, but was found to be relatively ineffective when applied as a delignification treatment. Manganese peroxidase (discovered in 1984) and laccases were found to be a better alternative since they gave significant delignification and improvements in brightness. However, both enzymes require the presence of  $H_2O_2$  to be stable and even then the manganese peroxide is quickly deactivated [43].

### 1.4.3 Chemical pretreatment methods

The National Renewable Energy Lab (NREL) and the United States Department of Energy (DOE) in the United States have spent a great deal of time and money to evaluate the effectiveness of a batch dilute acid pretreatment for enzymatic hydrolysis [2]. The general process uses a 0.5% (w/w) sulfuric acid solution which is added to the wood chips and heated to between 140 to 160°C for five to twenty minutes [26]. A diverse array of potential feedstock (ranging from wood to agricultural residues) have been evaluated using the process [2], the result being that most of the hemicellulose is hydrolyzed leaving a porous lignin-cellulose network that is more susceptible to attack by cellulase enzymes [26].

Steam explosion is also attractive as a pretreatment process because it produces a cellulose fraction that is easily hydrolyzed, the energy considerations are considerably less than dilute acid pre-hydrolysis and there is minimal environmental implications [15]. It is one of the most cost-effective processes for the pretreatment of hardwood and agricultural residues [15], and it allows for the recovery of most of the hemicellulose fraction [42]. In this process the lignocellulosic material is heated with steam to temperatures ranging from 185 – 210°C for 5 to 20 minutes, or 240 – 260°C for 20 seconds followed by explosive decompression [26]. It has further been shown that the digestibility of lignocellulosic material could be improved with the addition of a small amount of an acid (sulfuric acid or sulfur dioxide is normally used), and additionally, the process became more suited for use on softwood substrates [15]. In the steam explosion process, like acid pre-hydrolysis, the hemicellulose fraction is hydrolyzed and solubilized leaving a solid mass of partially depolymerized lignin and cellulose [26,29]. Grous *et al.* [28] was able to achieve a 90% conversion of wood chips treated by the steam explosion process compared to 15% for the untreated wood after 24 hours.

Ammonia Fibre Explosion (AFEX) pretreatment is a fairly recent development which is very similar to the steam explosion process. The lignocellulosic material to be treated is reacted with aqueous ammonia between 25 to 90°C under pressure (1 – 2 MPa) for less than 20 minutes followed by explosive expansion. The effects of this pretreatment process are very different from steam explosion and acid pre-hydrolysis since virtually none of the hemicellulose is hydrolyzed. However, the cellulose is decrystallized and the surface area available for enzymatic attack is

increased. Consequently, the hydrolysability of an AFEX treated lignocellulosic sample increases [29].

Since lignin is an organic polymer, it seems logical to employ a process utilizing an organic solvent to remove lignin from wood. Alcohol/water solvents have been used to successfully delignify lignocellulosic material for the production of pulp and it was discovered that a highly pure, highly digestible form of cellulose was left behind [30], implying that the process would be suitable as a pretreatment for enzymatic hydrolysis. Organosolv pretreatment is usually carried out at fairly high temperatures (185 – 210°C) in common solvent such as acetone, methanol, ethanol and ethylene glycol [15] in the presence of an acid as a catalyst. Chum *et al.* [30] were able to remove 88 – 92% of the lignin in a poplar sample using the organosolv pretreatment process.

### **1.5 The oxygen delignification process**

Oxygen delignification has been defined as a “method in which a substantial fraction of the residual lignin in unbleached pulp can be removed using oxygen and alkali” [11]. The ability of oxygen to delignify biomass has long been known, but the conditions needed to achieve significant levels of delignification led to severe cellulose degradation and loss of pulp viscosity and strength, parameters that are very important in the pulp and paper industry. However, installations of oxygen delignification systems in pulp mills has rapidly increased since the 1960s when it was discovered that the small amounts of magnesium salts could minimize the damage to cellulose [12,31,32,34,44]. Although the system demands a high initial capital cost, the operating cost is much lower than the conventional chlorine bleach plants since oxygen is cheap and readily available, and the required alkali loading is satisfied using oxidized white liquor from the alkali recovery system [31,32,44]. A more important advantage of using oxygen in an increasingly environmentally conscious society is that it can significantly decrease the chemical oxygen demand (COD), biological oxygen demand (BOD) and the adsorbable oxygen halides (AOX) of the bleach plant effluent [11,31,32].

The majority of new oxygen delignification units being installed and currently in use are medium consistency (10 – 14%) systems, operating at relatively high temperatures (85 – 105°C) for about

60 minutes and under pressure (700 - 800 kPa inlet pressure; 450 - 550 kPa outlet pressure). However, before 1983, practically all systems were high consistency (20 - 27%) where the three-phase mass transfer problem is minimized since most of the water is removed [11,31,32,41,44]. Mass transfer is very important to the effectiveness of the oxygen delignification system since there are three phases that must simultaneously interact before the delignification reaction will proceed. Oxygen must be transferred across the gas-liquid interface, through the liquid film surrounding the fibre and finally diffuse into the fibre wall. The low solubility of oxygen in aqueous sodium hydroxide increases the difficulty of the transport [31,37,38]. In high consistency systems, the thickness of the liquid layer through which the oxygen has to diffuse is smaller and the gas-liquid interfacial area is greatly increased meaning that the high consistency systems generally have lower chemical consumption and higher extents of delignification than medium consistency systems [31,33,34]. The trend towards using the medium consistency units stems from the fact that they represent a lower capital cost, there is greater ease of stock handling and pumping, and the selectivity is better than in the high consistency process [31,44]. Table 1.1 below shows the typical operating conditions for high and medium consistency systems.

Table 1.1 Comparing operating conditions of high and medium consistency oxygen delignification systems [32]

	High Consistency	Medium Consistency
Pulp Consistency, %	25-28	10-12
Delignification, %	45-50	40-45
Retention Time, minutes	30	50-60
Initial Temperature, °C	100-105	100-105
Pressure, kPa		
- inlet	500-600	700-800
- outlet	500-600	450-500
Steam consumption, kg/t		
- low pressure (450 kPa)	-	70
- medium pressure (1140 kPa)	75-100	200-300
- evaporator (450 kPa)	30-50	90-110
Power consumption, kWh/t	40-50	35-45
Alkali consumption, kWh/t	21-23	25-28
Oxygen consumption, kWh/t	20-24	20-24
Mg <sup>2+</sup> , kg/t	0.5	0.5

A typical flowsheet of a single-stage medium consistency process is shown in Figure 1.11. Unbleached pulp is washed and charged with caustic, preheated in a low-pressure steam mixer and transferred to a high shear mixture where oxygen is added. The mixture is fed into the base of the reactor which is designed to prevent channeling [31,32,44].

The high consistency system (shown in Figure 1.12) is markedly different from the medium consistency unit. The washed, unbleached pulp is first dewatered down to a consistency of about 30% by a twin-roll press at which point alkali and steam are added to the pulp and transferred to the reactor using a plug screw feeder or a thick-stock pump. Upon entering the reactor the pulp is fluffed and steam and oxygen are added to the top of the reactor to maintain the temperature and required oxygen partial pressure. Combustible gases such as carbon monoxide, methanol and some hydrocarbons are vented from the top of the reactor [31,32,44].



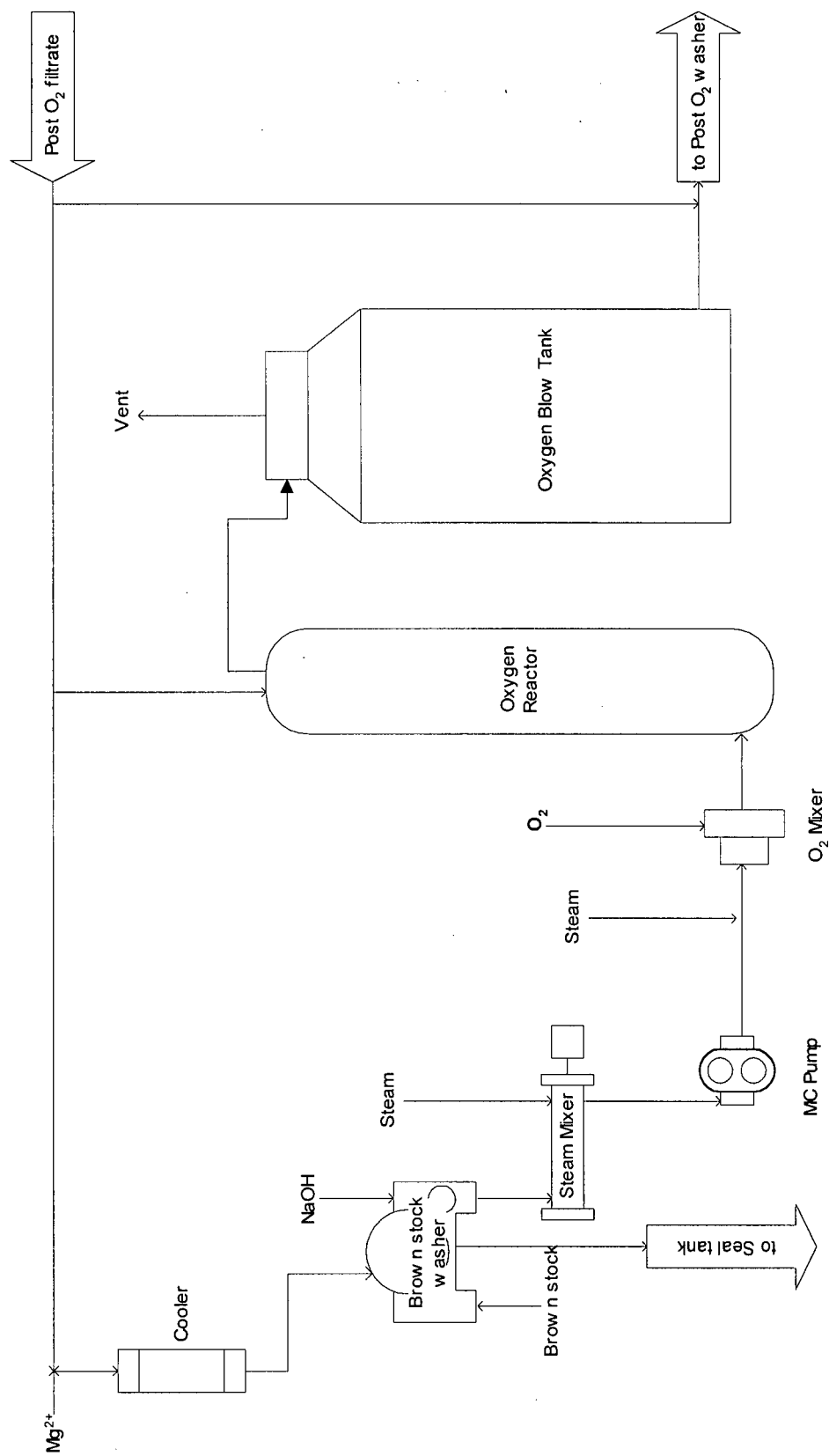


Figure 1.11 Flowsheet of a typical medium consistency oxygen delignification system [32]

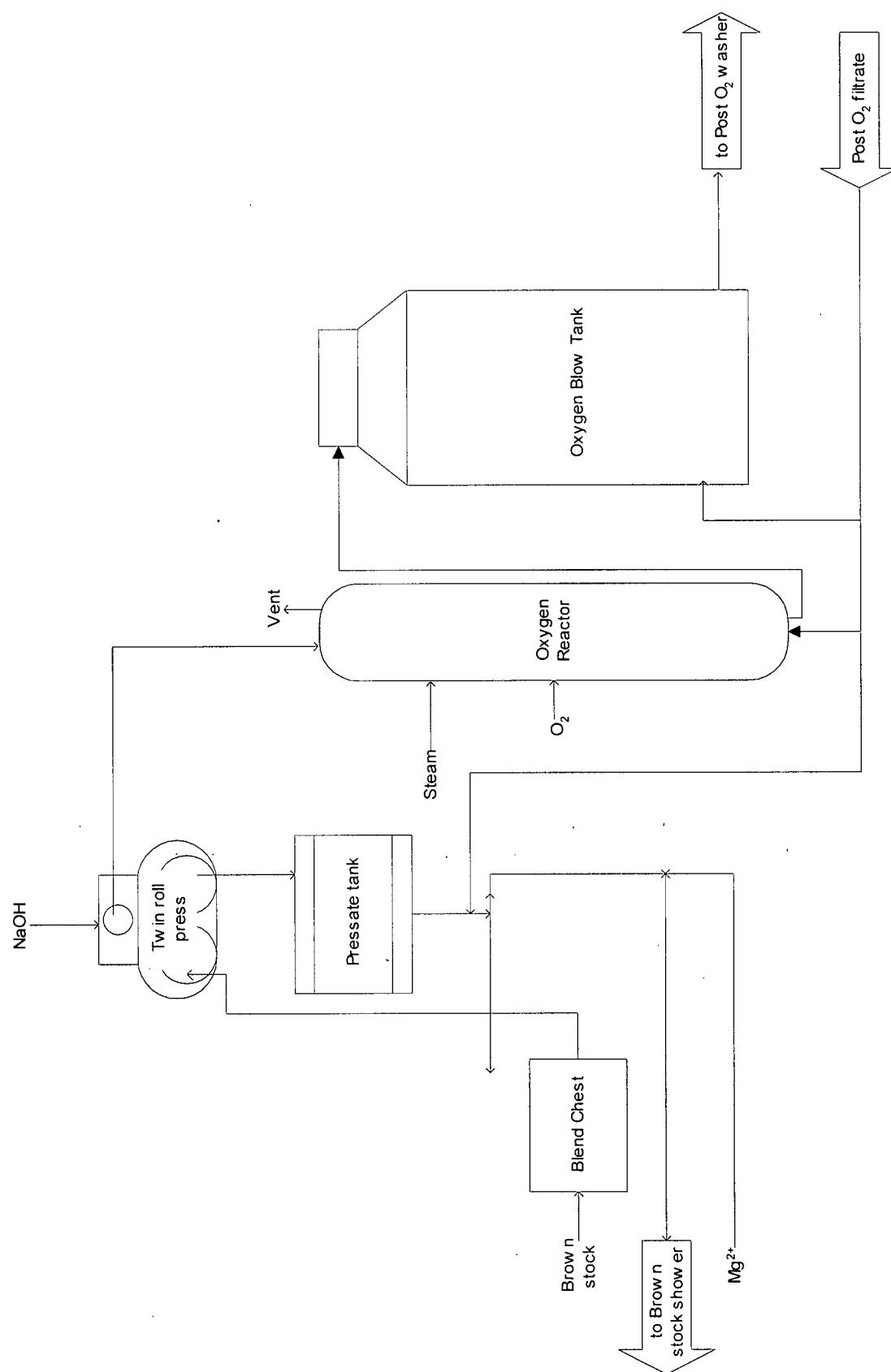


Figure 1.12 Flowsheet of a typical high consistency oxygen delignification system [32]

### 1.5.1 The chemistry of oxygen delignification

Although much research has been done on the chemistry of the oxygen delignification process, the individual reactions are still not yet fully understood. Oxygen molecules exist as a triplet in their ground state, but it is possible to excite the electrons so that there are two paired or unpaired electrons with anti-parallel spins. When oxygen reacts with a substrate, it may be reduced to water by a mechanism including four sequential one-electron transfer reactions giving rise to highly reactive radicals as intermediates [11,12,31].

