APPLICATION OF XYLANASES IN BLEACHING OF INDUSTRIAL PULPS

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Master of Technology in Biotechnology, at the ML Sultan Technikon, Durban. It has not been submitted before, for any degree or exam, at any other Technikon or University. Where use was made of the work of others, it has been duly acknowledged in the text.

Andreas Muzikababa Madlala

March 2000

This work is dedicated to my lovely mother

'Nombulelo KaHadebe Mkhize'

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CONTENTS

Acknowledgements.....(i)

List of Figures.....(viii)

List of Tables.....(xi)

CHAPTER 1: LITERATURE REVIEW.....1

1.1 INTRODUCTION.....1

1.2 WOOD HEMICELLULOSES.....5

1.2.1 Xylans.....7

1.2.1.1 Xylans from hardwood.....7

1.2.1.2 Xylans from softwood.....8

1.2.2 Glucomannans.....9

1.2.3 Other hemicelluloses.....10

1.3 PROCESSES INVOLVED IN WOOD PULPING.....10

1.3.1 Mechanical pulping process.....11

1.3.2 Chemical pulping process.....11

1.3.2.1 Kraft process.....12

1.3.2.2 Sulphite process.....14

1.3.2.3 Soda process.....14

1.4 MODIFICATION OF XYLANS DURING PULPING.....15

1.5 BLEACHING OF PULP.....16

1.5.1 Problems associated with chemical bleaching.....19

1.6 XYLAN-DEGRADING ENZYMES.....22

1.6.1 Endo-1,4-β-xylanase.....23

1.6.2 β-Xylosidase.....25

1.6.3 α-L-Arabinofuranosidase.....26

1.6.4 α-Glucuronidase.....27

1.6.5 Acetyl xylan esterases.....28

1.6.6 Ferulic and *p*-coumaric acid esterases.....28

1.7 APPLICATION OF ENZYME IN BLEACHING OF PULP.....29

1.7.1 Proposed mechanism of enzyme-aided bleaching.....30

1.7.2 Factors affecting the enzymatic pretreatment of pulp.....33

1.7.2.1 Operating conditions.....33

1.7.2.2 Raw material.....34

1.7.2.3 Pulping process.....35

1.7.3 Enzyme-aided bleaching using different bleaching sequences.....36

1.7.4 Industrial use of xylanases.....37

CHAPTER 2: CHARACTERISATION OF ENZYME AND PULP PROPERTIES.....39

2.1 ABSTRACT.....39

2.2 INTRODUCTION.....40

2.3 MATERIALS AND METHODS.....43

2.3.1 Enzyme source.....43

2.3.2 Crude enzyme preparation of *T. lanuginosus*.....43

2.3.3 Enzyme assay.....44

2.3.4 Characterisation of enzymes.....44

2.3.5 Preparation and characterisation of pulps.....45

2.4 RESULTS.....47

2.4.1 Enzyme properties.....47

2.4.2 Characteristics of pulp properties.....50

2.5 DISCUSSION.....52

CHAPTER 3: BIOBLEACHING EFFECTS OF *T. lanuginosus* SSBP XYLANASE ON PAPER PULPS.....54

3.1 ABSTRACT.....54

3.2 INTRODUCTION.....55

3.3 MATERIALS AND METHODS.....57

3.3.1 Enzyme preparation.....57

3.3.2 Enzymatic hydrolysis of pulp.....57

3.3.3 Analyses of hydrolysis products.....57

3.3.4 Chemical bleaching of pulps.....58

3.3.5 Analyses of pulps.....59

3.3.6 Evaluation of enzyme pretreatment effect on chlorine dioxide consumption.....59

3.4 RESULTS.....61

3.4.1 Release of chromophore material.....61

3.4.2 Release of reducing sugars.....64

3.4.3 Effect of enzyme pretreatment on Kappa number reduction.....66

3.4.4 Effect of enzyme pretreatment on pulp brightness.....67

3.4.5 Effect of enzyme pretreatment on pulp viscosity.....69

3.4.6 Effect of enzyme pretreatment on sugar composition.....71

3.4.7 Effect of enzyme pretreatment on chlorine dioxide consumption.....75

3.5 DISCUSSION.....78

CHAPTER 4: BIOBLEACHING EFFECTS OF A COMMERCIAL XYLANASE ON PAPER PULPS.....82

4.1 ABSTRACT.....82

4.2 INTRODUCTION.....83

4.3 MATERIALS AND METHODS.....85

4.3.1 Enzyme preparation.....85

4.3.2 Enzymatic hydrolysis of pulp.....85

4.3.3 Analyses of hydrolysis products.....85

4.3.4 Chemical bleaching of pulps.....85

4.3.5 Analyses of pulps.....86

4.3.6 Evaluation of enzyme pretreatment on chlorine dioxide consumption.....86

4.4 RESULTS.....87

4.4.1 Release of chromophore material.....87

4.4.2 Release of reducing sugars.....90

4.4.3 Effect of enzyme pretreatment on Kappa number reduction.....92

4.4.4 Effect of enzyme pretreatment on pulp brightness.....93

4.4.5 Effect of enzyme pretreatment on pulp viscosity.....96

4.4.6 Effect of enzyme pretreatment on sugar composition.....98

4.4.7 Effect of enzyme pretreatment on chlorine dioxide consumption.....102

4.5 DISCUSSION.....105

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS.....109

5.1 THE RESEARCH IN PERSPECTIVE.....109

5.2 FUTURE RESEARCH.....116

REFERENCES.....118

LIST OF FIGURES

Figure 1.1. Schematic of the organisation of different hemicelluloses in the secondary wall of wood fibres (Salmén and Olsson, 1998).....**6**

Figure 1.2. Composition of O-acetyl-4-O-methylglucuronoxylan (hardwood xylan). Numbers indicate the carbon atoms at which substitutions take place. Ac: acetyl group; α-4-O-Me-GlcA: α-4-O-methylglucuronic acid (Sunna and Antranikian, 1997).....**8**

Figure 1.3. Composition of arabino-4-O-methylglucuronoxylan (softwood xylan). Numbers indicate the carbon atoms at which substitutions take place. α-Araf: α-Arabinofuranose; α-4-O-methylglucuronic acid (Sunna and Antranikian, 1997).....**9**

Figure 1.4. Schematic illustration of the kraft pulping process (Nissen *et al.,* 1992).....**13**

Figure 1.5. Schematic illustration of the stages involved in the kraft pulp bleaching process (Nissen *et al.,* 1992).....**18**

Figure 1.6. **(A):** Xylanolytic enzymes involved in the degradation of xylan. Ac: Acetyl group; α-Araf: α-arabino-furanose; α-4-O-Me-GlcA: α-4-O-methylglucuronic acid

(B): Hydrolysis of xylooligosaccharide by β–xylosidase (Sunna and Antranikian, 1997) **22**

Figure 1.7. Hypothetical target substrates for xylanase during xylanase-aided bleaching xylan-derived chromophores; lignin-xylan complexes; xylan that physically entraps lignin; xylan that modifies fibre swelling. The diagrams illustrate how the attack

of these target substrates (L=lignin; X=xylan) on pulp fibres might affect subsequent chemical bleaching (Wong *et al.*, 1997b)...**32**

Figure 2.1. Effect of pH (A) and temperature (B) on the activity of *T. lanuginosus* SSBP xylanase.....**48**

Figure 2.2. Effect of pH (A) and temperature (B) on the activity of Xylanase P...**49**

Figure 3.1. Release of chromophore material at 280 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5.....**62**

Figure 3.2. Release of chromophore material at 465 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5.....**63**

Figure 3.3. Release of reducing sugars (RS) from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5.....**65**

Figure 3.4. Viscosity of **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp, pretreated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5, after DED-bleaching.....**70**

Figure 4.1. Release of chromophore material at 280 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0.....**88**

Figure 4.2. Release of chromophore material at 465 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0 .....**89**

Figure 4.3. Release of reducing sugars (RS) from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0.....**91**

Figure 4.4. Viscosity of **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp, pretreated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0, after DED-bleaching **97**

LIST OF TABLES

Table 1.1. Hemicellulose in softwood and hardwood (Sjöström, 1993).....**6**

Table 1.2. Annual production of wood-based kraft and sulfite pulp in the world (Suurnäkki *et al.,* 1997).....**13**

Table 1.3. Alternatives available to produce TCF or ECF pulps (Bajpai and Bajpai, 1997).....**21**