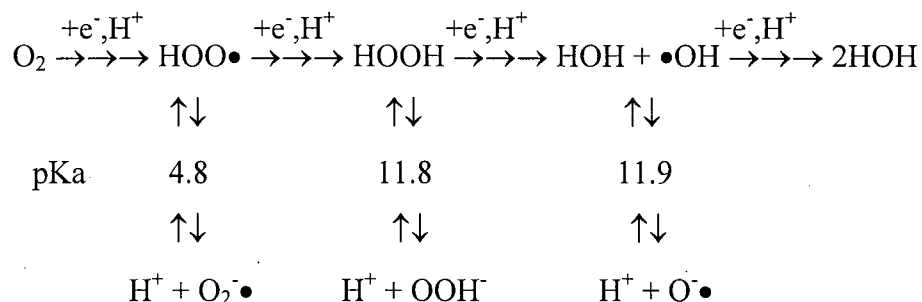
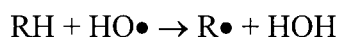
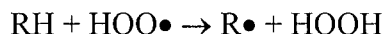
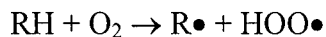
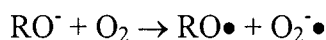


Figure 1.13 Reduction of oxygen to water by one-electron transfer mechanism [31]

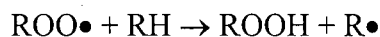
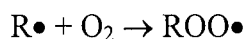
The hydroperoxyl radical ( $\text{HOO}\bullet$ ) is a weak acid ( $\text{pKa} = 4.8$ ) and the formation of its conjugate base is impeded in the alkaline medium of oxygen delignification, but since hydrogen peroxide ( $\text{HOOH}$ ) is a weak acid ( $\text{pKa} = 11.8$ ), both it and its anions are present in solution [11,31]. Finally, the hydroxyl radicals ( $\text{HO}\bullet$ ) formed from the reduction of oxygen are of particular importance since they are non-differentiating [31] and will attack lignin and cellulose to an equal extent.

Due to the number of reactive intermediates formed from the reaction of oxygen with a substrate, the chemistry of oxygen delignification is very complex, but it can be adequately described using the general mechanism of radical reactions [11,31].

#### INITIATION



#### PROPAGATION



#### TERMINATION



Figure 1.14 Mechanism of oxygen delignification reactions [11]

There is always a trace amount of transition metals in pulp (usually, iron, cobalt, copper or manganese) which catalyze the decomposition of hydrogen peroxide to the highly unselective hydroxyl radicals [11,12,31]. These hydroxyl radicals will attack both lignin and cellulose under the conditions of oxygen delignification so a protector compound is usually added to improve selectivity. It is believed that when magnesium salts are added to the pulp mixture during oxygen delignification, they precipitate out as magnesium hydroxide and either form inactive complexes with the transition metal ions, or adsorb them, thus effectively eliminating their catalytic abilities [11,31]. The mill survey performed by Bennington and Pinneault [33] suggested that a loading between 0.05% to 0.25%  $\text{MgSO}_4$  per gram dry weight of pulp is the commercial standard.

### 1.5.2 Lignin reactions during oxygen delignification

Under the alkaline conditions of oxygen delignification, lignin is activated so that a number of electron-rich and electron-deficient sites develop on the molecules. Although the exact mechanism of delignification is not fully resolved, it is clear that the reaction is initiated by the removal of an electron from a phenolic group by a suitable acceptor ( $O_2$  or one of its intermediate radicals) [11,12,31].

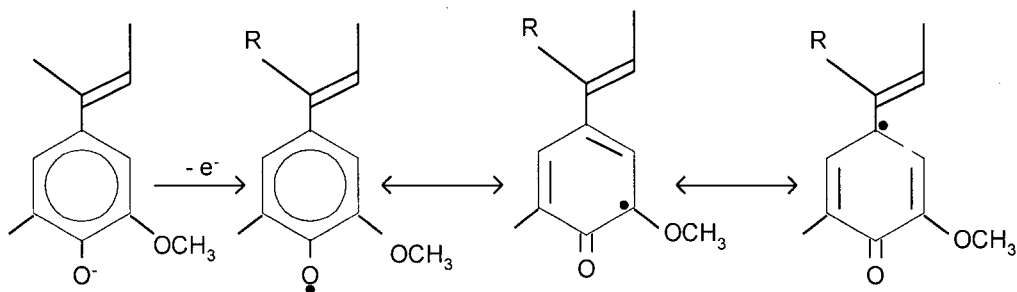


Figure 1.15 Initial lignin reactions leading to oxygen delignification [11]

### 1.5.3 Cellulose reactions during oxygen delignification

During oxygen delignification, cellulose molecules usually undergo two types of reactions: random chain cleavage, which may occur at any point on the cellulose chain and is the more significant reaction, and endwise peeling, where only the ends of the molecules are targeted [11,12,31].

The initial step in chain cleavage is the oxidation of a hydroxyl group to a carbonyl group, followed by a beta elimination reaction that breaks the glycosidic bond [11,31].

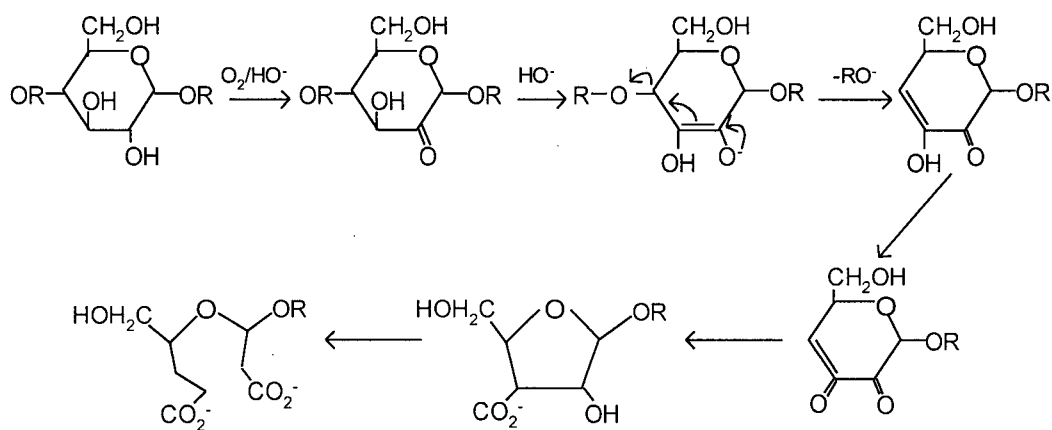


Figure 1.16 Mechanism of cellulose chain cleavage during oxygen delignification [11]

Endwise peeling only occurs on ends containing carbonyl groups (reducing ends), and since the reaction itself produces another carbonyl group, endwise peeling is self-propagating. Under the conditions of Kraft pulping, most of the end units are already stable and free from carbonyl groups and during oxygen delignification, any remaining carbonyl groups are readily oxidized to its alkali-stable form. Endwise peeling is only a problem when chain cleavage is excessive [11,31].

#### 1.5.4 Oxygen delignification process variables

A considerable amount of research has been done to determine the effects of reaction parameters on the degree of delignification and hence optimize the process for the pulp and paper industry. Oxygen delignification depends on five parameters: retention time, reaction temperature, pulp consistency, alkali concentration and oxygen partial pressure. Hartler *et al.* [35], Olm and Teder [36] and Liebergott *et al.* [34] investigated the effect of these parameters on kappa number and viscosity. Temperatures ranging from 80 – 130°C, reaction times of 5 – 180 minutes, caustic loadings between 0.8 – 4.8 % per oven-dried mass of substrate, consistencies between 0.2 – 30% and oxygen partial pressures of 0.1 – 1.0 MPa were employed.

Hartler *et al.* [35] and Olm and Teder [36] determined that there were two distinct phases of the delignification reaction - a rapid initial phase during which most of the lignin is removed, followed by a slower phase (Figure 1.17). This decrease in the rate of reaction has been attributed to caustic exhaustion i.e. less caustic is available to continue the reaction [35]. Previous research has also shown that delignification was accelerated by increasing the temperature [31,35,38] (Figure 1.18).

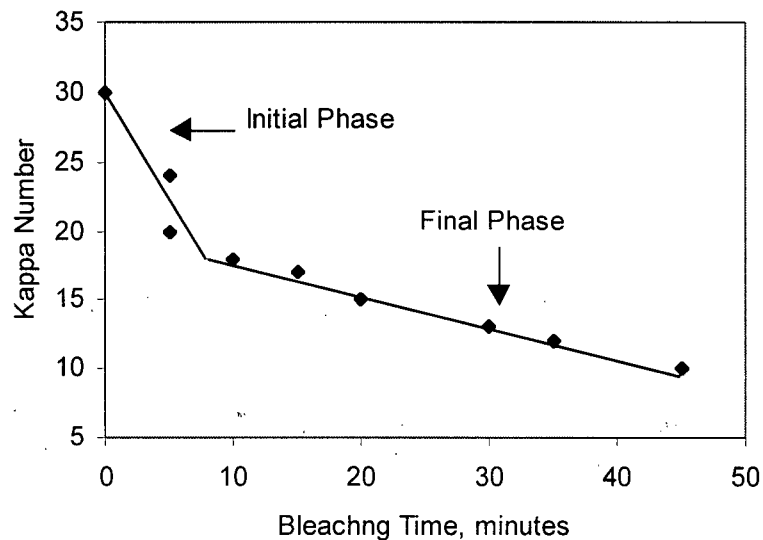


Figure 1.17 Effect of retention time on oxygen delignification as observed by Olm and Teder [36]

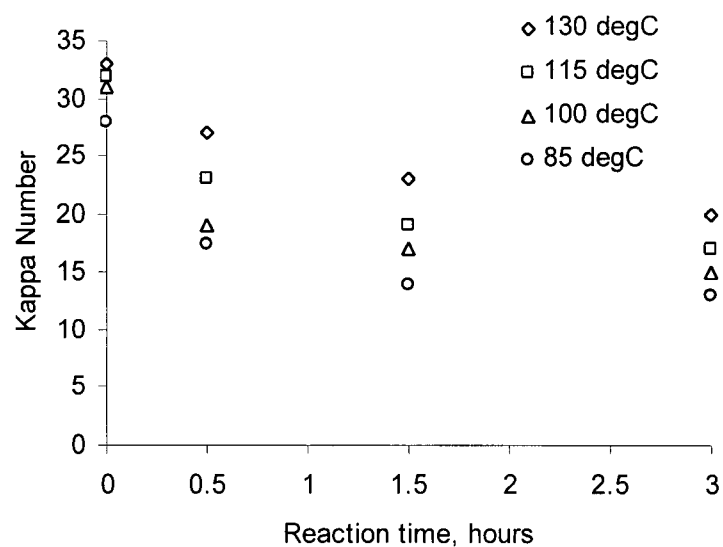


Figure 1.18 Effect of temperature on oxygen delignification as observed by Hartler *et al.* (3.2% NaOH per gram oven-dried pulp) [35]

Liebergott *et al.* [34] compared the effects of oxygen delignification at 7% and 25% consistency and observed that the higher consistency gave better delignification. A similar trend was observed by Olm and Teder [36] when they performed oxygen delignification experiments at 0.3% and 8% consistency. It has also been determined that, for a given reaction temperature, increasing the initial caustic loading caused the rate of delignification to increase (Figure 1.19) [34,35,36].



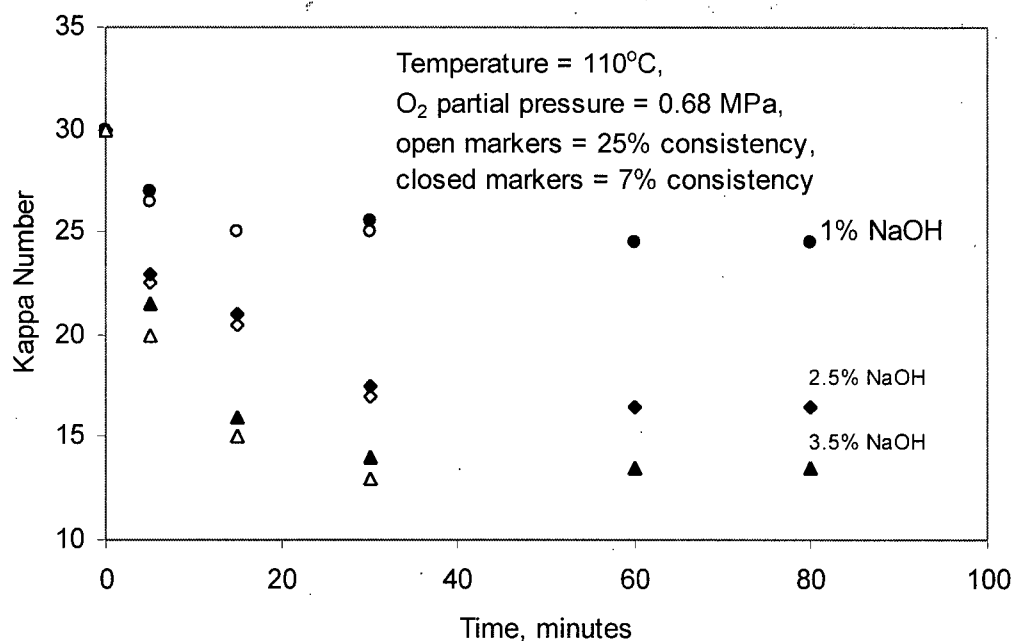


Figure 1.19 Effect of caustic loading and consistency on the rate of oxygen delignification as observed by Liebergott *et al.* [34]

Based on the data published by Olm and Teder [36], increasing the oxygen partial pressure from 0.1 to 1.0 MPa should cause an increase in the rate of delignification. Yet Liebergott *et al.* [34] found that, between 0.34 to 0.5 MPa, the rate of delignification decreased and they suggested the occurrence of a new phenomenon at those oxygen pressures (Figure 1.20).

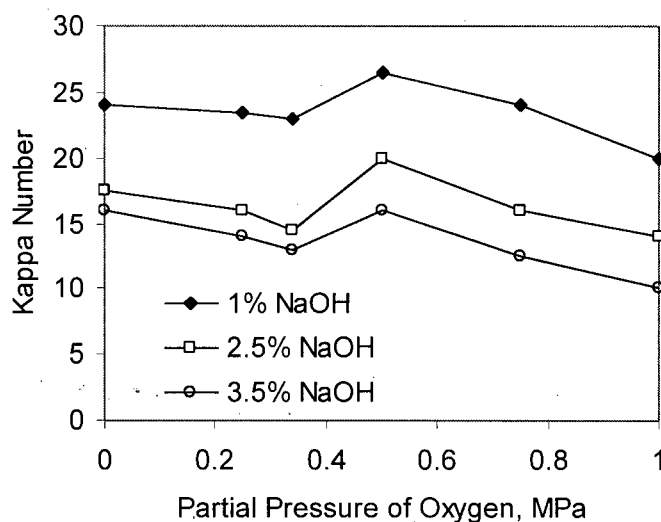


Figure 1.20 Effect of oxygen partial pressure on kappa number as observed by Liebergott *et al.* [34]

The effect of the reaction parameters on pulp viscosity was very similar to their effect on the kappa number [34,35,36]. The rate of carbohydrate degradation was also found to exhibit two distinct phases – a quick initial phase and a slower final phase [36] and Liebergott *et al.* [34] determined that a linear relationship existed between the kappa number of an oxygen delignified pulp sample and its viscosity (Figure 1.21). A regression analysis of the experimental data, performed at 7% consistency, produced the following equations (R-squared value was above 0.95 for both models):

$$\text{Kappa No.} = 53.06 - 1.32C - 0.19T - 0.076P - 0.213t - 0.0078CP + 0.0011t^2 + 0.0002tP + 0.0003P^2 \quad (1)$$

$$\text{Viscosity (mPa.s)} = 49.795 + 6.285C - 0.12t - 0.168P - 0.09CT + 0.0148CP + 0.00065t^2 + 0.00045P^2 \quad (2)$$

Where t = time (min),

T = temperature (°C),

P = oxygen partial pressure (MPa)

C = caustic concentration (% on pulp)

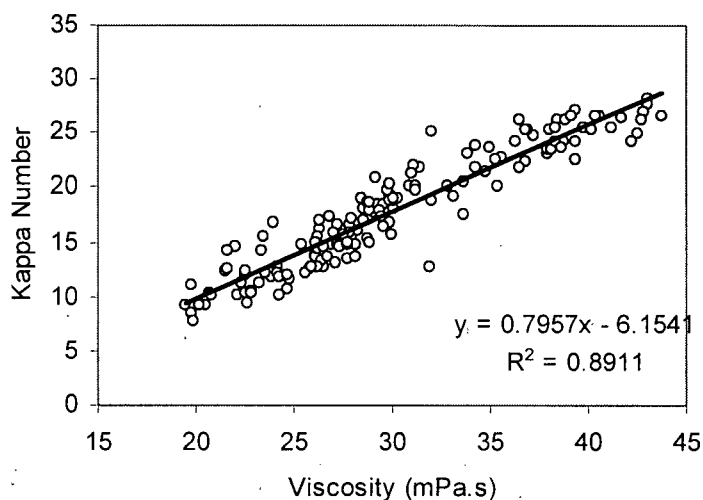


Figure 1.21 Relationship between kappa number and pulp viscosity (reconstructed from data published by Liebergott *et al.* [34])

The general conclusion from previous research on oxygen delignification suggests that increasing any of the parameters results in an increase in the extent of delignification, with the effects of partial pressure and consistency being minimal in comparison to the other parameters [34,35,36].

The oxygen delignification system has been optimized for use in the pulp and paper industry, where fibre strength and viscosity need to be maintained. Since a lower degree of polymerization is desirable in the bioconversion process, these conditions may not provide the highest yield of simple sugars during subsequent enzymatic hydrolysis and thus a new set of optimization parameters must be established.

Shimizu *et al.* (1987) [39] performed experiments to investigate the enzymatic hydrolysis of grasses that were pretreated using oxygen delignification. They determined that the lower the kappa number of the substrate, the easier it became to hydrolyze but when the lignin content fell

below 4%, the digestibility decreased by about 10% (Figure 1.22). They attributed this anomaly to a breakdown in the porous structure of the substrate i.e. a lower surface area available for attack by the enzymes.

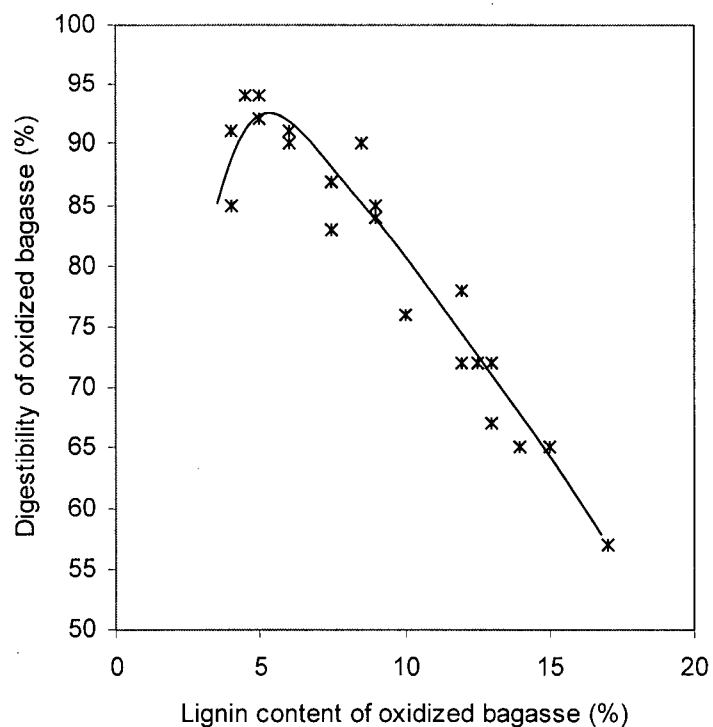


Figure 1.22 Effect of lignin content on the ease of hydrolysis of grasses pretreated using oxygen delignification [39]

## 2. OBJECTIVES

There has been a limited amount of research conducted to evaluate the usefulness of oxygen delignification as a pretreatment to enzymatic hydrolysis, despite the obvious potential of the process. Kraft pulp was chosen as the model substrate for this study because it represents lignocellulosic material in a simple state. Any information derived from a study using pulp may be applied to most woody substrates and modified thereafter to take into account the differences that exist between species and processing (e.g. AFEX, Steam explosion, etc).

The initial objective of the research was aimed at illustrating the effects of different oxygen delignification conditions on the model substrate. Four parameters were considered: the retention time, reaction temperature, caustic concentration and oxygen partial pressure over a range that was selected to take into account both limiting and excessive extremes. The data collected was used to formulate equations to describe the effects of those parameters on the kappa number and viscosity of the pulp.

A second purpose of the study was to determine the effect of oxygen delignification on the susceptibility of the substrate to enzymatic hydrolysis. Mathematical modeling used to develop empirical equations to relate the kappa number and pulp viscosity to the initial rate of saccharification and final sugar concentration. the hypothesis was that there would be some specific combination of kappa number and viscosity that translates to a maximum extent of hydrolysis.

The final objective replaced the model substrate with a more suitable example of a possible bioconversion feedstock, reject knots from a sulphite pulp mill. The new substrate had a higher starting kappa than the pulp with a larger, less uniform distribution of particles. It was hoped that the general trends observed when the pulp was subjected to oxygen delignification were mirrored by the new substrate before any further work can be done to investigate the possibility of using oxygen delignification as a pretreatment upstream of enzymatic hydrolysis.

### 3. MATERIALS AND METHODS

#### 3.1 Preliminary analysis of hemlock Kraft pulp samples from Howe Sound Pulp and Paper Ltd.

Two Hemlock pulp samples were obtained from Howe Sound Pulp and Paper Limited in Port Mellon, British Columbia. One sample was taken before the oxygen delignification unit and the other sample taken after the unit one hour later. The conditions for that particular run was as follows:

Slurry flow rate: 139 L/s

Consistency: 9.5%

Caustic concentration: 3.4%

Caustic flow rate: 4.49 L/s

Inlet temperature of reactor 1: 86°C

Outlet temperature of reactor 2: 92°C

Oxygen pressure in reactor 1: 539 kPa

Oxygen pressure in reactor 2: 246 kPa

Oxygen flow rate to reactor 1: 1007 kg/h

Oxygen flow rate to reactor 2: 495 kg/h

Magnesium Sulphate flow rate to reactor 1: 5.6 L/min

Incoming kappa number: 35.5

Predicted outlet kappa: 16.1

Howe Sound uses a two-stage delignification process, which has been proven to be more effective at removing lignin than a single stage unit. In a conventional single stage unit, delignification is usually of the order of 35 – 50%, while delignification can be increased to 60 – 70% using a two-stage unit with intermediate washing [11,40].

### 3.2 Laboratory-scale oxygen delignification

#### 3.2.1 Apparatus for performing laboratory scale oxygen delignification

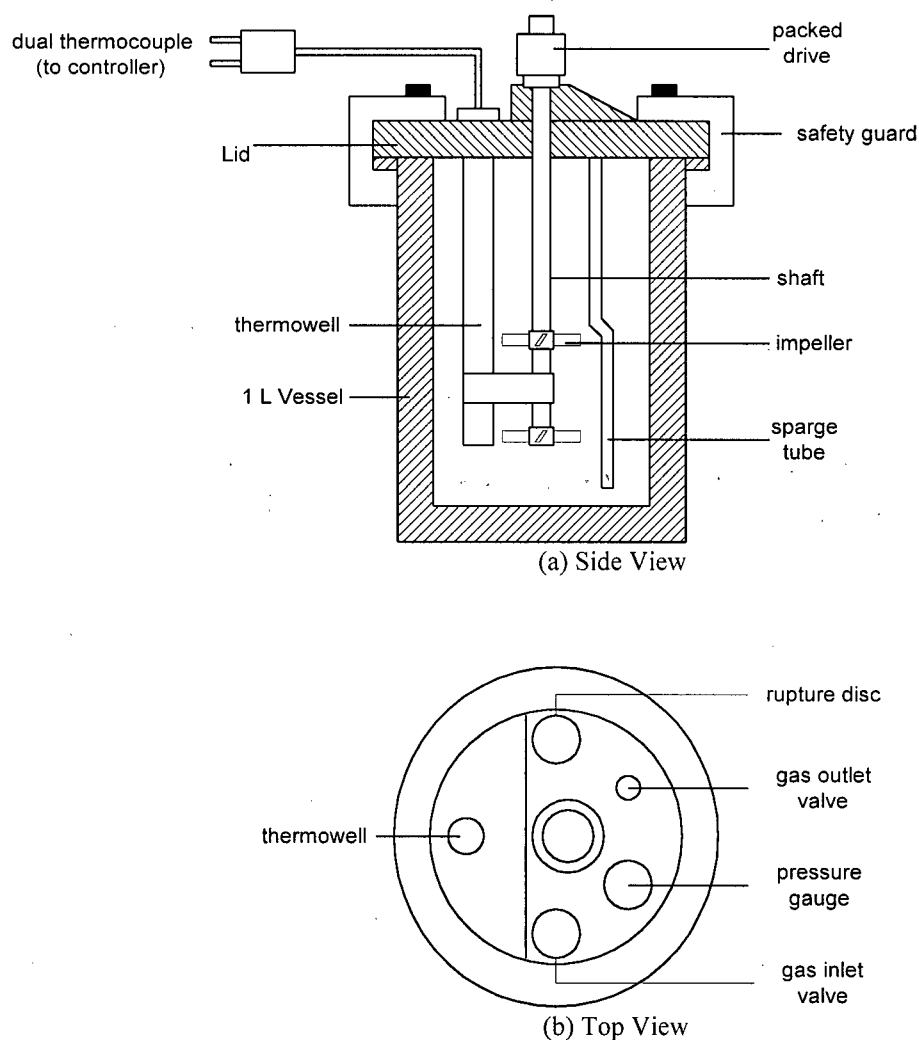


Figure 3.1 The lab-scale stirred tank reactor

The laboratory-scale oxygen delignification equipment (Figure 3.1) was meant to simulate the effectiveness of a commercial single-stage unit. A 1L stainless steel Parr reactor with a specially-made lid was used for this experiment. The lid was equipped with a thermowell for keeping a dual thermocouple, a gas outlet valve, a pressure gauge, a gas inlet valve, a rupture disc and a packed drive, all furnished by Parr Instruments. The contents of the reactor were heated and

mixed using a 4522 Parr pressure reactor unit with the temperature and motor speed being controlled by a 4842 Parr controller unit.

### **3.2.2 Procedure for performing oxygen delignification experiments**

All delignification cooks were done at 3% consistency to facilitate proper mixing. A sample of prepared pulp equivalent to 22.5 g oven-dried was weighed and placed into the reactor and oxygen delignification liquor, containing the desired quantity of sodium hydroxide, so that the total volume of liquid in the reactor was 750 mL. The reactor was sealed, placed in the heating element and connected to the temperature controller. The temperature was then set and the contents of the reactor allowed to reach the reaction temperature.

The experiments were carried out over a range of temperatures ( $90^{\circ}\text{C} - 165^{\circ}\text{C}$ ), reaction time (20 – 60 minutes), caustic concentration (1 – 3% gram NaOH per gram oven-dried pulp) and oxygen partial pressure (10 – 100 psig).  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was also added as a protector in a few experiments at a loading of 0.1% per gram dry weight of pulp. The exact combination of the parameters were determined by a factorial design.

Once temperature was attained, oxygen was introduced into the reactor via the gas inlet valve to give the desired oxygen partial pressure in the reactor. Combustible byproducts were removed from the reactor by keeping the outlet valve slightly opened. The reaction was allowed to proceed for the required time period after which it was quickly cooled in a bucket of ice and water. During cooling, the outlet valve was fully opened to hasten the reduction of pressure in the reactor.

When the pressure in the reactor fell to zero, the lid was removed and the pulp suspension filtered, under vacuum, through a Buchner funnel lined with FisherBrand coarse filter paper (Fisher Scientific, UK). The pulp was then thoroughly washed with 9 L of water until no more water could be withdrawn. The washed pulp was removed from the Buchner funnel, and half was allowed to dry overnight in a  $50^{\circ}\text{C}$  oven, while the other half was shredded and stored in an airtight plastic bag in a  $4^{\circ}\text{C}$  refrigerator.



### **3.3 Procedure for performing enzymatic hydrolysis**

Hydrolysis experiments were carried out in 125 mL Erlenmeyer flasks at 2% pulp consistency in 50 mM sodium acetate buffer (pH 4.8). Forty  $\mu\text{g/mL}$  tetracycline and 30  $\mu\text{g/mL}$  cycloheximide (Sigma Aldrich) were also added to eliminate bacterial growth. The flasks were preincubated at 45°C for 20 minutes in a shaker bath at a speed of 150 rpm.

Celluclast, a commercial cellulase mixture with an activity of 70 Filter Paper units (FPU) per milliliter, was loaded at 2 or 5 FPU per gram dry weight of pulp. Novozym 188, a  $\beta$ -Glucosidase solution with an activity of 565 Cellobiose units (CBU) per milliliter, was added so that the FPU:CBU ratio was 1:10. The high loading of Novozym 188 was to ensure that no end-product inhibition of Celluclast by cellobiose and other oligosaccharides would occur. Both enzyme solutions were obtained from Novozyme, North America.

The reaction was allowed to proceed at 45°C and a shaking speed of 150 rpm for 48 hours with 0.5 mL samples of the hydrolysate being withdrawn at 1, 2, 6, 24 and 48 hours after starting. The samples were centrifuged at 14 000 g for 5 minutes using a microcentrifuge and the supernatant removed and stored in 1.5 mL centrifuge tubes in a -80 °C freezer for future HPLC analysis.

The exact time-course for enzymatic hydrolysis is not necessary to determine the effectiveness of the process. The initial rate of hydrolysis was calculated as the total amount of sugar liberated per hour in the first six hours of the hydrolysis reaction (the rate of hydrolysis was constant during this period for each experiment), and can indicate the rate at which sugars are released into solution. The enzymatic conversion was calculated as the percent of the initial mass of carbohydrates found in the hydrolysate after 48 hours and demonstrates the maximum concentration of sugars that will be released at a given enzyme loading in that time.

### **3.4 Procedure for mechanical refining of the sulfite pulp mill rejects**

The rejects were first diluted to 2% consistency using tap water so that the refining would proceed quickly and easily. The distance between the refiner plates of the Sprout-Waldron mechanical refiner (Koppers Company Inc, USA) was initially set at 0.020" with the plate speed was set to 2500 rpm. The diluted rejects were fed through the refiner where they were broken

down into smaller, more uniform pieces by the plates and the resulting slurry was collected in large buckets. A constant flow of water was necessary to ease the friction between the plates and the rejects. The rejects were fed through the refiner two more times at a plate distance of 0.010" then 0.005" to further decrease the size of the particles. Once it was determined that the size of the particles was sufficiently small, the slurry was filtered under vacuum and sealed in airtight plastic bags.

## **4 ANALYSES**

### **4.1 Determination of the pulp composition**

The composition of the original pulps was determined using TAPPI method for Acid Insoluble Lignin (T 222 om-98).

The pulp was initially ground up to pass through a 40-mesh screen using a Wiley mill. A sample equivalent to 0.15 g of moisture-free ground pulp was weighed into a reaction flask. Three milliliters of cold 72% (w/w) sulfuric acid was added to the beaker containing the sample and the mixture was constantly stirred with a glass rod for two minutes. The mixture was subsequently stirred every ten minutes for two hours and the contents of the reaction flask was transferred to a serum bottle. The reaction flask was washed with deionised water and the wash transferred to the serum bottle so that the final acid concentration in the bottle was 4% (w/w) (this required about 115.5 mL of water). The serum bottle was sealed with a septa cap and autoclaved at 121°C for one hour.

Once cooled, the samples were suction filtered through labeled, pre-weighed, medium coarseness glass crucibles, ensuring all solids were recovered. The filtrate was collected to determine concentration of acid soluble lignin in the pulp sample based on the absorbance of the filtrate at 205 nm. The filtrate was also used to determine the concentration of carbohydrates in the pulp sample using HPLC (Section 4.2). The retentate (the acid-insoluble lignin) was washed with 100 mL deionised water to remove traces of the acid. The crucible with the lignin was dried overnight in a 105°C oven, dried in a dessicator, and weighed to determine the quantity of acid insoluble lignin in the pulp sample.

#### **4.2 Determination of reducing sugar composition of the hydrolysate**

The concentration of reducing sugars in a liquid sample was determined by high-performance liquid chromatography (HPLC) using a Dionex DX600 system (Dionex Corporation, Sunnyvale, CA).

The samples to be analyzed were appropriately diluted using deionised water and 50  $\mu\text{L}$  of a 5 mg/mL fucose solution was added as the internal standard. The diluted samples were filtered through 0.45  $\mu\text{m}$  filters into 1.5 mL glass, screw-top vials and capped with septa-lined lids.