Table 1.4. The aims of enzyme-aided bleaching using different bleaching sequences (Viikari *et al.,* 1994).....**37**

Table 2.1. Properties of crude and commercial enzyme preparations.....**50**

Table 2.2. Physical and chemical properties of unbleached bagasse, post-oxygen soda and post-oxygen kraft pulps (Batch 1).....**51**

Table 2.5. Physical and chemical properties of unbleached bagasse, post-oxygen soda and post-oxygen kraft pulps (Batch 2).....**51**

Table 3.1. Chemical bleaching conditions in D_1, E and D_2 stages.....**58**

Table 3.2. Chemical bleaching conditions of pulps used for evaluating the influence of enzyme pretreatment on chlorine dioxide consumption.....**60**

Table 3.3. Kappa number of bagasse, post-oxygen soda and post-oxygen kraft pulps pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5.....**66**

Table 3.4. Brightness of bagasse pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5.....**68**

Table 3.5. Brightness of post-oxygen soda pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5.....**68**

Table 3.6. Brightness of post-oxygen kraft pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5.....**69**

Table 3.7. Sugar composition of bagasse pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching.....**72**

Table 3.8. Changes in glucose and xylose concentrations of bagasse pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching.....**72**

Table 3.9. Sugar composition of post-oxygen soda pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching.....**73**

Table 3.10. Changes in glucose and xylose concentrations of post-oxygen soda pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching.....**73**

Table 3.11. Sugar composition of post-oxygen kraft pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching.....**74**

Table 3.12. Changes in glucose and xylose concentrations of post-oxygen kraft pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching.....**74**

Table 3.13. Reduction of chlorine dioxide charges in bleaching of bagasse pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5 .....**76**

Table 3.14. Reduction of chlorine dioxide charges in bleaching of post-oxygen soda pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5 ..... **76**

Table 3.15. Reduction of chlorine dioxide charges in bleaching of post-oxygen kraft pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5 ..... **77**

Table 4.1. Kappa number of bagasse, post-oxygen soda and post-oxygen kraft pulps pretreated with Xylanase P at 60°C and pH 5.0..... **92**

Table 4.2. Brightness of bagasse pulp pretreated with Xylanase P (X) at 60°C and pH 5.0 ..... **94**

Table 4.3. Brightness of post-oxygen soda pulp pretreated with Xylanase P (X) at 60°C and pH 5.0..... **94**

Table 4.4. Brightness of post-oxygen kraft pulp pretreated with Xylanase P (X) at 60°C and pH 5.0..... **95**

Table 4.5. Sugar composition of bagasse pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching..... **99**

Table 4.6. Changes in glucose and xylose concentrations of bagasse pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching..... **99**

Table 4.7. Sugar composition of post-oxygen soda pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching..... **100**

Table 4.8. Changes in glucose and xylose concentrations of post-oxygen soda pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching..... **100**

Table 4.9. Sugar composition of post-oxygen kraft pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching.....**101**

Table 4.10. Changes in glucose and xylose concentrations of post-oxygen kraft pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching.....**101**

Table 4.11. Reduction of chlorine dioxide charges in bleaching of bagasse pulp pretreated with Xylanase P at 60°C and pH 5.0.....**103**

Table 4.12. Reduction of chlorine dioxide charges in bleaching of post-oxygen soda pulp pretreated with Xylanase P at 60°C and pH 5.0.....**103**

Table 4.13. Reduction of chlorine dioxide charges in bleaching of post-oxygen kraft pulp pretreated with Xylanase P at 60°C and pH 5.0.....**104**

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The ever-increasing demand for a wide variety of paper products has led to the pulp and paper industry becoming one of the largest industries in the world. In 1988 the United States alone produced almost 71 million metric tonnes of paper and pulp board (Jeffries, 1992). South Africa has also become one of the major international producers of pulp and paper products. Since 1970, the production of paper and board by the South African industry achieved an average growth rate of 5.2% per annum, and in 1997 South Africa was the twelfth largest producer of pulp and 24th biggest supplier of paper and board in the world (Molony, 1999).

Pulp is produced from the conversion of wood other lignocellulosic materials such as sugarcane and wheat straw using physical and/or chemical pulping processes (Biermann, 1993). The kraft process is the major pulping process that is regarded as the most cost-effective, versatile and efficient wood delignification method available representing over 70% of the world's annual pulp production (Bajpai and Bajpai, 1997) that is estimated at more than 160 million metric tonnes (Garg *et al.,* 1996). Wood and pulp contain high amounts of structurally different hemicelluloses, which are polymeric constituents of the plant cell wall together with lignin and cellulose. In the pulp and paper industry these hemicellulosic polysaccharides play an important role in determining the chemical interactions between fibres, water and the pulping chemicals in kraft pulp (Viikari *et al.,* 1993). The hemicellulose in softwood kraft pulps is composed of xylan and glucomannan, whereas in hardwood kraft pulps xylan is the main hemicellulosic component (Sjöström,

1993). About 90% of the constituent lignin, which is deposited in wood as a polyphenolic matrix surrounding and encrusting the cellulosic and hemicellulosic polysaccharides, is removed during the pulping process (Chen *et al.,* 1997). The remaining lignin (about 10%) which is covalently bound to carbohydrates moieties, however, is believed to be primarily responsible for imparting the dark brown colour to kraft pulp and papers (Bajpai and Bajpai, 1997). To remove this residual lignin the resulting pulp has to undergo bleaching in order to get a white pulp for paper production. The traditional bleaching involves the use of a chlorine-based bleaching sequence, where chlorine gas is used during prebleaching to chlorinate lignin for subsequent extraction under alkaline conditions, and the chlorine dioxide is used to oxidise the remaining residual lignin to brighten the pulp (Wong *et al.,* 1997a).

The effluents resulting from bleaching of pulp with chlorine-based chemicals have been shown to have the potential of causing serious environmental problems as they are said to contain the chlorolignins which are highly coloured and some of these compounds are in part toxic and mutagenic (Christov and Prior, 1998). Dioxins and volatile organochlorine compounds, such as chloroform, and other chlorinated organic compounds known as adsorbable organic halides (AOX) are also produced during the chlorine bleaching process. Trichloroacetic acid (TCA) and dichloroacetic acid (DCA), which are potential carcinogens, have also been detected downstream of some pulp mills (Juuti *et al.,* 1996). This situation has resulted into the pulp and paper industry becoming one of the major contributors to both air and water pollution. Due to increasing pressure from environmental organisations against the use of chlorine and for the industry to reduce discharges into rivers and streams, the pulp and paper industry has to reconsider their chlorine-based bleaching techniques.

This has led to a need for production of elemental chlorine-free (ECF) and totally chlorine-free (TCF) pulps which means using other environmentally friendly bleaching agents in place of chlorine-containing bleaching chemicals or developing new pulping and bleaching technologies. These include the introduction of oxygen delignification as a pretreatment step and the extension of the cooking time for additional lignin removal prior to bleaching (Christov and Prior, 1998). Other alternatives are substituting chlorine with chlorine dioxide or replacing chlorine-based chemicals with hydrogen peroxide and/or ozone. All these techniques are good in lowering the levels of AOX but require high investment and can also cause a loss in pulp viscosity and strength in the final paper product (Nissen *et al.,* 1992; Yang *et al.,* 1992). Enzymes therefore remain as the only possible answer to reduce or eliminate chlorine-based chemicals utilised in bleaching of pulp.

Several applications have been investigated for enzymatic treatments of pulp, which include modification of pulp properties and enhanced bleaching. Xylanases have been the most widely used of all the hemicellulases and have been found to enhance the extractability of lignin by different bleaching chemicals (Kantelinen *et al.*, 1993). A combination of two mannanases with a xylanase preparation from *Sclerotium rolfsii* increased brightness of softwood kraft pulp by up to 2.8% ISO compared to the individual enzymes (Gübitz *et al.,* 1996). The same pulp was shown to gain brightness increase of 3.0% ISO when treated only with a xylanase from *Penicillium simplicissimum* (Gübitz *et al.,* 1997a). Oxygen delignified softwood pulp produced by modified continuous cooking process gained a brightness increase of 1.9 ISO units after being pre-treated with an acid tolerant xylanase from *Aspergillus kawachii* at 100 $nkat.g^{-1}$ (Tenkanen *et al.*, 1997). Haarhoff *et al.* (1999) characterised a *Themomyces lanuginosus* strain MED2D1 that was able to reduce Kappa number of Eucalyptus kraft pulp by 10.5%. The use of xylanases in bleaching of eucalypt kraft pulp led to chlorine dioxide savings of up to 40 % without

affecting the final brightness (Vicuña *et al.*, 1997; Vidal *et al.*, 1997). In a comparative study by Garg *et al.* (1998) crude xylanase was shown to be effective as commercial xylanase preparations used in that study and had the advantage of preserving paper strengths properties compared to those commercial xylanases.

In addition application of xylanase preparations from *Aureobasidium pullulans* and *T. lanuginosus* SSBP were effective bleach boosters on sulphtite pulps (Christov and Prior, 1997; Balakrishnan *et al.*, 1998). A combination of xylanase and mannanase preparations from *T. lanuginosus* and *S. rolfsii,* respectively, were able to solubilize 50% more mannan and 11% more xylana from softwood sulphite dissolving pulps (Gübitz *et al.,* 1997b). An increase in the brightness of bagasse pulp and wheat straw pulp produced by alkaline delignification was also observed after enzyme treatment with *T. lanuginosus* SSBP xylanase and commercially available xylanase preparations, respectively (Bissoon *et al.,* 1998; Spiridon e*t al.,* 1998).

This study was thus designed to evaluate the effect of crude *T. lanuginosus* SSBP xylanase preparation and commercial available xylanase, Xylanase P (Iogen Corp., Canada), in biobleaching of three industrial pulps: unbleached bagasse, post-oxygen soda and post-oxygen kraft, produced by SAPPI (South African Pulp and Paper Industries). To achieve this, the following objectives were pursued:

i Production and determination of optimum dose and treatment time at which the crude preparation of *T. lanuginosus* SSBP xylanase was most effective in biobleaching of three different paper pulps;

ii Determination of optimum dose and treatment time at which Xylanase P was most effective in biobleaching of three different paper pulps; and

iii Evaluation of the effect of *T. lanuginosus* SSBP xylanase and Xylanase P in chlorine dioxide consumption in a D_1ED_2-bleach sequence.

1.2 WOOD HEMICELLULOSES

Plant cell wall, which is the main polysaccharide-containing renewable resource in nature, is composed of three polymeric constituents: (i) cellulose, an insoluble polymer composed of β-D-glucopyranosyl residues linked by β-1,4-glycosidic bonds; (ii) hemicellulose, a series of heteropolysaccharides; and (iii) lignin, a complex polyphenol (Biely, 1993). Cellulose is the most abundant of the components, generally representing 40-45 % of the wood dry weight (Sjöström, 1993). In plant fibres cellulose determines the character of the fibre and permits its use in papermaking. The principal role of lignin which is about 15-25 % % of the wood dry weight is to form the middle lamella, the intercellular material that cements the fibres together. Depending on the wood species, hemicellulose constitutes about 20-30 % of the wood dry weight (Suurnäkki *et al.*, 1997). In pulps, hemicelluloses play an important role in fibre morphology and paper physics and also in determining the chemical interactions between fibres, water and the pulping chemicals (Viikari *et al.,* 1993).

The structure of various types of hemicelluloses depends on the type of plant, and may even vary between different parts of the same plant. The hemicelluloses are composed of both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid which may be acetylated or methylated and most

hemicelluloses contain two to six of these sugars (Eriksson *et al.*, 1990). The hemicelluloses are usually classified according to the main sugar residues in the backbone, for example, xylans, glucomannans, galactans and glucans (Buchert *et al.*, 1994). The two main hemicelluloses in wood are xylans and glucomannans, both of which are present in softwood kraft pulp whereas in hardwood pulps xylan is the main hemicellulose component, as shown in Table 1.1 (Sjöström, 1993). According to Salmén and Olsson (1998) the glucomannan is more closely attached to the cellulose, making up a layer of arranged molecules closely to the cellulose microfibrils with the xylan arranged in a mixture with the lignin surrounded by the glucomannan layer (Figure 1.1).

Table 1.1. Hemicellulose in softwood and hardwood (Sjöström, 1993)

Wood species	Component	Dry weight of component (%)
Pine	Xylan	5 – 11
	Glucomannan	14 – 20
Birch	Xylan	22 – 30
	Glucomannan	1 – 4

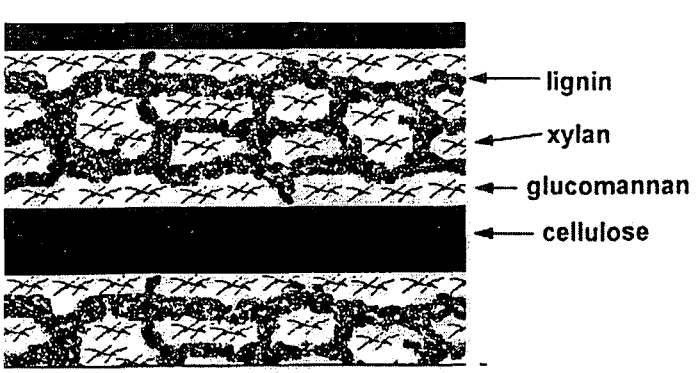

Figure 1.1. Schematic of the organisation of different hemicelluloses in the secondary wall of wood fibres (Salmén and Olsson, 1998)

1.2.1 Xylans

Xylan is the most common hemicellulosic polysaccharide, representing more than 30% of the dry weight in plant cell walls. Xylans comprise up to 30% of the cell wall material of annual plants, 15-30% of hardwoods and 7-10% of softwoods (Viikari *et al.*, 1993). They occur as heteropolysaccharides containing different substituent groups in the backbone chain and in the side chain, and the common substituents present are acetyl, arabinosyl, and glucuronosyl residues (Sunna and Antranikian, 1997).

1.2.1.1 Xylans from hardwood

Hardwood xylan is O-acetyl-4-O-methyl glucuronoxylan (Figure 1.2). It consists of at least 70 α-xylopyranose residues (average degree of polymerization (DP) between 150 and 200), linked by β-1,4-glycosidic bonds (Sunna and Antranikian, 1997). A total of 60-70% of the xylose units are esterified with acetic acid at the hydroxyl group of carbon 2 and/or 3 and there is a 4-O-methylglucuronic acid attached to the 2 position of xylose on every tenth xylose unit (Viikari *et al.*, 1993).

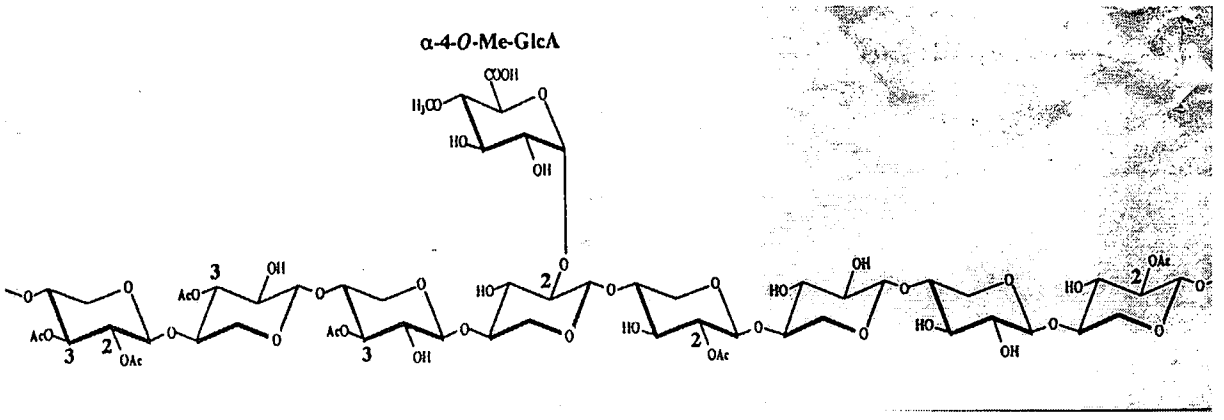

Figure 1.2. Composition of O-acetyl-4-O-methylglucuronoxylan (hardwood xylan). Numbers indicate the carbon atoms at which substitutions take place. Ac: acetyl group; α-4-O-Me-GlcA: α-4-O-methylglucuronic acid (Sunna and Antranikian, 1997)

1.2.1.2 Xylans from softwood

The xylan of softwood is mainly arabino-4-O-methyl glucuronoxylan, which in addition to 4-O-methyl glucuronic acid is also substituted by L-arabinofuranoside units linked by α-1,3-glycosidic bonds to the xylan backbone (Figure 1.3). These xylans have a higher 4-O-methylglucuronic acid content than hardwood xylans with the 4-O-methylglucuronic acid residues are attached to the C-2 position and are less branched and shorter than hardwood xylans, with a DP between 70 and 130, (Sunna and Antranikian, 1997). The average molar ratio of arabinose:4-O-methyl-glucuronic acid:xylose sugar units in softwood xylan is 1.3:2:10 (Sjöström, 1993).