An Dionex AS50 Autosampler was used to load a 20  $\mu\text{L}$  sample and the sugars (fucose, arabinose, galactose, glucose, xylose and mannose) were separated using a Dionex CarboPac PA1 column. Degassed, deionised water, flowing at a rate of 1.0 mL/min, was used as the mobile phase.

Each sample was completely analyzed in 60 minutes, with the reducing sugars emerging as peaks on a chromatogram recorded by a Dionex ED50 electrochemical detector. The area of each peak was recorded and a spreadsheet was used to find the corresponding concentration of each sugar.

#### **4.3 Determination of the kappa number**

The lignin content of all the pulps was determined using TAPPI method for Micro Kappa Number (UM 246).

A sample of air-dried pulp was weighed out and dispersed in a beaker containing 80 mL deionised water. The mixture is continuously stirred to keep the pulp suspended in the liquid. Ten milliliters of 4 N sulfuric acid was mixed with 10 mL of 0.1 N potassium permanganate in a 50 mL Erlenmeyer flask and then added to the pulp-water mixture. The reaction was allowed to proceed for exactly 10 minutes (the temperature of the solution is measured after 5 minutes) then 2 mL potassium iodide was added to stop the reaction. The resulting mixture was titrated using

0.1 N sodium thiosulphate and the amount of thiosulphate consumed was recorded. A blank was done using the same method without the addition of pulp.

The results are most accurate when the permanganate consumption is 50% and the temperature of the reaction is 25°C, but the equation for calculating kappa number can be modified to take into account permanganate consumption ranging from 10% to 70% and temperatures between 20 and 30°C:

$$K = (100f/wn) (1+0.013 (25-T)) \quad (3)$$

Where K = kappa number

T = reaction temperature measured at 5 minutes, °C

n = amount of 0.1 permanganate solution used in the test, mL

w = weight of moisture-free pulp in the sample, mg

f = factor for correction to 50% permanganate consumption

#### **4.4 Filter paper assay for determining cellulase activity**

The cellulase activity was determined using NREL method for Measurement of Cellulase Activities (LAP-006).

One milliliter of 50 mM sodium acetate buffer (pH 4.8) was added to each of three test tubes, each containing 500 µL of a dilute Celluclast solution. The test tubes were placed in the constant temperature water bath at 50°C for 10 minutes to equilibrate. One 50 mg strip of Whatman No. 1 filter paper was immersed into each test tube and the samples incubated for one hour at 50°C. Enzyme and substrate blanks were also included in the assay. Three milliliters of Dinitrosalicylic Acid (DNS) reagent was added to stop the reaction, and then the test tubes were placed in the boiling bath for five minutes to develop the color of the DNS (DNS reacts with glucose molecules to produce a color when heated). Twenty milliliters of deionised water was added to each tube and the absorbance measured at 540 nm to determine the concentration of glucose in solution. The reducing sugar concentration is determined from a standard graph relating glucose in solution (mg) to the absorbance.

A filter paper unit (FPU) is defined as the amount of enzyme needed to liberate 2 mg glucose in one minute from 50 mg Whatman No. 1 filter paper under the conditions of the assay.

#### **4.5 $\beta$ -Glucosidase assay using p-Nitrophenyl- $\beta$ -D-glucoside**

One milliliter of a 5 mM p-Nitrophenyl- $\beta$ -D-glucoside in 0.05 M sodium acetate buffer was pipetted into a test tube containing 1.8 mL of acetate buffer. The mixture was equilibrated at 50°C in a water bath followed by the addition of 200  $\mu$ L of a diluted enzyme solution. Enzyme and substrate blanks were also included in the assay. The reaction was allowed to proceed for thirty minutes after which time 4 mL of glycine buffer was added to stop the reaction (p-Nitrophenol turns yellow in glycine buffer). The absorbance was measured at 430 nm and the concentration of p-Nitrophenol was determined from a standard graph relating micromoles of p-Nitrophenol in solution to the absorbance.

The CBU unit of activity is defined as the amount of enzyme required to release 1  $\mu$ mol p-Nitrophenol in one minute under the conditions of the assay [45].

#### **4.6 Determination of pulp viscosity**

The viscosity of the pulps was determined using TAPPI method T 230 om-99: Viscosity of pulp (capillary viscometer method). The method determines the viscosity of a 0.5% cellulose solution using a capillary viscometer and a 0.5 M cupriethylenediamine as the solvent.

A sample of air-dried pulp equivalent to  $0.2500 \pm 0.0005$ g of moisture-free pulp was weighed into a dissolving bottle containing eight 6-mm diameter glass beads. Twenty-five milliliters of distilled water was transferred into a reaction bottle using a pipette and the bottle was capped and shaken to disperse the pulp fibres in the distilled water. The bottle was allowed to stand for 2 minutes then 25 mL of 0.5M cupriethylenediamine solution was pipetted into the water-pulp suspension. Air in the bottle was evacuated by purging with nitrogen for about 1 minute. The cap was replaced and the bottle was shaken until all the pulp fibres were completely dissolved. When the shaking was completed, the bottles were lain on their sides to allow the solution to degas.

The viscometer was secured in a 25°C constant-temperature water bath so that it was within 1° of vertical. Ten milliliters of the solution was withdrawn from the dissolving bottle using a pipette and transferred to the viscometer through the larger arm. Once the solution in the viscometer achieved the same temperature as the water bath (about 5 minutes), the solution was drawn up through the measuring arm of the viscometer and allowed to drain down to wet the inner surfaces of the viscometer. The solution was then drawn above the upper line of the viscometer and the time taken for the liquid level to pass between the two marks was recorded.

The time taken to pass between the lines was determined three times for each pulp sample analyzed and the viscosity was calculated using the following equation:

$$V = CTD \quad (4)$$

Where V = viscosity of 0.5 M cupriethylenediamine solution at 25°C (mPa.s)

C = viscometer constant (mPa/gcm<sup>-3</sup>)

D = density of the pulp solution (= 1.052 gcm<sup>-3</sup>)

T = average efflux time (s)

#### 4.7 Statistical analysis and development of the model equations

EXCEL is equipped with many tools to analyze data and develop model equations. Once linear regression analysis was completed, only parameter estimates with a P-value [ 0.1 were judged to be adequate. The analysis of variance (ANOVA) function (95% confidence level) was used to determine whether the differences between experimental results were statistically significant. Where applicable, data is presented as the mean plus or minus one standard deviation.

## 5 RESULTS AND DISCUSSION

### 5.1 Preliminary analysis of pulp samples from Howe Sound Pulp and Paper

Pulp was used as an ideal substrate from which other, more complex substrates can be modeled since it represents the basic building block of all lignocellulosic material. The Howe Sound pulp samples were analyzed for their composition (carbohydrate and lignin content), viscosity and hydrolysability to establish a basis for comparing the results of the lab-scale experiments.

#### 5.1.1 Composition of pulps from the Howe Sound Pulp and Paper

The composition of the Howe Sound pulp samples was determined using the Acid Insoluble Lignin (Klason) analysis described in Section 4.1, and is presented in Table 5.1.

Table 5.1 Chemical composition of hemlock pulp samples before and after oxygen delignification (from Howe Sound Pulp and Paper Ltd.)

Component	Composition (%)	
	Before delignification	After delignification
Arabinose	$0.61 \pm 0.062$	$0.48 \pm 0.048$
Galactose	$0.48 \pm 0.047$	$0.24 \pm 0.016$
Glucose	$77 \pm 0.52$	$80 \pm 1.6$
Xylose	$6.7 \pm 0.28$	$6.8 \pm 0.48$
Mannose	$7.4 \pm 0.042$	$7.20 \pm 0.15$
Lignin	$5.8 \pm 0.34$	$2.4 \pm 0.17$

In addition to glucose (corresponding to the cellulose content in the substrates), the Howe Sound pulp samples also contained a small amount of galactose, arabinose, xylose and mannose (the hemicellulose fraction), as well as lignin. The oxygen delignified pulp contained 59% less lignin and 2.6% and 1.3% more glucose and xylose respectively than the undelignified pulp. Additionally, a significant portion of the hemicellulose was removed: the arabinose, galactose and mannose contents decreased by 21%, 48% and 2%, respectively. This is not an indication

that the process is selective, since it is impossible to conclude from this information if the viscosity of the cellulose was affected.

### **5.1.2 Kappa number and viscosity of the pulp samples from Howe Sound Pulp & Paper**

Kappa number is another method used to measure the lignin content of a pulp sample and it correlates to the total percent of lignin by the following equation:

$$\% \text{ Lignin} = 0.15 \times \text{Kappa} \quad (5)$$

The post-oxygen delignification pulp sample was found to have a kappa number of 16 ( $\cong$  2% lignin), approximately 53% lower than that of the pre-oxygen delignification pulp sample which was found to be 34 ( $\cong$  5% lignin). These results correspond almost perfectly to results obtained from the Klason analysis (Table 5.1) where the total lignin in the pre-delignified sample was 5.8% while the post-delignified sample was determined to be 2.4%.

The pre- and post-delignification pulp samples had viscosities of 35 mPa.s and 18 mPa.s respectively. The sharp decrease in the viscosity of the oxygen-delignified pulp is due to cellulose degradation, primarily random chain cleavage [11,12,31].



### 5.1.3 Enzymatic hydrolysability of the pulp samples from Howe Sound Pulp and Paper Ltd.

The results of the enzymatic hydrolysis of the Howe Sound pulps are summarized in Table 5.2 below.

Table 5.2 Initial rates and final conversion from enzymatic hydrolysis of Howe Sound pulp samples taken before and after the oxygen delignification unit

Sample Name	2FPU Celluclast Loading		5FPU Celluclast Loading	
	Conversion (%)	Initial Rate (mg/ml.h)	Conversion (%)	Initial Rate (mg/ml.h)
Pre-O <sub>2</sub> delignified pulp	26 ± 0.74	0.31	51 ± 0.59	0.59
Post-O <sub>2</sub> delignified pulp	47 ± 3.5	0.56	89 ± 0.96	0.96

The higher enzyme loading gave both greater conversions and initial rates of hydrolysis than the lower enzyme loading. It follows from the general rules of reaction that increasing the amount of reactants will result in an increase in product formation - the larger the enzyme loading, the more active sites that can be attacked over the course of the reaction and the greater the rate and extent of the reaction. However, the increase was not linear i.e. the output from the 5 FPU-loaded hydrolysis experiment was not 2.5 times higher than the 2 FPU-loaded experiment, a result that is likely a function of substrate limitations.

One reason for this nonlinearity is that hydrolysis depends on the number of reaction sites on the substrate and it is possible that there were not enough sites to accommodate all the enzyme molecules once the loading was increased. Another possibility that was mentioned before, occurs when the enzyme occupies a large portion of the surface area of the substrate. Steric hindrance prevents access of the enzyme to all active sites so that the rate of hydrolysis is not proportional to the enzyme loading [17,23].

It has also been suggested that during the course of hydrolysis, amorphous regions of cellulose are generally hydrolyzed first since it is easier to digest [17,19,29]. A higher enzyme loading will increase the rate at which the amorphous cellulose is degraded, and therefore the 5 FPU

enzyme loading will generally face an increasingly more crystalline substrate than the 2 FPU loading. The difference in recalcitrance of the substrate may be another reason why the concentration of sugar released in the 5 FPU loading experiments is less than 2.5 times the quantity released in the 2 FPU experiments. However, leaving the reaction for extended times generally result in similar degrees of saccharification.

The initial rate and final conversion of the oxygen delignified sample were approximately 84% higher than the undelignified sample at 2 FPU enzyme loading and approximately 66% higher at the 5 FPU loading. These results strongly suggest that oxygen delignification would be well suited to be a pretreatment for enzymatic hydrolysis although it doesn't indicate whether the improvement in hydrolysis is due to the lower degree of polymerization or the lower lignin content of the oxygen-delignified sample.

## **5.2 Effect of temperature and oxygen partial pressure on kappa number, viscosity and yield of oxygen-delignified hemlock Kraft pulp**

Previous work indicated that significant improvements in lignin removal could be achieved by increasing the reaction temperature over the range of 80°C to 130°C [36,37,38]. Since viscosity loss is not a concern in this work, the temperature was varied from 125°C to 165°C. The maximum practical temperature was determined by the substrate yield – a loss of less than 10% of the initial mass of substrate was considered acceptable. The results are summarized in Table 5.3.

Table 5.3 Pulp characteristics after oxygen delignification at temperatures between 125 – 165°C, 10 – 100 psig, 60 minutes and 3% NaOH

Temperature (°C)	O <sub>2</sub> pressure (psig)	Yield (%)	Kappa Number	Viscosity (mPa.s)	Carbohydrate lost (%)	Lignin lost (%)
125	10	95	13 ± 0.14	20 ± 0.48	2.0	63
125	40	97	12 ± 0.093	18 ± 0.78	0.57	67
125	70	92	9.8 ± 0.39	16 ± 0.47	4.3	73
125	100	96	9.4 ± 0.60	17 ± 0.61	0.66	73
145	10	91	9.6 ± 0.13	14 ± 0.51	5.8	74
145	40	93	6.1 ± 0.14	14 ± 0.38	3.1	83
145	70	92	5.4 ± 0.083	14 ± 0.62	3.6	85
145	100	96	4.9 ± 0.13	13 ± 0.34	0.063	86
165	10	90	4.7 ± 0.033	9.6 ± 0.23	6.1	87
165	40	91	3.4 ± 0.18	9.6 ± 0.18	4.3	90
165	70	95	3.0 ± 0.078	9.7 ± 0.26	0.33	92
165	100	92	2.9 ± 0.054	9.7 ± 0.20	3.7	92

### 5.2.1 Effect of temperature and oxygen partial pressure on kappa number

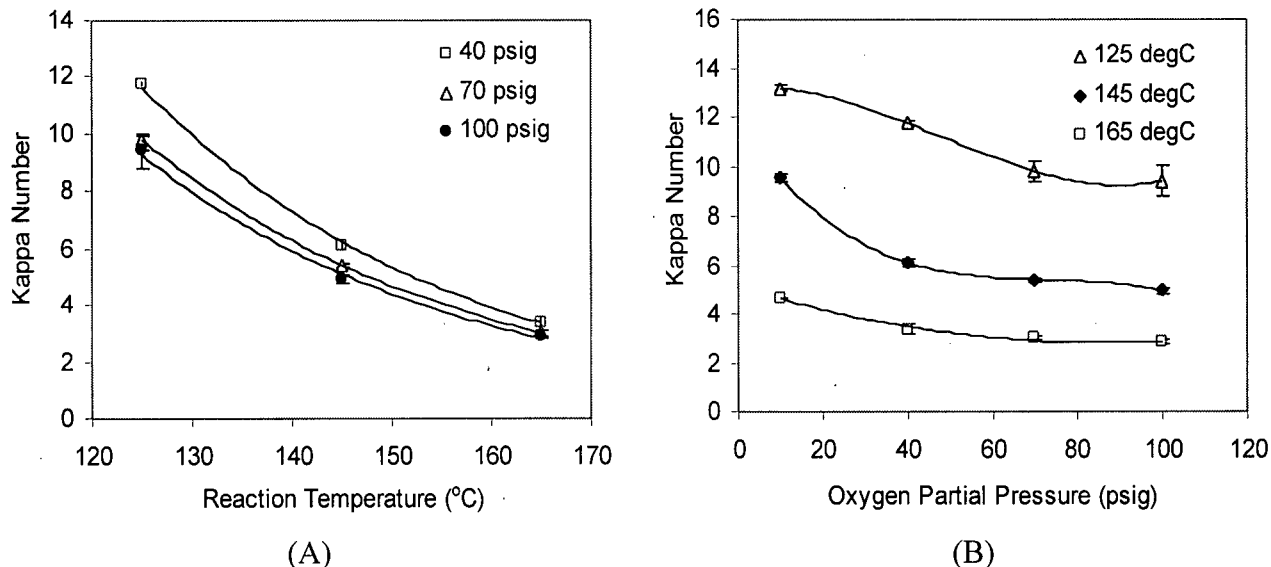


Fig 5.1 Effect of (A) temperature and (B) oxygen partial pressure on kappa number

Generally, as the temperature of the reaction increased, the extent of delignification also increased (Figure 5.1A), which is in agreement with the findings from previous research [35,38]. Increasing the temperature from 125 - 165°C increased the amount of lignin removed from the pulp from 63 – 73% to 86 – 92%, respectively. This corresponded to a decrease in kappa number from between 9 – 13 at 125°C to less than 5 at 165°C. As the temperature was increased from 125°C to 165°C, the effect of temperature on the kappa number diminished (Figure 5.1A).

There was an overall decrease in kappa number as the oxygen partial pressure was increased (Figure 5.1B). The decrease in kappa number was also not linear - the kappa numbers decreased by 29 – 48% when the oxygen partial pressure increased from 10 psig to 40 psig. However, between 40 – 70 psig, it was apparent that further increases in the partial pressure had no effect on delignification.

Since the lignin structure has to be activated by caustic in the liquor before delignification will occur [11,12,31], it should follow that the number of active sites created on the lignin structure is fixed by the amount of caustic in solution. Increasing the partial pressure of oxygen at a given

caustic concentration increases the number of free radicals in the reaction and hence, the degree of delignification, as long as there are still sites to be activated. Since little improvement was observed in the degree of delignification by increasing the oxygen partial pressure from 40 – 70 psig, it is possible that the number of active sites was limiting.

### 5.2.2 Effect of temperature and oxygen partial pressure on pulp viscosity

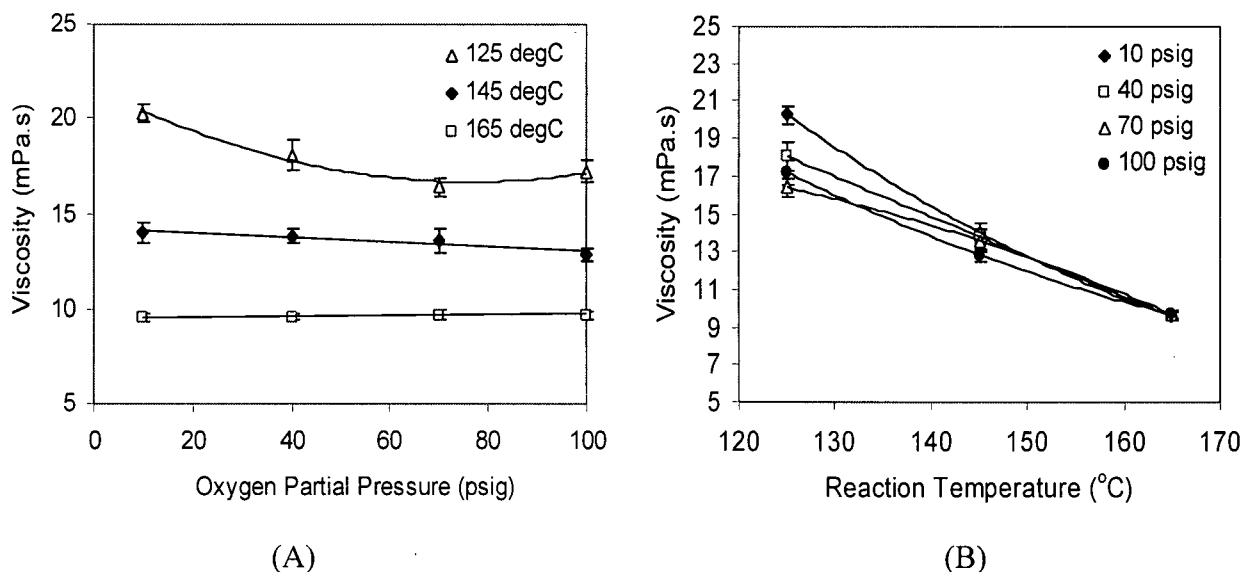


Fig 5.2 Effect of (A) oxygen partial pressure and (B) temperature on pulp viscosity

The effects of oxygen partial pressure was most visible at 125°C - the viscosity decreased steadily from 13 to 9 mPa.s as the oxygen partial pressure increased from 10 to 100 psig (Figure 5.2A), and is concurrent with the trends observed by Olm and Teder [36]. At 145°C and 165°C, it appears that cellulose degradation is relatively unaffected by the oxygen partial pressure. Cellulose degradation, which increases as the temperature increases [34,36], was found to have two distinct phases during oxygen delignification - a quick initial phase and a slower final phase [36]. Since previous research was carried out at milder temperatures (80 – 130°C) than the data presented in Figure 5.2A, it is possible that all the viscosity data collected at 145°C and 165°C fall within the final phase, which would make it difficult to observe any effects of partial pressure.

The data presented in Figure 5.2B agrees with the conclusions from previous research - cellulose degradation increases as the temperature increases [34,36]. As the temperature of the reaction was increased from 125°C to 165°C, the viscosity the pulp decreased steadily from between 16.4 – 20.2 mPa.s to 9.6 – 9.7 mPa.s, respectively (Figure 5.2B).

### **5.2.3 Effect of temperature and oxygen partial pressure on yield**

The yield after delignification, calculated as the percentage of the original dry weight of pulp recovered after the oxygen delignification experiment, is given in Table 5.3. The data randomly fluctuated between 89 and 97%, which made it impossible to observe any concrete relationships between the temperature and oxygen partial pressure of the reaction and the yield.

### **5.3 Effect of reaction time and caustic concentration on kappa number, viscosity and yield of oxygen-delignified hemlock Kraft pulp**

The effect of reaction time and caustic concentration on the kappa number was also investigated at a constant temperature (125°C) and oxygen partial pressure (75 psig). The lowest cooking temperature from the previous set of experiments was used so that any effect of time and caustic concentration would be more obvious. The results are summarized in Table 5.4.

Table 5.4 Pulp characteristics after oxygen delignification at temperatures between 125°C, 75 psig, 20 - 60 minutes and 1 - 3% NaOH

Time (mins)	NaOH (%)	Yield (%)	Kappa Number	Viscosity (mPa.s)	Carbohydrate lost (%)	Lignin lost (%)
60	3	92	9.8 ± 0.39	16 ± 0.19	4.3	73
40	3	96	9.7 ± 0.35	17 ± 0.59	0.85	73
20	3	97	12 ± 0.14	19 ± 0.37	-0.05	65
60	2	96	11 ± 0.47	20 ± 0.50	0.97	69
40	2	96	13 ± 0.37	20 ± 0.71	0.99	64
20	2	97	15 ± 0.31	22 ± 0.63	-0.29	57
60	1	96	17 ± 0.14	26 ± 0.24	0.92	52
40	1	97	17 ± 0.16	26 ± 0.52	0.25	52
20	1	99	19 ± 0.76	27 ± 0.79	-1.7	44

### 5.3.1 Effect of reaction time and caustic loading on kappa number

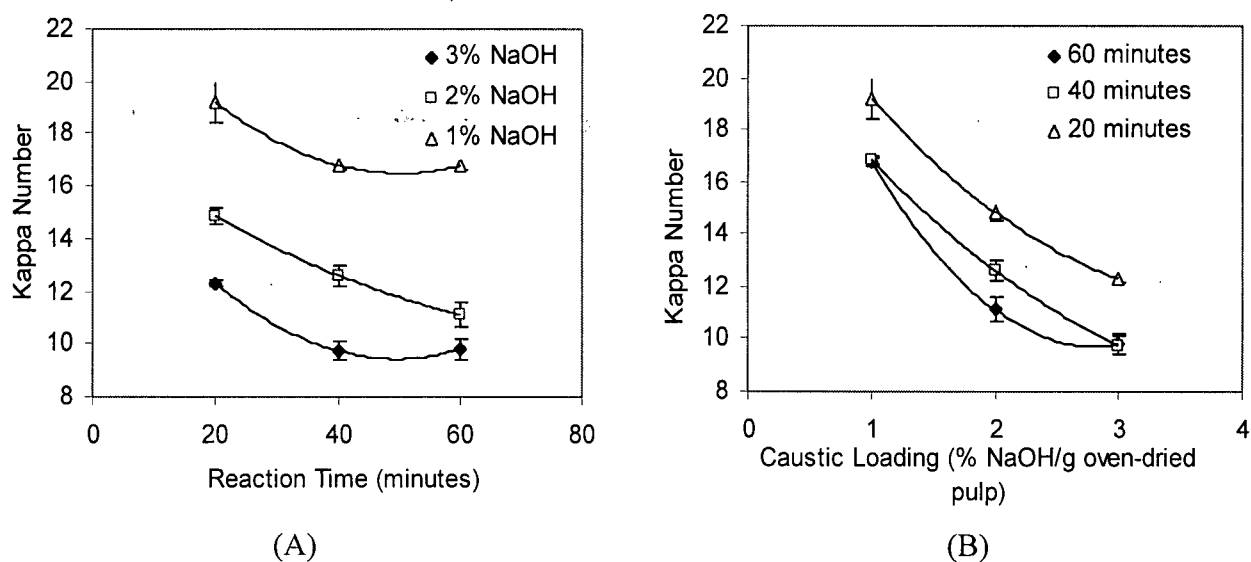


Figure 5.3 Effect of (A) reaction time and (B) caustic concentration on kappa number

Generally, reaction times greater than 40 minutes had no significant effect on kappa number. However, when the reaction time was increased from 20 to 40 minutes, there was a 2.2 – 2.6 decrease in kappa number (Figure 5.3A). Most of the lignin in the pulp was removed during the initial rapid phase of delignification which appeared to occur within the first 40 minutes of the reaction, similar to the graphical results presented by Liebergott *et al.* [34] and Hartler *et al.* [35]. The decrease in the rate of delignification between 40 to 60 minutes is likely due to caustic limitations (most of the caustic is consumed early in the reaction) or, less likely, because fewer active sites are available on the lignin molecule.

From Figure 5.3B, it can be seen that as the caustic concentration was increased, the extent of delignification increased. At a given reaction time, a 6.88 – 7.07 drop in kappa number was observed when the caustic concentration was increased from 1% – 3%. As mentioned previously, the delignification reaction is initiated when the lignocellulosic material is activated by the presence of caustic and then attacked by the oxygen and hydroxyl radicals. This indicates that the greater the amount of caustic present, all other process parameters being kept constant, the more sites that can be activated and hence the greater the extent of delignification.

### 5.3.2 Effect of reaction time and caustic loading on pulp viscosity

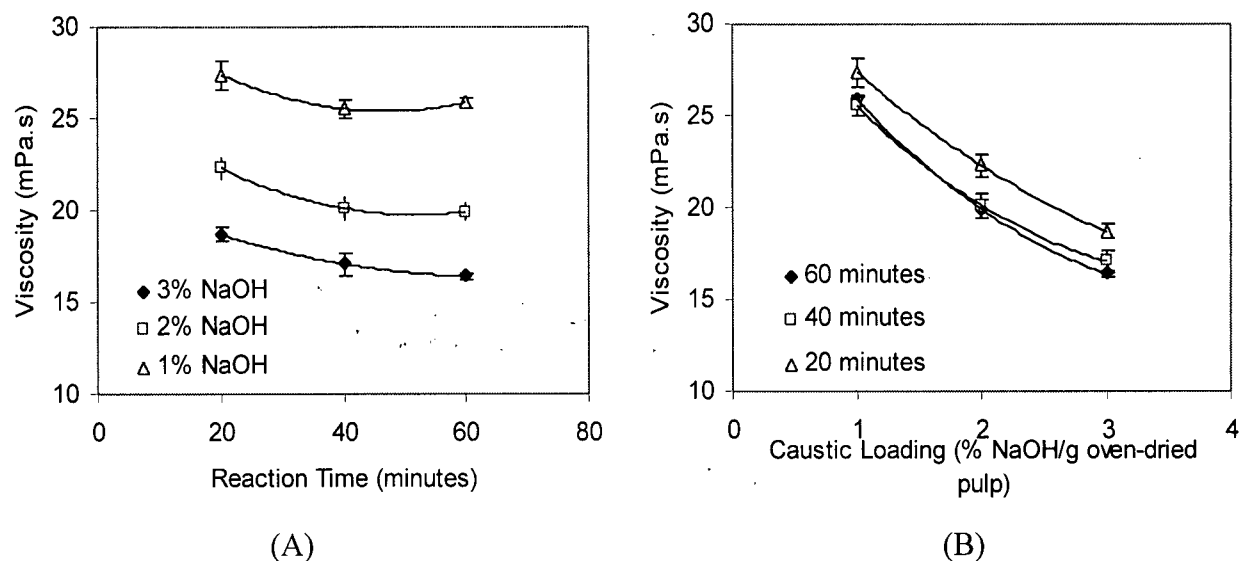


Figure 5.4 Effect of (A) reaction time and (B) caustic concentration on viscosity



Cellulose degradation has been shown to follow a similar time course to delignification [31,34,36] so the trends on Figure 5.4A should be very similar to Figure 5.3A. During the initial rapid phase (approx. 40 minutes), the viscosity dropped by 1.6 – 2.2 mPa.s, while between 40 and 60 minutes, when the reaction entered the slower final phase, the drop in the viscosity was minimal (less than 1 mPa.s).

Increasing the caustic concentration from 1% to 3% at a given reaction time resulted in 8.4 – 9.4 mPa.s decrease in viscosity (Figure 5.4B). As previously discussed, the chain cleavage reaction is the most significant cellulose degradation reaction and it is initiated by the presence of hydroxyl radicals [11,31]. Increasing the caustic concentration results in an increase in the number of hydroxyl radicals in solution, which in turn results in an increase in the chain cleavage reaction.

### 5.3.3 Effect of reaction time and caustic loading on yield

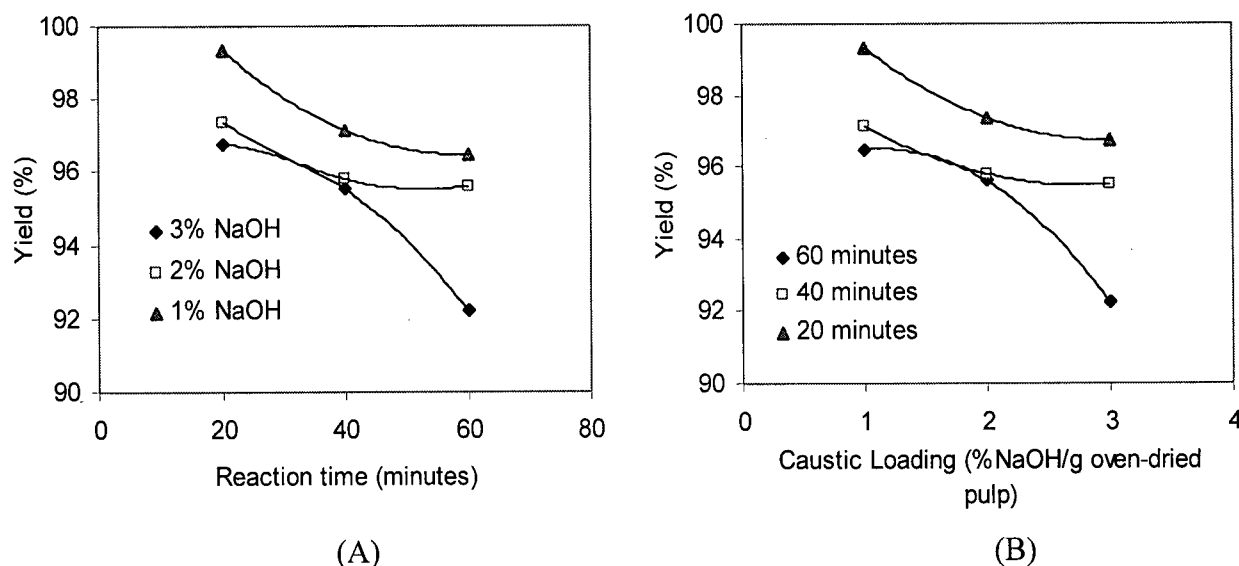


Figure 5.5 Effect of (A) reaction time and (B) caustic concentration on yield after delignification

The general trend is that increasing the reaction time (Figure 5.5A) and/or the caustic concentration (Figure 5.5B) causes the yield after delignification to decrease. Since lignin contributes to the weight of the sample, when it is removed, the overall mass of the sample will decrease.