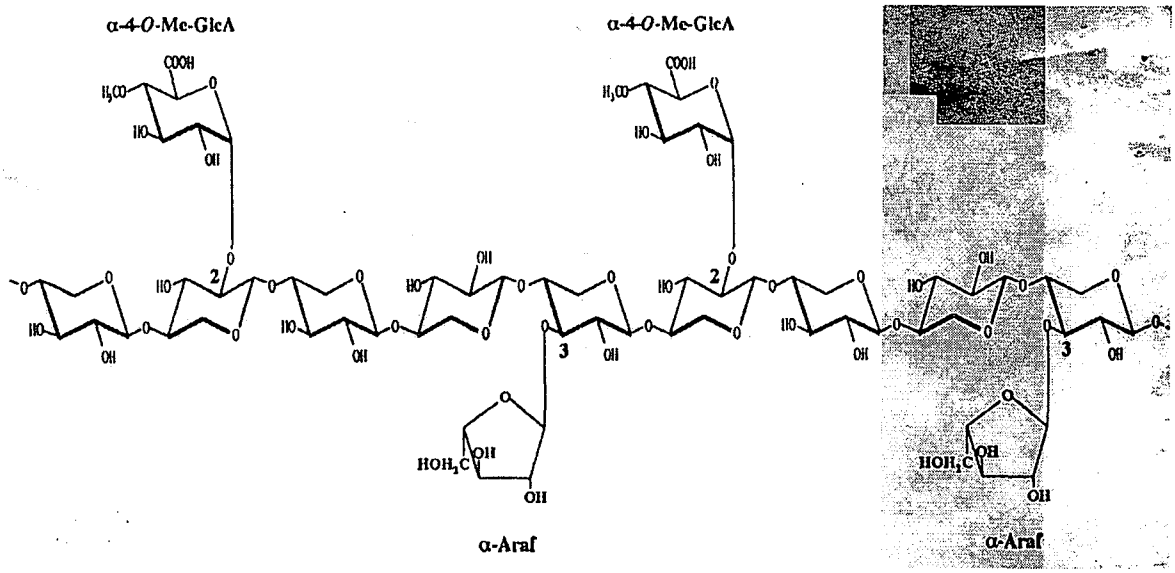

Figure 1.3. Composition of arabino-4-O-methylglucuronoxylan (softwood xylan). Numbers indicate the carbon atoms at which substitutions take place. α-Araf: α-Arabinofuranose; α-4-O-methylglucuronic acid (Sunna and Antranikian, 1997)

1.2.2 Glucomannans

The main group of mannans in the cell walls of higher plants is galactoglucomannans with the backbone composed of 1,4-β-linked D-glucose and D-mannose units, which are distributed randomly within the molecule. D-Galactose side groups are attached to mannose or glucose units, which are partially substituted by acetyl groups, via β-1,6 bonds (Viikari *et al.*, 1994). Softwood glucomannan has a backbone composed of β-1,4-linked D-glucopyranose and D-mannopyranose units which is partially substituted by α-galactose and acetyl units (Sjöström, 1993). The principal hemicelluloses in softwoods are O-Acetyl-galactoglucomannans, about 20%, which are divided into two main fractions (Viikari *et al.*, 1994). These fractions differ in their average molar ratio of galactose: glucose: mannose. The low galactose content fraction, glucomannan, has a ratio of 0.1:1:4 and the

corresponding ratio in the high-galactose content fraction, galactoglucomannan, is 1:1:3 (Sjöström, 1993). Hardwoods contain a small amount of glucomannan, 2-5%, and have no galactose or acetic acid side groups present (Viikari *et al.*, 1994). The ratio of glucose: mannose varies between 1:1 and 1:2 (Sjöström, 1993).

1.2.3 Other hemicelluloses

In addition to xylan and glucomannan, both softwoods and hardwoods contain minor amounts of other hemicelluloses such as pectin, galactan and arabinan (Sjöström, 1993). Softwood contains approximately 10% 1,4-β-D-galactan and 2% - 5% 1,3-β-D-glucan (Eriksson *et al.*, 1990).

1.3 PROCESSES INVOLVED IN WOOD PULPING

Pulp consists of wood or other lignocellulosic materials that have been broken down physically and/or chemically such that discrete fibres are liberated and can be dispersed in water and reformed a web. This is achieved by pulping processes that are divided into four broad categories in order of increasing mechanical energy required to separate fibres and decreasing reliance on chemical action. These are chemical, semi-chemical, chemi-mechanical and mechanical pulping processes (Biermann, 1993). Of these processes, mechanical and chemical pulping are the commonly used pulping processes with the chemical process being the more popular of the two.

1.3.1 Mechanical pulping process

Mechanical pulp is produced by using only mechanical attrition to pulp lignocellulosic materials without any chemicals. Lignin is retained in the pulp resulting into a total yield of about 90 – 98%. Residual lignin interferes with hydrogen bonding between fibres when paper is made resulting into low strength pulp and paper and imparts yellow colour to pulps when exposed to air and light (Biermann, 1993). This pulping process is capable of converting up to 95% of the dry weight of the wood into pulp of good quality, but requires amounts of energy. Mechanical pulps are commonly produced from softwood species as hardwoods have smaller, thinner fibres which are damaged easily during conventional mechanical pulping and yield pulp of poor quality (Smook, 1992).

1.3.2 Chemical pulping process

Chemical pulping involves the cooking of wood chips cooked with appropriate chemicals in an aqueous solution at elevated temperature and pressure in order to degrade and dissolve away the lignin and leave behind most of the cellulose and hemicelluloses in the form of intact fibres (Smook, 1992). This pulping process is mainly used to separate the wood fibres from each other in order to render them suitable for further industrial processing and is carried out by either the kraft (alkaline) process or the sulfite (acidic) process (Suurnäkki *et al.,* 1997).

1.3.2.1 Kraft process

The kraft process is the predominant and the most widely used pulping method in the world, representing over 70% of the world's annual pulp production, as illustrated in Table 1.2 (Bajpai and Bajpai, 1997). It is a full chemical pulping method using sodium hydroxide and sodium sulfide at pH above 12, at 160 – 180°C, corresponding to about 800kPa steam pressure, for up to 3 h to dissolve much of the lignin wood fibres (Biermann, 1993). The first part of the process involves debarking and chipping of the wood logs followed by a strong alkaline cooking where the main part of lignin is dissolved and washed away, as illustrated in Figure 1.4 (Nissen *et al.,* 1992). The resultant pulp is strongly coloured and has to be bleached in order to get a white pulp for paper production.

The kraft pulping process has advantage over other pulping processes in that it produces pulp with highest strength properties, utilises proven technology for efficient chemical recovery, has more flexibility with regard to wood species and can tolerate bark in the pulping process (Smook, 1992). The disadvantages are the difficulty with which the pulps are bleached resulting into consumption of large volumes of various pulping and bleaching chemicals and also results into low yields due to carbohydrate loss (Biermann, 1993).