#### 5.4 Modeling the effect of oxygen delignification conditions on a pulp sample using linear regression

The data from all the oxygen delignification experiments was combined and subjected to a linear regression analysis. Since the oxygen delignification reaction has four input parameters and two outputs, a multiple linear regression model of the form,

$$Y = k_1X_1 + k_2X_2 + k_3X_3 + \dots + k_p \quad (6)$$

was developed. In this model, a single response,  $Y$ , depends on  $n$  independent variables ( $X_i$ ) with  $n+1$  unknown parameters ( $k_i$ ). The kappa number and the pulp viscosity were found to depend on the retention time, reaction temperature, caustic concentration and oxygen partial pressure as indicated by equations 6 and 7 below.

$$K = 49.73 - 0.19T - 3.09S - 0.05t - 0.05O \quad (7)$$

$$V = 55.91 - 0.03t - 0.19T - 4.06S - 0.02O \quad (8)$$

Where,  $K$  = kappa number

$V$  = viscosity (mPa.s)

$t$  = retention time (mins)

$S$  = caustic loading (% NaOH per gram dry weight of pulp)

$T$  = reaction temperature ( $^{\circ}\text{C}$ )

$O$  = oxygen partial pressure (psig)

The two equations can be used to predict the kappa number and the pulp viscosity of a sample of hemlock Kraft pulp with an initial kappa number of 34. Equations 7 and 8 above do not resemble the models put forward by Liebergott *et al.* [34] (equations 1 and 2) and it is possible that differences in the consistency, type of substrate, equipment and/or operating procedures used may have also contributed to the different results.

### 5.5 Relationship between residual kappa number and viscosity of oxygen-delignified hemlock Kraft pulp

A comparison of Figures 5.1 and 5.2 as well as Figures 5.3 and 5.4 show very similar trends between the effect of the reaction parameters on the delignification reaction and carbohydrate degradation. This suggested that there is a likely correlation between the kappa number and viscosity.

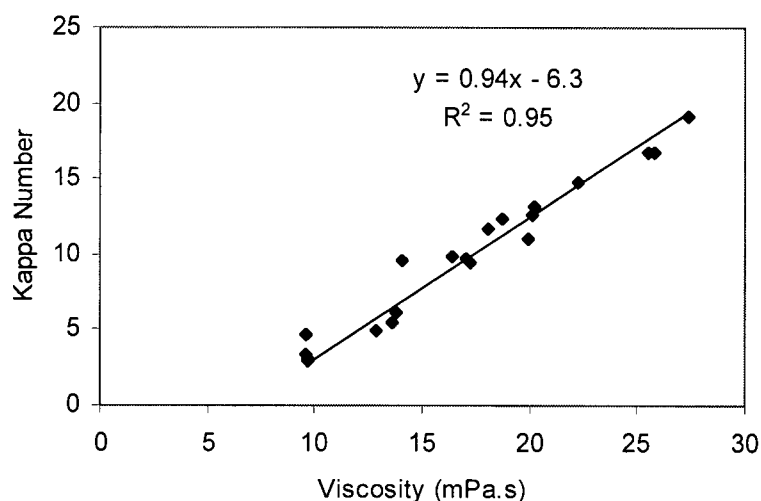


Figure 5.6 Relationship between kappa number and pulp viscosity for the lab-scale oxygen delignification experiments

Our linear relationship (Figure 5.6) clearly concurs with the previously published relationship established by Liebergott *et al.* [34] experiments (Figure 1.21). The differences in the coefficients may be due to the type of substrate used and/or differences in operating conditions.

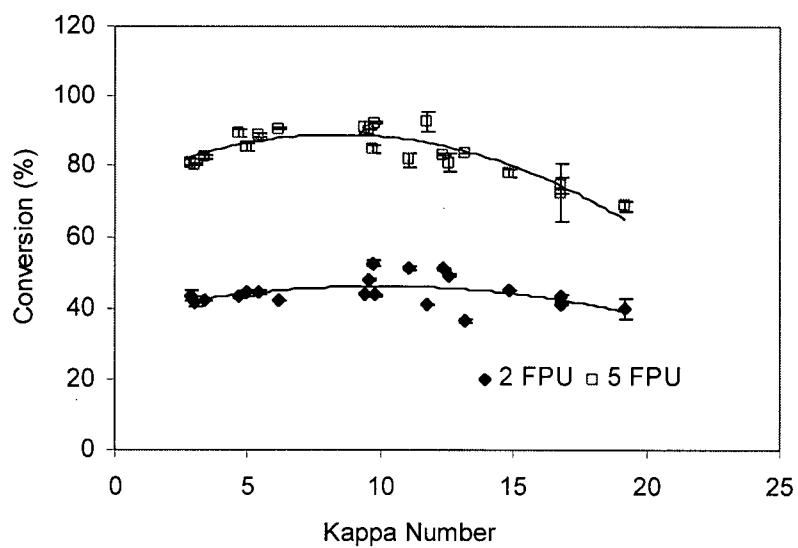
## **5.6 Effect of kappa number and pulp viscosity on enzymatic hydrolysis**

In order to determine the usefulness of the oxygen delignification process as a pretreatment parameter upstream of enzymatic hydrolysis, it was necessary to determine the effect of kappa number and viscosity on the conversion and initial rate of hydrolysis.

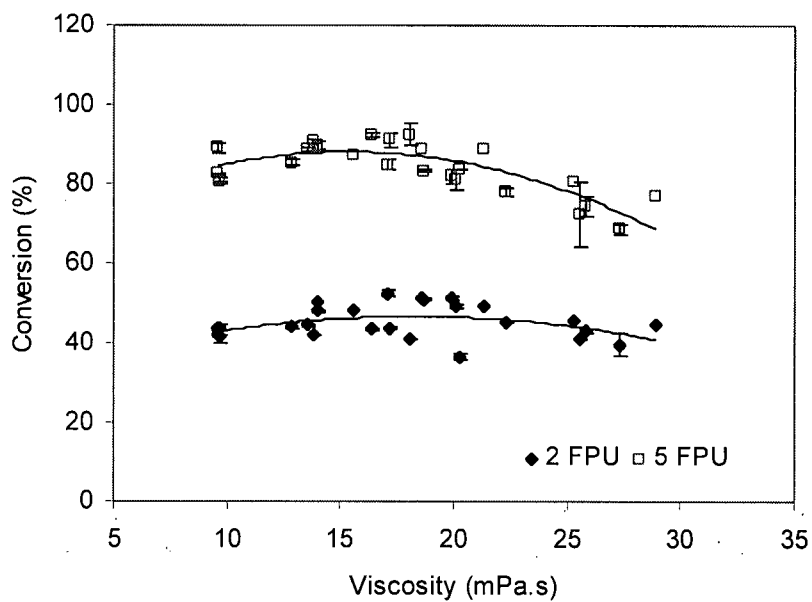
The first major assumption of this approach was that any changes in the structure and reactivity of the pulp fibres are directly proportional to the kappa number and pulp viscosity i.e. it was assumed that the kappa number and viscosity were sufficient to describe the effect of oxygen delignification on hydrolysis.

A second assumption that can be derived from the one mentioned above is that, since the physical properties of the pulp are the same at a given kappa number, then at a given enzyme loading, two pulps with the same kappa number will have similar conversions and rates of hydrolysis.

On the basis of these two assumptions, all the data collected from the experiments were combined to give Figures 5.7 and 5.8.

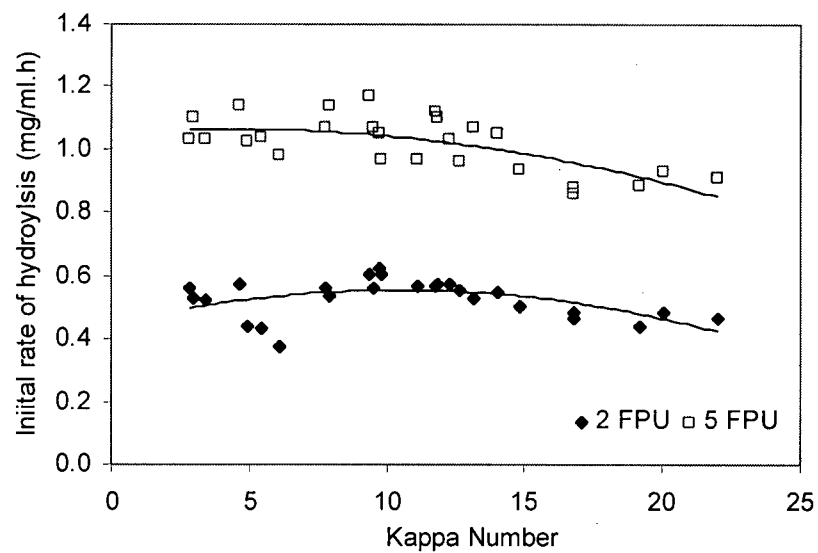


(A)

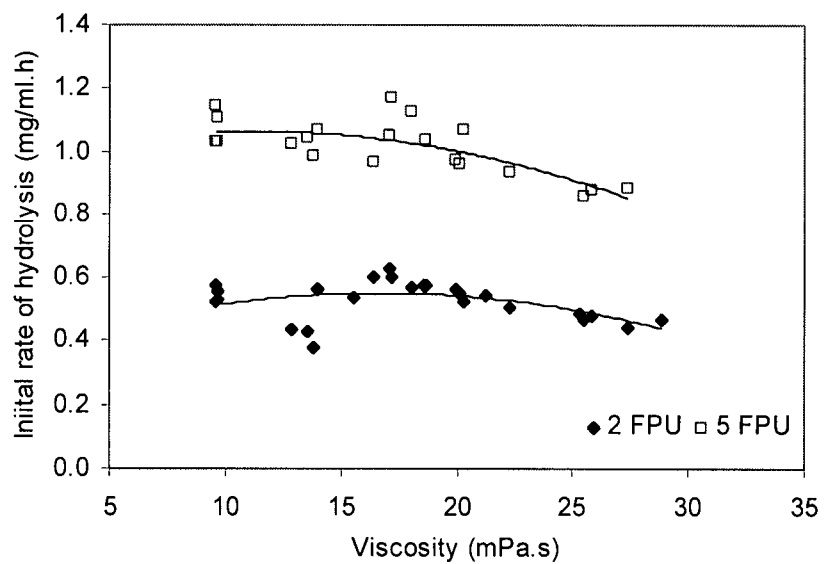


(B)

Figure 5.7 Effect of (A) kappa number and (B) viscosity on enzymatic conversion



(A)



(B)

Figure 5.8 Effect of (A) kappa number and (B) viscosity on the initial rate of hydrolysis

At the 2 FPU loading, the effects of kappa number and viscosity are barely discernible and is likely due to enzyme limitations.

As kappa number decreased from 19 to 10, which corresponded to an associated decrease in viscosity from 27 to 18 mPa.s, the conversion at the 5 FPU loading increased from 69% to 92% (Figure 5.7). Previous research has shown that increasing the accessibility of the cellulose fibres increase the extent of enzymatic hydrolysis [9,17,18,19,20,]. This suggests that as the kappa number is decreased, there is less inhibition due to the presence of lignin, and the rate and extent of enzymatic hydrolysis should increase. This trend is clearly visible in Figures 5.8, which shows that as the kappa number decreased from 19 to 3 (a viscosity decrease from 27 to 10 mPa.s), the initial rate of hydrolysis increased from 0.9 to 1.1 mg/ml.h at the 5 FPU loading.

The total conversion appears to reach a maximum of 92% between kappa numbers of 6 - 10 (a viscosity between 13 – 16 mPa.s) and further decreases in the kappa number to 3 resulted in a decrease in the conversion to 80%. A similar trend was observed by Shimizu *et al.* [39] when they investigated the enzymatic hydrolysis of grasses pretreated by oxygen delignification. The digestibility of the oxygen delignified grasses increased from approximately 43% at a kappa number of 18, to 93% at a kappa number of 5, then decreased to 84% when the kappa number was further decreased to 4. They suggested that the decrease in digestibility was due to a breakdown in the porous structure of the cellulose fibres when the degree of delignification reaches a certain value, which would result in less surface area available for attack by the enzyme and the resultant loss in conversion.

### **5.7 Effect of magnesium addition on response variables**

The discovery that there was a definite relationship between kappa number and pulp viscosity introduced the possibility of adding catalysts to the reaction to alter this relationship. It would then be possible to determine which parameter, kappa number or viscosity, is more suitable as a determinant of susceptibility to hydrolysis.

### 5.7.1 Effect of addition of magnesium heptasulphate on kappa number and viscosity

Table 5.5 Effect of addition of 0.1%  $\text{Mg}^{2+}$  on kappa number and pulp viscosity

Conditions		Kappa Number	Viscosity (mPa.s)
Temp ( $^{\circ}\text{C}$ )	Mg (%)		
90	0.0	$20 \pm 0.50$	$25 \pm 0.31$
90	0.1	$22 \pm 0.71$	$29 \pm 1.0$
110	0.0	$12 \pm 0.39$	$19 \pm 0.50$
110	0.1	$14 \pm 0.49$	$21 \pm 0.38$
145	0.0	$8.0 \pm 0.13$	$14 \pm 0.51$
145	0.1	$7.8 \pm 0.55$	$16 \pm 0.31$

There was a 3.61, 2.74 and 1.54 mPa.s increase in viscosity at  $90^{\circ}\text{C}$ ,  $110^{\circ}\text{C}$  and  $145^{\circ}\text{C}$ , respectively (Table 5.5) due to the addition of the magnesium salt during oxygen delignification. The effectiveness of the fibre protector appears to diminish as the temperature increased from  $90^{\circ}\text{C}$  to  $145^{\circ}\text{C}$  (Figure 5.9), and it would be expected that if the temperature were increased further, there would come a point when the addition of magnesium was ineffective.

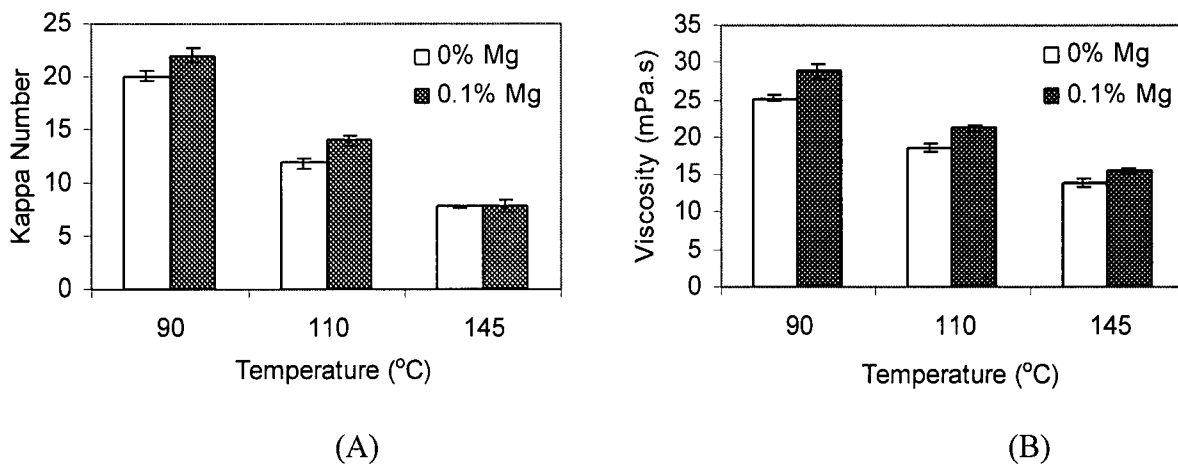


Figure 5.9 Dependence of (A) kappa and (B) viscosity on temperature when 0.1%  $\text{Mg}^{2+}$  is added



The addition of the magnesium salt also caused an increase in the kappa number: at 90°C and 110°C, the kappa number increased by 2 when the magnesium was added while, at 145°C, the increase was only 0.2. It is possible that in maintaining the fibre lengths, more lignin remains trapped within those fibres and is inaccessible to the chemicals affecting delignification.

In general, the magnesium had no discernible effect on the kappa-viscosity relationship. The data collected from the experiments where magnesium was added coincides with the data from the experiments without magnesium (Figure 5.10).

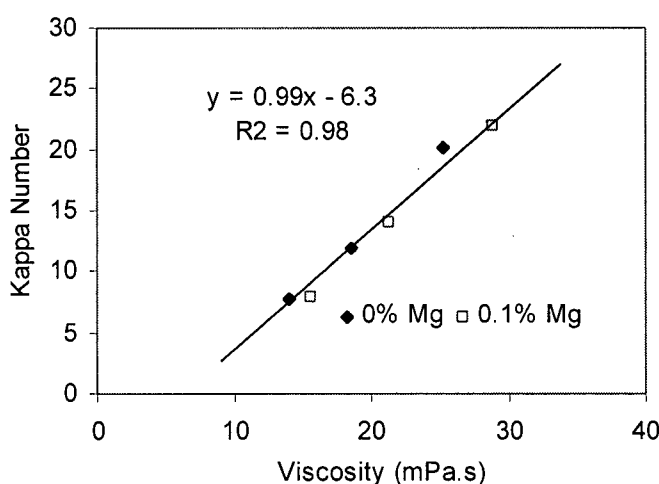


Figure 5.10 Relationship between kappa number and viscosity with and without the addition of 0.1%  $Mg^{2+}$

It is possible that magnesium was already added to the pulp sample that provided by Howe Sound Pulp and Paper Ltd. This would mean that further addition of magnesium in the lab would have little effect on the kappa-viscosity relationship.

### 5.7.2 Effect of magnesium on enzymatic hydrolysis

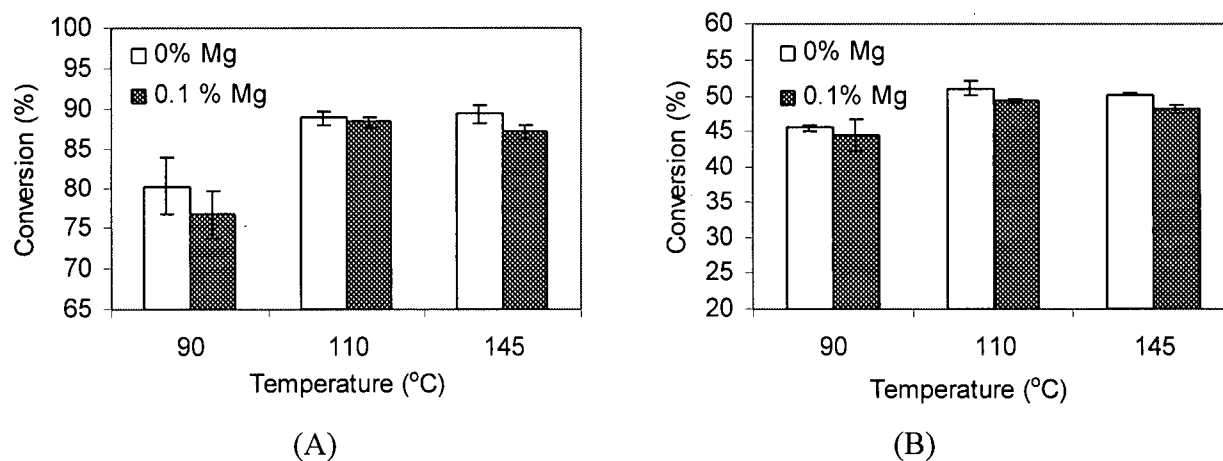


Figure 5.11 Effect of addition of magnesium on conversion at (A) 5:50 FPU:CBU and (B) 2:20 FPU:CBU loading

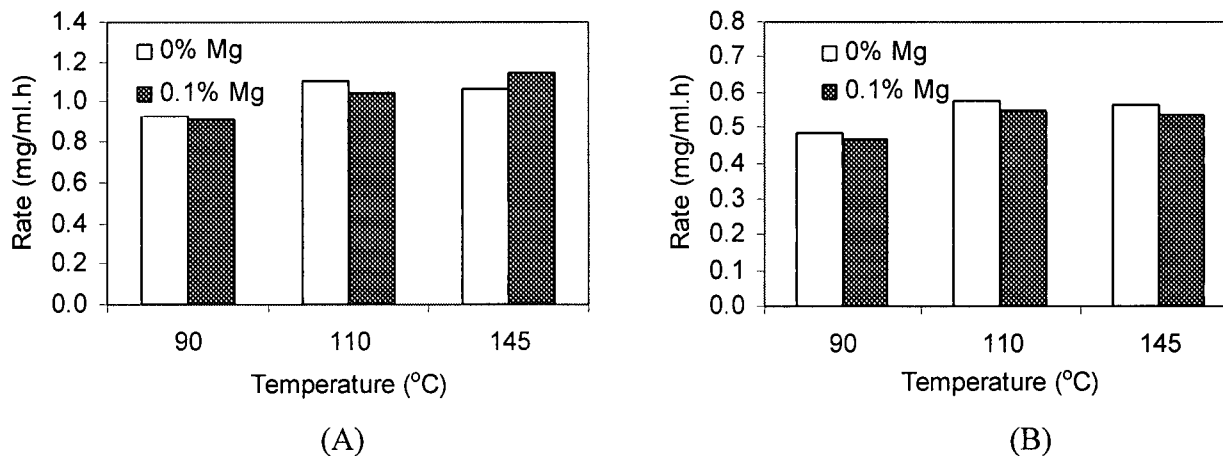


Figure 5.12 Effect of addition of magnesium on initial rate of hydrolysis at (A) 5:50 FPU:CBU and (B) 2:20 FPU:CBU loading

The addition of the magnesium salt, which caused an increase in kappa number and viscosity, resulted in a decrease in the conversion and initial rate of hydrolysis (Figures 5.11 and 5.12). In the range of kappa numbers from 10 to 20, increasing the kappa number was shown to cause a decrease in the rate and extent of hydrolysis (Figures 5.7 and 5.8).

## 5.8 Development of empirical equations to describe the effect of kappa number and pulp viscosity on enzymatic conversion

The linear regression function in EXCEL was used to develop empirical equations that would describe the effect of the kappa number and viscosity on conversion and the initial rate of hydrolysis. It was determined that either the kappa number or the pulp viscosity was sufficient to develop an equation with useful parameter estimates (i.e. a p-value < 0.1). When both kappa number and viscosity were used simultaneously, the p-values of all the parameter estimates were always much greater than 0.1. The resulting model equations were all second-order polynomials (equations 9 to 12) with R-squared values ranging from 0.79 to 0.49 at the 5 FPU loading and 0.27 to 0.17 at the 2 FPU loading (See Appendix II for full statistical analysis).

At 5:50 FPU:CBU loading:

$$\text{Conversion (\%)} = 74.25 + 3.49 \text{ Kappa} - 0.21 \text{ Kappa}^2 \quad (9)$$

$$\text{Rate (mg/ml.h)} = 1.02 - 1.22 \times 10^{-3} \text{ Kappa}^2 \quad (10)$$

$$\text{Conversion (\%)} = 54.47 + 4.47 \text{ Viscosity} - 0.15 \text{ Viscosity}^2 \quad (11)$$

$$\text{Rate (mg/ml.h)} = 0.97 - 7.80 \times 10^{-4} \text{ Viscosity}^2 \quad (12)$$

At 2:20 FPU:CBU loading:

$$\text{Conversion (\%)} = 37.84 + 1.66 \text{ Kappa} - 0.08 \text{ Kappa}^2 \quad (13)$$

$$\text{Rate (mg/ml.h)} = 0.42 + 0.03 \text{ Kappa} - 1.49 \times 10^{-3} \text{ Kappa}^2 \quad (14)$$

$$\text{Conversion (\%)} = 28.43 + 2.01 \text{ Viscosity} - 0.06 \text{ Viscosity}^2 \quad (15)$$

$$\text{Rate (mg/ml.h)} = 0.32 + 0.03 \text{ Viscosity} - 8.40 \times 10^{-4} \text{ Viscosity}^2 \quad (16)$$

It wasn't possible to develop one model to describe the results at both 2 and 5 FPU loadings due to differences in the hydrolysis reaction rates. This is likely due to the reaction kinetics and nonlinearity in hydrolysis.

By substituting equations 7 and 8 into equations 9 - 16, it was possible to develop 3-D graphical illustrations of the dependence of the conversion and initial rates of hydrolysis on the oxygen

delignification conditions (Figures 5.13 and 5.14). The graphs depicted here represent oxygen delignification runs carried out in an excess of oxygen (100 psig) for one hour. Since the maximum possible conversion and rate of hydrolysis are not restricted to a particular temperature or caustic concentration, the process is very flexible and may be easily modified.

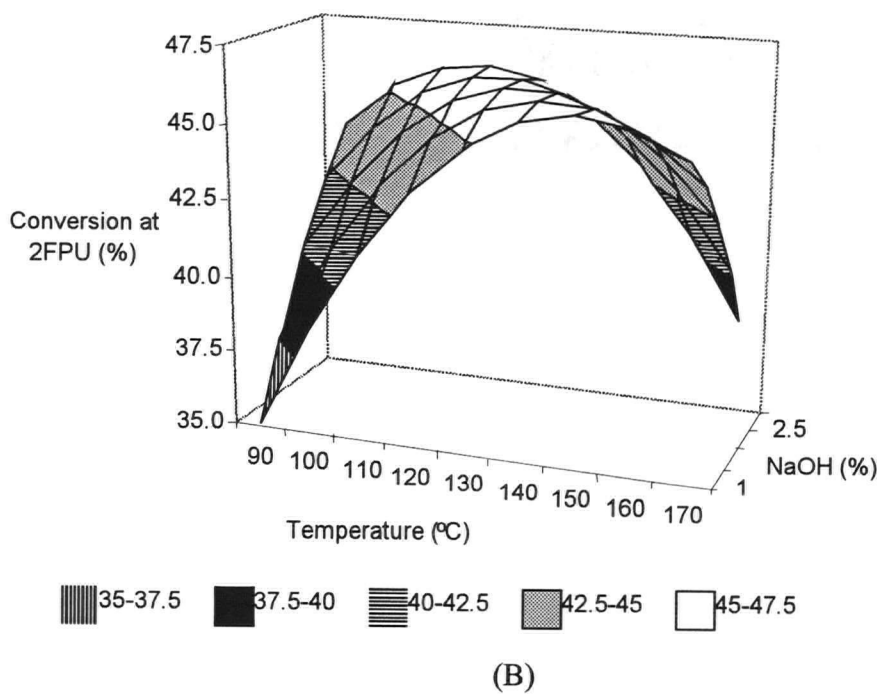
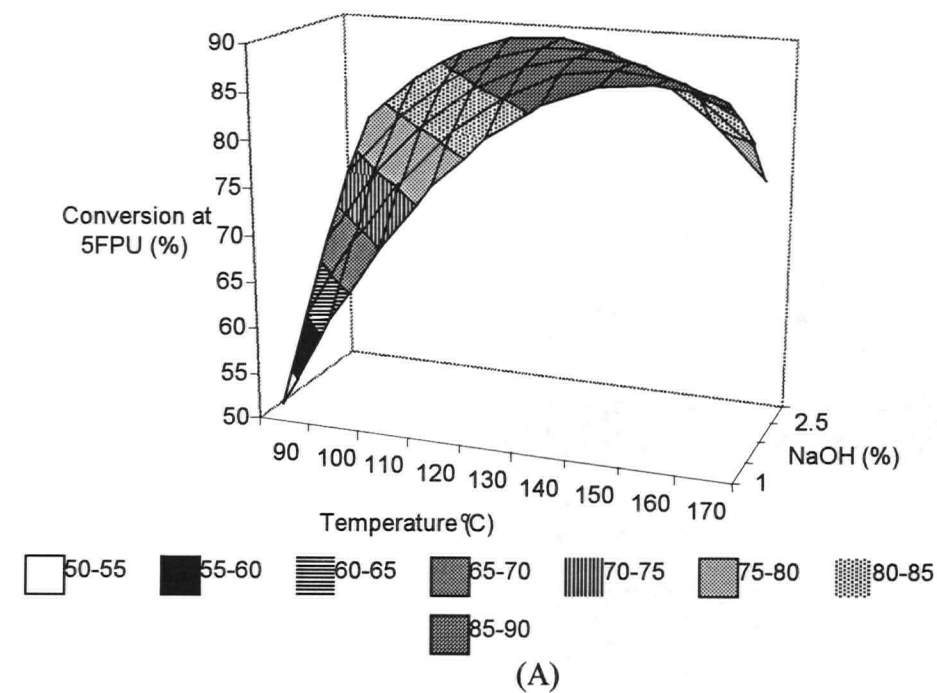


Figure 5.13 Dependence of conversion on temperature and caustic concentration at (A) 5:50 FPU:CBU loading and (B) 2:20 FPU:CBU loading (100 psig  $O_2$  partial pressure and 60 minutes, temperatures ranging from 90 – 170°C and caustic loading from 1 – 3%)

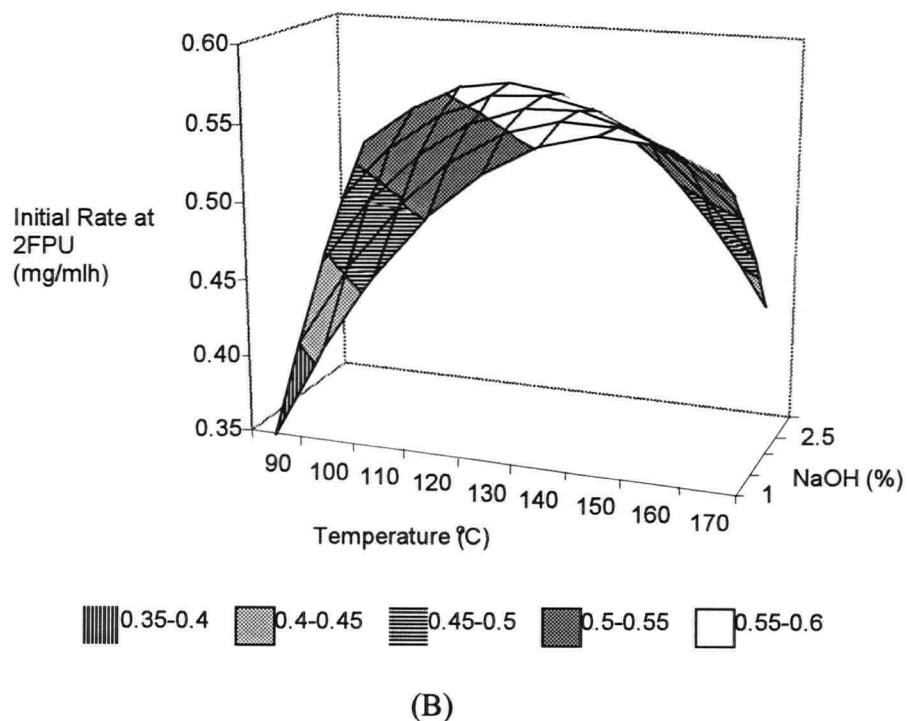
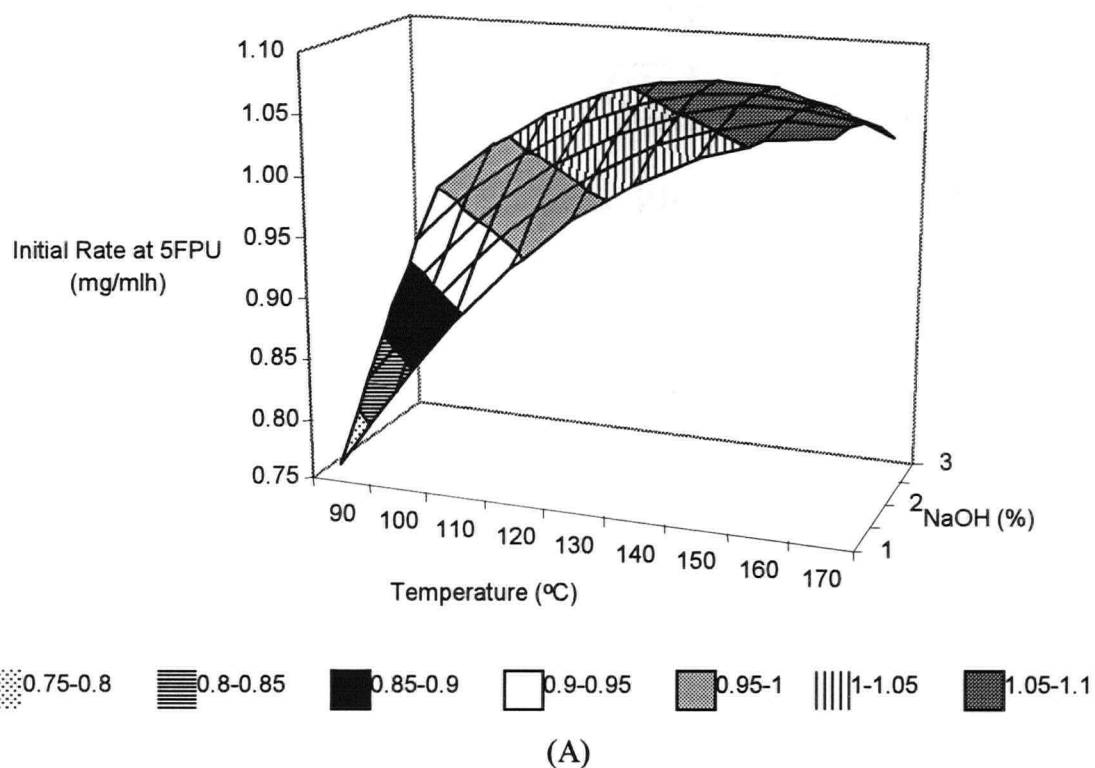


Figure 5.14 Dependence of initial rate of hydrolysis on temperature and caustic concentration at (A) 5:50 FPU:CBU loading and (B) 2:20 FPU:CBU loading (100 psig  $O_2$  partial pressure and 60 minutes, temperatures ranging from 90 – 170°C and caustic loading from 1 – 3%)

### 5.9 The effect of oxygen delignification on the enzymatic hydrolysis of refined and unrefined pulp mill rejects

The promising results obtained with the model substrate prompted the evaluation of the effect of oxygen delignification on one of the possible sources of lignocellulosic material for use in bioconversion. Tembec, a sulfite pulp mill located in Témiscaming, Quebec, produces about 20 - 30 tonnes of knots and fines annually, classified as rejects, which could be converted to fuel ethanol. A portion of the rejects collected from the Tembec mill was mechanically refined until the particle sizes were very small and fairly uniform, and both the refined and unrefined substrates were subjected to oxygen delignification.