Table 1.2. Annual production of wood-based kraft and sulphite pulp in the world (Suurnäkki *et al.*, 1997)

Region	Pulping method	Pulp production[a] (10^6 t[b]/a)
Northern America	Kraft	62.7
	Sulfite	8.2
Scandinavia	Kraft	13.3
	Sulfite	0.9
Europe	Kraft	8.1
	Sulfite	2.4
Latin America	Kraft	9.1
	Sulfite	0.9
Asia	Kraft	6.1
	Sulfite	0.2
Oceania	Kraft	1.2
	Sulfite	0.1
Africa	Kraft	0.9
	Sulfite	0

[a]Total production (unbleached, bleached)
[b]Metric tons of air dry pulp

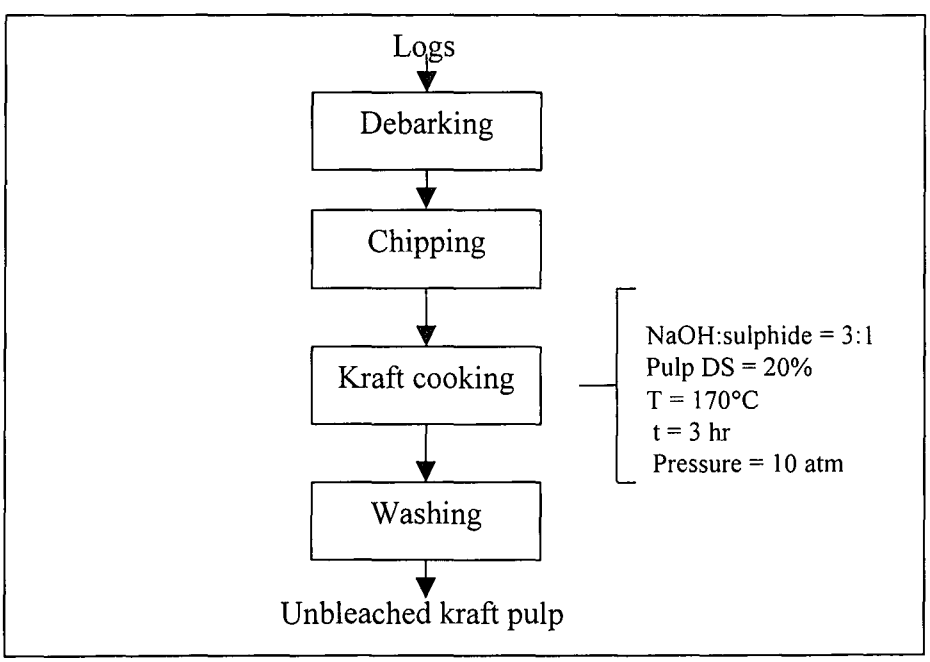

Figure 1.4. Schematic illustration of the kraft pulping process (Nissen *et al.*, 1992)

1.3.2.2 Sulphite process

Sulphite pulping is usually carried out in acidic conditions using mixtures of sulphurous acid and its alkali salts to solubilise lignin through the formation of sulfonate functionalities and cleavage of lignin bonds (Biermann, 1993). Pulps produced by this process are lighter in colour than kraft pulps and can be bleached more easily using fewer chemicals, hence it is still preferred in other countries for environmental reasons and in those countries involved in the manufacture of dissolving pulp (Suurnäkki *et al.,* 1997; Balakrishnan *et al.,* 1998). One of the disadvantages of this process is that the paper sheets produced are weaker than equivalent kraft sheets. Sulphite pulping is also sensitive to wood species and has difficulty in chemical recovery (Smook, 1992).

1.3.2.3 Soda process

This pulping process, though not extensively used, is applied to easily pulped materials like straws and some hardwoods as is the case at Sappi Enstra pulp mill. Anthraquinone may be used as a pulping additive to decrease carbohydrate degradation. Oxygen bleaching is also incorporated which is not specific to delignification compared to other bleaching methods, but fairly specific to delignification relative to other pulps (Biermann, 1993). In one study by Idarraga *et al.* (1999), a soda-ethanol process was used in pulping of blue agave waste, from production of tequila, and was shown to be superior in terms of delignification and pulp properties in comparison to the soda and ethanol organosolv processes.

1.4 MODIFICATION OF XYLANS DURING PULPING

Hemicelluloses in pulps play an important role both in fibre morphology and paper physics. Retention of hemicelluloses increases the pulp yield, improves pulp strength and affects fibre quality. In kraft pulp, they are concentrated on the outer surfaces of microfibrils and this enables them to determine the chemical interactions between fibres, water and the pulping chemicals (Viikari *et al.*, 1993). Because of its crystallinity and linearity, wood cellulose is resistant to the harsh conditions used in chemical pulp production compared to hemicellulose components (Suurnäkki *et al.,* 1997).

During the heating period of the kraft cooking, when the alkali concentration is comparatively high, xylan in wood is partly solubilised by the alkaline cooking liquor. As the cook proceeds, the alkali concentration decreases, and degraded, short-chain xylan precipitates in a more or less crystalline form on the surface of cellulose microfibrils. The configuration of the xylose units allows close contact of the xylan chains with the cellulose. After removal of its substituents part of the xylan co-crystallises with or become adsorbed or reprecipitated onto the cellulose. During kraft pulping the reprecipitation of xylan is followed by the reprecipitation of dissolved lignin (Viikari *et al.*, 1994). The amount of xylan readsorbed during the cooking depends on the wood species used in pulping. High amounts of xylan have been found to locate on the surface of birch kraft fibres whereas in pine kraft fibres the concentration of xylan on the fibre surfaces has not been observed to be higher than in the whole fibres (Suurnäkki *et al.,* 1997).

In sulphite cooking, xylans are rather extensively solubilised to monomeric and oligomeric compounds and no reprecipitation takes place. Thus, the distribution of residual xylans is

constant across the cell fibres. The sulphite pulps have a lower pentosan content and greater solubility of the xylan constituents, which contain some acetyl groups, than kraft pulps (Viikari *et al.*, 1993). Due to the reprecipitation, xylans are concentrated on the outer surface of microfibrils. They are generally regarded to have a positive effect on interfibre bonding in papermaking. Thus the removal of xylans is not expected to lead to an improvement of the bonding properties (Viikari *et al.*, 1994). In wood, lignin is most concentrated in the middle lamella and the cell corners but most is dispersed in the secondary wall. The bulk of xylan is also in the secondary wall, with the highest concentration in the outer layer, while relatively low concentrations are in the primary wall and the middle lamella (Wong *et al.,* 1997b).

1.5 BLEACHING OF PULP

The purpose of processing wood is to get rid of the lignin which is responsible for the brownish colour characteristic of pulp, but doing as little damage as possible to the other wood constituents, cellulose and hemicellulose (Nissen *et al.,* 1992). Lignin removal is generally stopped before other fibre properties deteriorate to unacceptable levels. The amount of residual lignin remaining in conventional kraft pulps constitutes 2-4% of the total dry mass, generally less in hardwood than in softwood pulps (Wong *et al.,* 1997b). The residual lignin removal phase during kraft pulping, which occurs at about 90% delignification, is characterised by slow delignification, believed to be caused by the presence of alkali-stable lignin carbohydrate bonds, coupled with rapid carbohydrate degradation reactions. In kraft pulping, initial delignification occurs preferentially in the secondary wall and at about 50% delignification, the lignin in the middle lamella and cell

corner areas dissolve rapidly, leaving the residual lignin in the secondary wall (Jacobs *et al.,* 1998).

The residual lignin becomes more difficult to remove as the reaction progresses, and cannot be removed without unacceptably large yield losses. Extending digestion removes cellulose and results in unacceptably low pulp yields, so more specific methods of removing residual lignin and colour must be employed (Jeffries, 1992). Kraft pulp is subsequently bleached in a multi-step chlorine-based chemical sequence (Buchert *et al.,* 1994). The primary goal of bleaching is to remove the residual lignin from the pulp as selectively as possible without degrading the pulp carbohydrates, especially cellulose, which would lead to a decrease in viscosity (Suurnäkki *et al.,* 1997).

Current bleach processes are achieved through a continuous sequence of process stages utilising different chemicals and conditions in each stage, usually with washing between stages. The initial stages of a sequence are used primarily to delignify the pulp and can be considered as a continuation of the delignification process, which starts with cooking. The later stages employ oxidising agents to scavenge and destroy the residual colour (Smook, 1992). The bleaching procedure is chosen with respect to the pulp type in order to attain the target brightness with retention of the strength properties. In a conventional five-stage bleach sequence the main bleaching chemical during the first stage will be elemental chlorine (C), but part of it can be replaced with the milder chlorine dioxide (D). An alkaline extraction (E) follows in order to remove the dissolved lignin. This is succeeded by two chlorine dioxide treatments, with an alkaline extraction in between, to remove the last traces of lignin (Nissen *et al.,* 1992). This bleach sequence would be designated as CEDED, as illustrated in Figure 1.5.

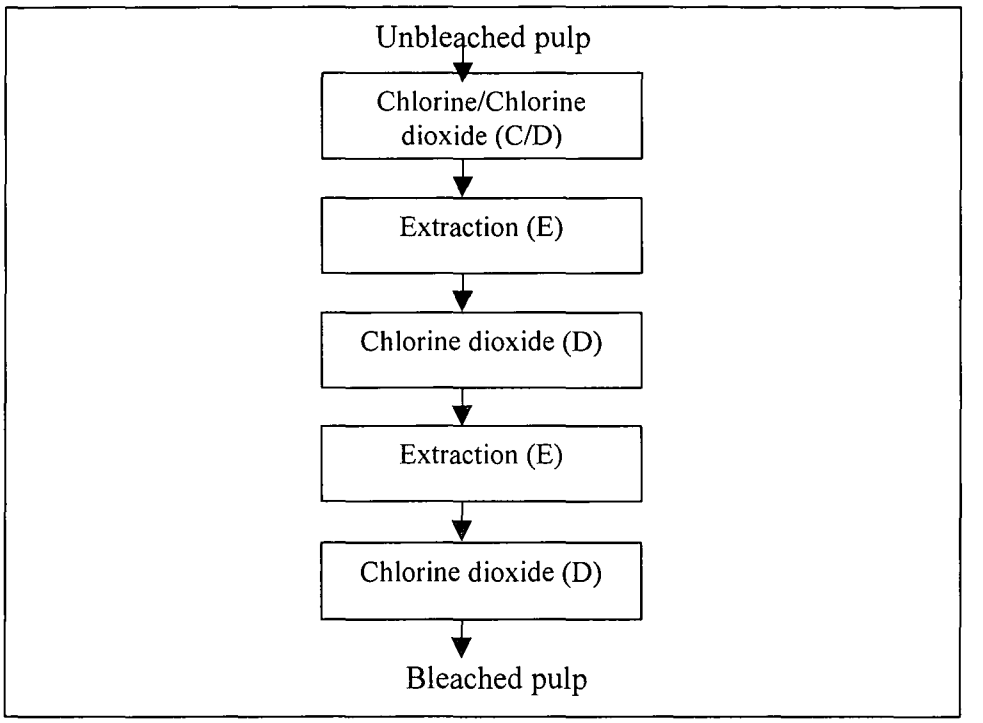

Figure 1.5. Schematic illustration of the stages involved in the kraft pulp bleaching process (Nissen *et al.,* 1992)

The most reactive bleaching chemicals are elemental chlorine, ozone and peroxy acids, which react with all the aromatic structures of lignin (Buchert *et al.,* 1994). Chlorine reacts with lignin primarily by means of substitution and oxidation, rendering the non-carbohydrate constituent water soluble or soluble in alkaline media. In substitution, elemental chlorine replaces hydrogen on organic molecule with simultaneous formation of a hydrogen chloride (HCl) molecule. Oxidation can be seen as forming elemental oxygen, which reacts with the pulp. Oxidation-type reactions can have a relatively more degrading effect on the cellulose (Smook, 1992). Chlorine dioxide and oxygen react with lignin structures, which have a free phenolic hydroxyl group. The most effective bleaching can be achieved by the action of different types bleaching chemicals active on different sites in lignin. Bleaching chemicals used in final bleaching depend on target brightness and the end-use of the pulp (Buchert *et al.,* 1994).

1.5.1 Problems associated with chemical bleaching

During pulp chlorination, toxic and carcinogenic compounds such as dioxins and furans and a host of other chlorinated organic compounds, the AOX are formed and conventionally bleached pulps contained 10-15 $\mu g.g^{-1}$ of these AOX (Garg *et al.,* 1996). Chloroform has been found to be the main volatile organochlorine compound formed during the bleaching of pulp with agents containing chlorine. When chloroform and other volatile organochlorine compounds (VOCC) drift into wastewater treatment plants with the bleaching effluent, most of them are stripped away in aerated basins, and thereby transported into the immediate environment. They may also contribute to the long-range transport of organic compounds. Together with dichloroacetonitrile and bromodichloromethane, chloroform has been shown to be a potential carcinogen while trichloronitromethane and 1,2-dichloropropane were found to exhibit irritating effects in humans (Juuti *et al.,* 1996).

The growing public concern regarding the environmental impact of the wastewaters from pulp bleaching has created a strong driving force for intensive research into new bleaching techniques. Since the chlorinated phenolic compounds produced during conventional pulp bleaching are toxic and very resistant to biodegradation, it thus seems very urgent to reduce or eliminate the use of elemental chlorine in pulp bleaching (Yang *et al.,* 1992). To achieve this, alternative-bleaching techniques that do not give rise to formation of compounds hazardous to the environment have to be developed. There is now also a consumer-led demand for ECF, TCF and total effluent free (TEF) pulps. This has offered opportunities for alternative bleaching methods, including enzymes, which provide a simple and cost-effective way to reduce the use of bleaching chemicals and do not give rise to formation of hazardous compounds (Bajpai and Bajpai, 1997).

These alternative techniques include oxygen delignification and replacement of elemental chlorine with chlorine dioxide, which substantially reduced the release of AOX to receiving waters (Yang *et al.,* 1992). Hydrogen peroxide and ozone were also said to have good bleaching performance but can cause a loss of strength in the final paper product as they are capable of degrading cellulose (Nissen *et al.,* 1992). The common problem with these techniques is that they require very high financial investment compared to enzymes, which have greater potential for specific modifications of pulp for the development of environmentally safe processes as summarised in Table 1.3.

Table 1.3 Alternatives available to produce TCF or ECF pulps (Bajpai and Bajpai, 1997)

Alternatives	Advantages	Disadvantages
(a) Substitution of chlorine dioxide for chlorine.	Lower AOX.	High bleaching costs. High investment may be required to satisfy increased demand for chlorine dioxide.
(b) Oxygen-delignification to reduce kappa prior to bleaching.	Lower AOX. Lower bleaching costs.	High investment.
(c) Hydrogen peroxide to replace chlorine-based chemicals.	Lower AOX.	High bleaching costs. Risk of pulp viscosity and strength.
(d) Ozone to replace chlorine-based chemicals.	Lower AOX.	Very high investment. Risk of pulp viscosity and strength.
(e) Extended cooking to reduce the kappa number before bleaching.	Lower AOX. Lower bleaching costs.	High investment.
(f) Enzymes	Lower AOX. Reduced use of bleaching chemicals Minimal capital investment. Improved strength and brightness.	Chances of reduction in yield due to some loss of hemicellulose.

1.6 XYLAN-DEGRADING ENZYMES

Xylan which is the most abundant component of the hemicelluloses in wood, is extremely complex and composed of a backbone of β-1,4-linked xylose units, which are substituted with arabinose and acetate residues (Ratanakhanokchai *et al.,* 1999). Its complete hydrolysis requires the action of a complex enzyme system (Figure 1.6), which is usually composed of β-1,4-endoxylanase, β-xylosidase, and enzymes which cleave side chain sugars from the xylan backbone, such as α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, and phenolic acid esterases. All these enzymes act co-operatively to convert xylan to its constituent sugar (Sunna and Antranikian, 1997).