Since kappa number determination of lignin content is only useful for pulp (there are numerous pieces of wood in the unrefined samples), the amount of lignin in the rejects was determined using Klason analysis. It was also impossible to determine the viscosity of the unrefined samples using the capillary viscometer method since that analysis is only useful for pulp containing no more than 5% lignin. The percent lignin before and after delignification is given in Table 5.6.

Table 5.6 The lignin contents of refined and unrefined rejects from the Tembec mill and their effect on enzymatic hydrolysis

Sample	% Lignin	2:20 FPU:CBU loading		5:50 FPU:CBU loading	
		Conversion (%)	Initial rate (mg/ml.h)	Conversion (%)	Initial rate (mg/ml.h)
Refined only	22 ± 0.50	35 ± 0.70	0.69	51 ± 0.026	1.4
Refined/oxygen delignified	16 ± 0.12	37 ± 0.61	0.75	67 ± 8.2	1.6
Unrefined only	26 ± 0.30	33 ± 0.49	0.61	43 ± 1.9	1.2
Unrefined/oxygen delignified	21 ± 0.078	33 ± 0.11	0.67	51 ± 0.042	1.4

The difference between the initial lignin content of the refined and unrefined knots is probably due to the fact that in the refined knots, there is a more even distribution of high and low lignin-containing components. However, for the unrefined knots, the percent lignin depends on the sample that was taken to do the analysis, which may have had more lignin than was representative of the sample. The lignin content of the rejects was reduced by about 20% for the unrefined sample and 25% for the refined sample, suggesting that oxygen delignification is affected by the particle size of the substrate.

The rejects were subsequently subjected to enzymatic hydrolysis under standard conditions and the final conversion and the initial rate of hydrolysis were measured (Table 5.6). When the unrefined sample was oxygen delignified, there was no change in the conversion at the 2:20 FPU:CBU loading but a 19% increase in conversion was observed at the 5:50 FPU:CBU loading. The initial rate of hydrolysis increased by 10 – 29%. Mechanical refining, with no oxygen delignification, increased the initial rate of hydrolysis and final conversion of the rejects by 16 - 22% and 5 - 21%, respectively, providing further evidence that smaller particle sizes increase enzymatic hydrolysis. The oxygen-delignified refined rejects gave 12 - 58% greater conversion and caused 11 – 13% increase in the initial rate of hydrolysis when compared to the unrefined sample.

These results clearly demonstrate the potential of oxygen delignification to be an effective means of pretreating lignocellulosic substrates for enzymatic conversion. Greater kappa reductions may be possible with higher temperatures and higher caustic loadings, which should translate to a greater extent of hydrolysis.



## 6. CONCLUSIONS

It was determined that, when subjected to oxygen delignification, the residual kappa number and viscosity of a hemlock Kraft pulp sample with an initial kappa number of 34 and a viscosity of 35 mPa.s depend on the reaction time, temperature, caustic concentration and oxygen partial pressure as follows:

$$K = 49.73 - 0.19T - 3.09S - 0.05t - 0.05O$$

$$V = 55.91 - 0.03t - 0.19T - 4.06S - 0.02O$$

Where, K = kappa number

V = viscosity (mPa.s)

t = retention time (mins)

S = caustic loading (% NaOH per gram dry weight of pulp)

T = reaction temperature (°C)

O = oxygen partial pressure (psig)

Increasing any one of the reaction parameters resulted in an increase in delignification and cellulose degradation, and hence caused a decrease in the kappa number and viscosity.

The kappa number and viscosity were found to share a linear relationship:

$$Kappa = 0.94 Viscosity - 6.33$$

And were found to have an impact on the initial rate of hydrolysis and conversion as follows:

At 5:50 FPU:CBU loading:

$$Conversion (\%) = 74.25 + 3.49 Kappa - 0.21 Kappa^2$$

$$Rate (mg/ml.h) = 1.02 - 1.22 \times 10^{-3} Kappa^2$$

$$\text{Conversion (\%)} = 54.47 + 4.47 \text{ Viscosity} - 0.15 \text{ Viscosity}^2$$

$$\text{Rate (mg/ml.h)} = 0.97 - 7.80 \times 10^{-4} \text{ Viscosity}^2$$

At 2:20 FPU:CBU loading:

$$\text{Conversion (\%)} = 37.84 + 1.66 \text{ Kappa} - 0.08 \text{ Kappa}^2$$

$$\text{Rate (mg/ml.h)} = 0.42 + 0.03 \text{ Kappa} - 1.49 \times 10^{-3} \text{ Kappa}^2$$

$$\text{Conversion (\%)} = 28.43 + 2.01 \text{ Viscosity} - 0.06 \text{ Viscosity}^2$$

$$\text{Rate (mg/ml.h)} = 0.32 + 0.03 \text{ Viscosity} - 8.40 \times 10^{-4} \text{ Viscosity}^2$$

Decreasing the kappa number (and the pulp viscosity) caused an increase in the digestibility of the pulp up to a maximum of 92% between kappa numbers of 6 - 10. When the kappa number is decreased further, the digestibility started to decrease.

When sulphite pulp mill rejects were subjected to oxygen delignification, the rate and extent of hydrolysis increased by 19% and 29%, respectively. Refining the rejects before oxygen delignification caused even greater increases in the digestibility of the substrate - the initial rate of hydrolysis increased by 13% and the conversion increased by 58%.

The results from the experiments show when a substrate was oxygen-delignified before being subjected to enzymatic hydrolysis, both the initial rate and conversion of the substrate increased. In conclusion, oxygen delignification should be given more consideration as a pretreatment process.

## 7. POSSIBLE FUTURE WORK

The work presented in this thesis is the first step in the development of the oxygen delignification technology for use as a pretreatment process upstream of enzymatic hydrolysis. It was shown that, when a hemlock Kraft pulp and the rejects from a sulphite mill were subjected to oxygen delignification, the rate and extent of hydrolysis was greatly improved.

In order to establish a general model for hydrolysis of Kraft pulps, many more experiments are necessary to fully define the effect of the residual kappa number and viscosity on enzymatic hydrolysis. Hemlock Kraft pulps with different initial kappa number and viscosities could be oxygen delignified under a range of conditions and the residual kappa number and viscosity measured. Subsequent hydrolysis would indicate the effect of starting lignin content and degree of polymerization on the conversion and initial rate of hydrolysis. This can be repeated for Kraft pulp from other lignocellulosic species, and then it may be possible to determine whether there is a general range of kappa numbers for which the hydrolysis of any Kraft pulp is maximized.

The kappa number and viscosity data could be also used to investigate whether the relationship between the residual kappa number and viscosity is substrate specific. This would be useful to the Pulp and Paper industry since many researchers are currently trying to develop catalysts to improve the selectivity in the oxygen delignification unit. A general correlation between the kappa number and the viscosity aid in developing a more accurate measure of the effectiveness of a particular catalyst.

Once the effect of oxygen delignification on the enzymatic hydrolysis of Kraft pulp has been thoroughly investigated, the next likely step would be to use a number of different substrates of various particle sizes. In this case, the relationship between kappa number and particle size would have to be determined. The effect of particle size on oxygen delignification and hydrolysis can be combined with the information obtained from the analysis of the various Kraft pulps, and may give a general model to relate delignification and hydrolysis to caustic concentration, temperature, oxygen pressure, time, initial kappa number and particle size. Such an equation

would be applicable to any substrate for use in an oxygen delignification – enzymatic hydrolysis system.

Additionally, it would be useful to determine what causes the decrease in the extent of hydrolysis at very low kappa numbers and make adjustments to the oxygen delignification reaction to combat these negative effects. Experiments would have to be performed to determine the extent of the effects of fibre characteristics such as porosity and crystallinity on hydrolysis. This may indicate some critical values for porosity or crystallinity at which hydrolysis is maximized.

## 8. NOMENCLATURE

$\alpha$	Alpha
$\beta$	Beta
$\mu\text{g}$	microgram(s)
$\mu\text{L}$	microliter(s)
$\mu\text{m}$	micrometer(s)
$^{\circ}\text{C}$	degree Celsius
CBU	cellobiose units
cm	centimeter(s)
DNS	Dinitrosalicylic Acid
FPU	filter paper units
L	liter(s)
m	meters
mg	milligram(s)
mL	milliliter(s)
mM	millimolar
mPa	milliPascal
min	minute(s)
M	molar
psi	pounds per square inch (gauge)
nm	nanometers
N	normal
s	second(s)
g	gram(s)
rpm	revolutions per minute
w/w	weight per weight

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## APPENDIX - Linear regression analysis of experimental data

Development of equation 7, describing the effect of oxygen delignification parameters on Kappa Number

<i>Regression Statistics</i>	
Multiple R	0.978721
R Square	0.957894
Adjusted R Square	0.950238
Standard Error	1.172209
Observations	27

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	49.72773	1.782172	1.15E-18	46.03173	53.42374
t	-0.052184	0.02018	0.016865	-0.094035	-0.010332
T	-0.191576	0.012241	2.08E-13	-0.216962	-0.166191
N	-3.087608	0.403605	1.23E-07	-3.924633	-2.250582
O	-0.04759	0.009032	2.75E-05	-0.066321	-0.028859

Development of equation 8, describing the effect of oxygen delignification parameters on Viscosity

<i>Regression Statistics</i>	
Multiple R	0.984726
R Square	0.969686
Adjusted R Square	0.964174
Standard Error	1.00233
Observations	27

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	55.91449	1.5238953	3.146E-21	52.75413	59.074863
t	-0.03279	0.0172557	0.0705671	-0.06858	0.0029938
T	-0.18677	0.0104667	1.425E-14	-0.20847	-0.1650606
N	-4.05564	0.3451132	5.934E-11	-4.77136	-3.3399197
O	-0.01903	0.0077229	0.0220277	-0.03505	-0.003013

Development of equations 9 and 11, describing the effect of kappa and viscosity on the Conversion at 5:50 FPU:CBU loading

Kappa dependence:

<i>Regression Statistics</i>	
Multiple R	0.889002
R Square	0.790324
Adjusted R Square	0.767027
Standard Error	3.285087
Observations	21

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	74.24566	3.083882	3.84E-15	67.76666	80.72466
K	3.491043	0.660107	4.99E-05	2.104208	4.877878
K <sup>2</sup>	-0.20744	0.031388	3.32E-06	-0.27338	-0.14149

Viscosity dependence:

<i>Regression Statistics</i>	
Multiple R	0.881588
R Square	0.777197
Adjusted R Square	0.752442
Standard Error	3.386358
Observations	21

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	54.46699	7.500991	9.46E-07	38.70798	70.226
V	4.466954	0.88698	8.59E-05	2.603477	6.330431
V <sup>2</sup>	-0.14655	0.024677	1.28E-05	-0.19839	-0.09471

Development of equations 13 and 15, describing the effect of kappa and viscosity on the Conversion at 2:20 FPU:CBU loading

Kappa dependence:

<i>Regression Statistics</i>	
Multiple R	0.4834
R Square	0.233676
Adjusted R Square	0.148529
Standard Error	3.712156
Observations	21

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	37.83579	3.484793	2.48E-09	30.51451	45.15708
K	1.656219	0.745923	0.039471	0.089093	3.223346
K <sup>2</sup>	-0.08276	0.035469	0.031439	-0.15727	-0.00824

Viscosity dependence:

<i>Regression Statistics</i>	
Multiple R	0.441582
R Square	0.194995
Adjusted R Square	0.105549
Standard Error	3.80469
Observations	21

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	28.42636	8.427622	0.003388	10.72057	46.13215
V	2.00647	0.996552	0.059271	-0.08721	4.10015
V <sup>2</sup>	-0.05754	0.027725	0.052544	-0.11579	0.000704

Development of equations 10 and 12, describing the effect of kappa and viscosity on the initial rate of hydrolysis at 5:50 FPU:CBU loading

Kappa dependence:

<i>Regression Statistics</i>					
Multiple R	0.699822				
R Square	0.489751				
Adjusted R Square	0.433057				
Standard Error	0.064376				
Observations	21				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	1.020379	0.060433	1.75E-12	0.893414	1.147344
<b>K</b>	<b>0.014044</b>	<b>0.012936</b>	<b>0.291947</b>	<b>-0.01313</b>	<b>0.041221</b>
K <sup>2</sup>	-0.00122	0.000615	0.062421	-0.00251	7.04E-05

Viscosity dependence:

<i>Regression Statistics</i>					
Multiple R	0.730624				
R Square	0.533811				
Adjusted R Square	0.482012				
Standard Error	0.061534				
Observations	21				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	0.970954	0.136301	1.23E-06	0.684595	1.257312
<b>V</b>	<b>0.017083</b>	<b>0.016117</b>	<b>0.303213</b>	<b>-0.01678</b>	<b>0.050944</b>
V <sup>2</sup>	-0.00078	0.000448	0.098293	-0.00172	0.00016

Development of equations 14 and 16, describing the effect of kappa and viscosity on the initial rate of hydrolysis at 2:20 FPU:CBU loading

Kappa dependence:

<i>Regression Statistics</i>					
Multiple R	0.521088				
R Square	0.271533				
Adjusted R Square	0.190592				
Standard Error	0.060643				
Observations	21				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	0.415405	0.056928	8.85E-07	0.295803	0.535007
K	0.029483	0.012186	0.026351	0.003882	0.055084
K <sup>2</sup>	-0.00149	0.000579	0.019356	-0.00271	-0.00027

Viscosity dependence:

<i>Regression Statistics</i>					
Multiple R	0.417911				
R Square	0.17465				
Adjusted R Square	0.082944				
Standard Error	0.064549				
Observations	21				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	0.321341	0.142981	0.037387	0.020949	0.621733
V	0.027955	0.016907	0.115575	-0.00757	0.063476
V <sup>2</sup>	-0.00084	0.00047	0.089234	-0.00183	0.000143