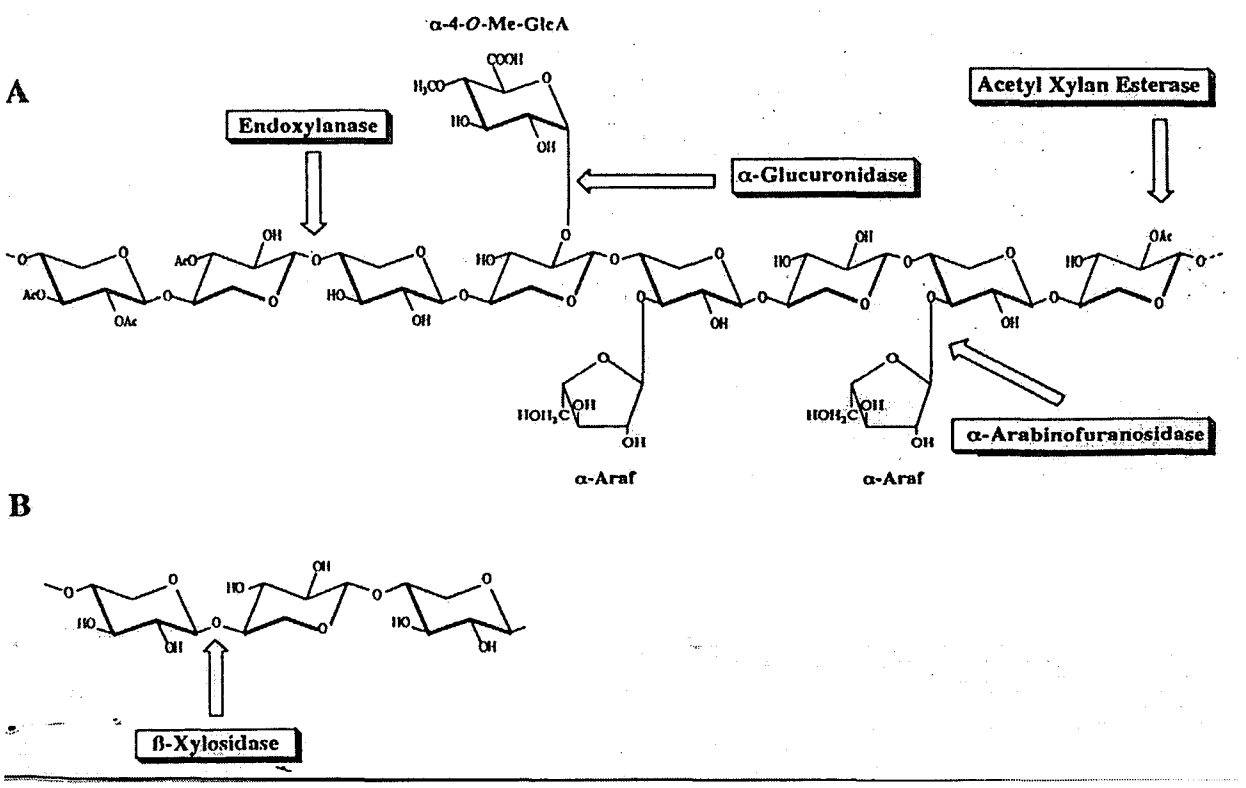

Figure 1.6. (A): Xylanolytic enzymes involved in the degradation of xylan. Ac: Acetyl group; α-Araf: α-arabinofuranose; α-4-O-Me-GlcA: α-4-O-methylglucuronic acid.

(B): Hydrolysis of xylooligosaccharide by β–xylosidase (Sunna and Antranikian, 1997)

The endoxylanases launch an endwise attack on the backbone of xylans to produce both substituted and non-substituted shorter oligomers, xylobiose, and xylose (Eriksson *et al.*, 1990). Xylosidases are essential for the complete breakdown of xylans as they hydrolyse xylooligosaccharides to xylose (Poutanen and Puls, 1988). The enzymes, arabinosidase, glucuronidase, and acetylxylan esterase, act in synergism with the endoxylanases and xylosidases by releasing the substituents on the xylan backbone to achieve a total hydrolysis of xylans to monosaccharides (Eriksson *et al.*, 1990). Of all the xylanolytic enzymes, the endoxylanases are the best characterised and the most widely studied (Biely *et al.,* 1992; Nakamura *et al.,* 1993; Alam *et al.,* 1994; Singh *et al.,* 1995; Balakrishnan *et al.,* 1998; Ratanakhanokchai *et al.,* 1999; Ximenes *et al.,* 1999).

1.6.1 Endo-1,4-β-xylanase

Endo-1,4-β-xylanase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.8) cleaves the internal glycosidic linkages of the heteroxylan backbone, resulting in a decreased DP of the substrate. The attack of the substrates is not random, and the bonds to be hydrolysed depend on the nature of the substrate. During the early course of hydrolysis of xylan, the main products formed are xylooligosaccharides. As hydrolysis proceeds, these oligosaccharides will be further hydrolysed to xylotriose, xylobiose, and xylose, which are the end products of xylan used to differentiate the endo-acting xylanases (Sunna and Antranikian, 1997).

Xylanases may be classified as non-debranching (arabinose non-liberating) or debranching (arabinose-liberating) enzymes (Sunna and Antranikian, 1997), and can be grouped into two major families of glycosyl hydrolases, Family 10 (F) and 11(G), based on hydrophobic

cluster analysis and sequence homology. Of these, the family F xylanases are somewhat larger and have a molecular mass of approximately 35 kDa, while family G xylanases have a molecular mass of only about 20 kDa (Jeffries, 1996). Endo-1,4-β-xylanases are produced by both fungi and bacteria, with those of the former having been the more extensively studied.

Most xylanases studied are active in slightly acidic conditions between pH 4 and 6 and temperatures below 70°C (Suurnäkki *et al.*, 1997). The pH optimum for xylan hydrolysis is around 5 for most fungal xylanases and they are normally stable at pH 2-9. Most of the fungi produce xylanases which tolerate temperatures below 50°C (Viikari *et al.*, 1993). Thermophilic fungi have been shown to produce highest levels and most stable forms of β-xylanase. *Thermomyces lanuginosus* and *Thermoascus aurantiacus* produced xylanases that were most active at 70°C, but at pH 6.0 and 5.0, respectively, showing no loss of activity at 50°C for 24 h (Alam *et al.,* 1994). Purkarthofer *et al.* (1993) also reported a xylanase from *T. lanuginosus* with activity levels as high as 36 200 nkat.ml^{-1}. Cellulase-free xylanases were produced by two strains of *T. lanuginosus,* RT9 and MH4, when grown on xylan and wheat bran at 50 or 55°C (Hoq and Deckwer, 1995). Culture supernatants of two *T. lanuginosus* strains, MED4B1 and MED2D, were found to have crude xylanase activity with a temperature optimum of 70°C at pH optima of 6.0 and 6.5, respectively (Haarhoff *et al.,* 1999). Another strain of *T. lanuginosus,* strain SSBP, had a high cellulase-free β-xylanase activity of 59 600 nkat.ml^{-1} when cultivated on a medium containing coarse corn cobs and yeast extract (Singh *et al.,* 1998). Optimal temperature and pH conditions for β-xylanase production by strain SSBP were 50°C and 6.5, respectively, while 70°C and 6.5 were found to be optimal for β-xylanase activity and stable at a pH range of 5.5-9.5 at 50°C.

Bacterial xylanases which have attracted huge interest to most researchers are those of the *Bacillus* spp. and *Streptomyces* spp. (Nakamura *et al.,* 1993; He *et al.,* 1994;.Bataillon *et al.,* 1998; Ratanakhanokchai *et al,* 1999). The pH optima of bacterial xylanases are generally slightly higher than the pH optima of fungal xylanases (Viikari *et al.*, 1993). Alkalophilic *Bacillus* sp. and alkalophilic actinomycetes have been reported to produce xylanases with relatively high activity at alkaline pH values. *B. subtilis* produced xylanase which had an optimum pH and temperature of 7.0 and 55°C, respectively (Khanongnuch *et al.,* 1998). Production of xylanases has also been detected in several thermophilic bacteria (Suurnäkki *et al.*, 1997). The most thermophilic xylanases so far described are produced by an extremely thermophilic bacteria *Thermotoga* sp. A thermostable xylanase from *T. maritima* was characterised and found to be active at 100°C for several hours (Chen *et al.,* 1997). The pH and temperature stability of xylanases is vital for pulp bleaching as the bleaching process is normally performed at almost alkaline conditions at very high temperatures.

Most studies have concentrated more on endoxylanases which are the main xylan-degrading enzymes, than on those that cleave the side chain sugars from the xylan backbone in that way facilitating the complete hydrolysis of the xylan component to its constituent sugar. These enzymes include xylosidase, arabinofuranosidase, glucuronidase, acetylxylan esterase and phenolic acid esterases.

1.6.2 β-Xylosidase

β-D-Xylosidases (β-D-xyloside xylohydrolase; EC 3.2.1.37) are exoglycosidases that hydrolyse short xylooligosaccharides and xylobiose from the nonreducing end to liberate

xylose. They are large enzymes with molecular weights between 60 and 360 kDa, and they may be mono- or dimeric proteins (Sunna and Antranikian, 1997). Most microbial hemicellulolytic systems contain β-xylosidase, which has been purified and characterised from many fungi and some bacteria. Poutanen and Puls (1988) purified and characterised the β-xylosidase of *T. reesei*. The β-xylosidase activity reached its maximum at pH 4.0 and decreased gradually with increasing pH. This enzyme was relatively thermostable, having a temperature optimum at 60°C in the 10 min incubation assay and being stable up to 55°C when incubated in a buffer of pH 5.0 for 24 hours. The thermophilic fungus *Thermoascus* sp. produced a β-xylosidase with optimum activity at pH 4.5 and 55°C that was stable up to 60°C at pH 4.5 for one hour (Matsuo *et al.,* 1998). Two strains of *T. lanuginosus* were also shown to produce β-xylosidases which had temperature optima of 40°C and 30°C with associated pH optima of 6.5 and 6.0 respectively (Haarhoff *et al.*, 1999).

1.6.3 α-L-Arabinofuranosidase

L-Arabinose residues are widely distributed in heteropolysaccharides and glycoconjugates, although the quantities of L-arabinose and L-arabinan in living tissues are relatively small. The α-L-arabinofuranosyl side chains of xylans are usually α-1,3-linked to the α-1,4-xylopyranosyl backbone. Although the frequency of L-arabinose side chains is generally relatively low, they restrict the enzymatic hydrolysis of hemicelluloses by xylanases (Eriksson *et al.*, 1990).

There are two types of arabinases, the exo-acting α-L-arabinofuranosidase (EC 3.2.1.55), which is active against *p*-nitrophenyl-α-L-arabinofuranosides and on branched arabinans,

and the endo-1,5-α-L-arabinase (EC 3.2.1.99), which is active only toward linear arabinans. Endoarabinases hydrolyse 1,5-α-L-arabinans, but are not able to hydrolyse the chromogenic substrate phenyl-α-L-arabinofuranoside or gum arabic. The size of native arabinofuranosidases may reach up to 495 kDa and are found in mono-, di-, tetra, hexa-, and octameric forms (Sunna and Antranikian, 1997).

Only a few enzymes of this nature have been isolated and characterised, despite the important role they play. Bezalel *et al.* (1993) characterised an extracellular α-L-arabinofuranosidase (EC 3.2.1.55) from *B. stearothermophilus* L1 strain. This enzyme had temperature optimum of 70°C and pH optimum of 7.0, and retained 50% of its maximum activity at pH 8.0. The purified enzyme consisted of two subunits with molecular mass (M_rs) of 52 500 and 57 500. A thermostable α-L-arabinofuranosidase, produced by *B. stearothermophilus* T6 strain, was most active at 70°C in the pH range of 5.5 to 6.0 (Gilead and Shoham, 1995). Kaneko *et al.* (1998) purified two α-L-arabinofuranosidases, I and II, from a culture filtrate of *A. awamori* IFO4033 strain. These enzymes had a pH optimum of 4.0 and a temperature optimum of 60°C and they exhibited stability at pH values from 3 to 7 and at temperatures up to 60°C.

1.6.4 α-Glucuronidase

α-D-Glucuronidases hydrolyse the α-1.2 linkages between glucuronic acid and xylose residues in glucuronoxylan. The substrate specifities of α-glucuronidases differ according to the enzyme source. The enzymes from *Agaricus bisporus* and *S. olivochromogenes* require a low-molecular-weight glucuronoxylan substrate. They release 4-O-

methylglucuronic acid from 4-O-methylglucuronose-substituted xylooligomers, but not from the polymer. α-Glucuronidases produced by *A. niger* and *Schizophyllum commune* were shown to be able to liberate 4-O-methylglucuronoxylan (Sunna and Antranikian, 1997).

1.6.5 Acetylxylan esterases

The glucuronoxylans of hardwoods are acetylated, with 60 to 70% of the xylose residues esterified at the hydroxyl group of carbon 2 or 3 in the xylopyranose ring (Poutanen and Sundberg, 1988). Acetylxylan esterases (EC 3.1.1.6) remove the *O*-acetyl substituents at the C-2 and C-3 positions of xylose residues in acetylxylan (Sunna and Antranikian, 1997). As compared to plant and animal esterases, the fungal esterases had high specific activities towards acetylated xylan (Poutanen and Sundberg, 1988).

1.6.6 Ferulic and *p*-coumaric acid esterases

Ferulic and *p*-coumaric acids are linked to xylan by ester bonds. Ferulic acid esterase cleaves the ester linkages between arabinose side chains and ferulic acids in xylan. Similarly, *p*-coumaric acid esterase cleaves the ester linkage between arabinose and *p*-coumaric acid (Sunna and Antranikian, 1997). According to McKenzie and co-workers (as cited by Sunna and Antranikian, 1997), the best substrate for the production of the ferulic acid esterases by *Streptomyces olivochromogenes* is oat spelt xylan.

1.7 APPLICATION OF ENZYMES IN BLEACHING OF PULP

The basic idea of enzyme-aided bleaching was first published in 1986 by Viikari and co-workers (as cited by Viikari *et al.,* 1994), and originally developed at VTT Biotechnical Laboratory in co-operation with the Finnish Pulp and Paper Research Institute. The first aim was to investigate the utilization of enzymes capable of degrading either lignin or hemicellulose as a means of developing a pulp bleaching process involving no chlorine. Due to increasing environment awareness the gradual phasing out of chlorine and chlorine-based chemicals in the pulp and paper industry globally is fast approaching reality. The use of xylanases in biobleaching of pulps has become one of the leading areas of research to date.

According to Kibblewhite and Wong (1999), the objective of treating pulp with enzymes is to enhance certain properties while retaining others, in particular fibre strength. The idea of using a hemicellulase treatment to promote pulp bleaching arose at the time when the lignin-degrading enzymes were attracting major research interest. When acting alone, the lignin modifying enzymes were not able to improve bleachability of oxygen delignified pine kraft pulps and had to be combined with xylanases to achieve this (Niku-Paavola *et al.,* 1994). The use of white-rot fungi as a pretreatment of wood chips prior to biomechanical pulping also resulted in the darkening of the pulp, due to the presence of the fungus, and pulps had to be treated with xylanases from thermophilic fungi to improve bleachability (Meinhold *et al.,* 1998).

Positive results were obtained using crude enzymes from various sources with xylanase as the main component. Xylanase treatment of a commercial radiata pine kraft pulp improved

the tear index-apparent density and tear index-tensile index properties of handsheets with the retention of fibre strength (Kibblewhite and Wong, 1999). Even with crude enzymes, identification of the sugars released in the enzymatic treatments confirmed that xylanase displayed major activity positively affecting the bleachability (Suurnäkki *et al.,* 1997).

Most studies on xylanase pretreatment have been conducted on kraft pulp and positive results on bleachability of the pulp were obtained (Yang *et al.,* 1992; Yu *et al.,* 1994; Garg *et al.,* 1996; Chen *et al.,* 1997; Wong *et al.,* 1997c; Roncero *et al.,* 1998; Ximenes *et al.,* 1999). Xylanases enhanced extractability of lignin by different bleaching chemicals (Kantelinen *et al.*, 1993) and in bleaching of eucalypt kraft pulp, they led to a maximum of 40% and 37% savings in chlorine dioxide or hydrogen peroxide, respectively, without affecting the final brightness (Vicuña *et al.*, 1997; Vidal *et al.*, 1997).

Xylanase treatment has also been demonstrated to have a bleach-boosting effect on other pulp types such as sulphite pulp, bagasse pulp and wheat straw pulp. Xylanase preparations of *A. pullulans* and *T. lanuginosus* brought about the bleach-boosting effects on sulphite pulps (Christov and Prior, 1997; Balakrishnan *et al.*, 1998). An increase in brightness of bagasse pulp and wheat straw pulp was observed after enzyme treatment with a *T. lanuginosus* SSBP xylanase and commercially available xylanase preparations, respectively (Bissoon *et al.,* 1998; Spiridon *et al.,* 1998).

1.7.1 Proposed mechanism of enzyme-aided bleaching

The effect of hemicelluloses in bleaching is not direct attack of residual lignin but on the modification of pulp hemicelluloses, enhancing the removal of lignin in chemical bleaching

(Suurnäkki *et al.,* 1997). A number of other hypotheses have been proposed to explain the effect that xylanase pretreatment has on subsequent bleaching of kraft pulp (Buchert *et al.,* 1994; Viikari *et al.,* 1994). Kantelinen *et al.* (1993) proposed that the reprecipitated and readsorbed alkali-resistant xylan, possibly together with lignin, form a physical barrier against extraction of residual lignin molecules from the fibres. Hemicellulases, especially when used in rather low amounts, apparently act first on the fraction of xylan located on the surface of the fibres. Gerber *et al.* (1999) found that the ionic strength and pH of treatment influence the fibre surface charge and adsorption behaviour of the enzymes. They also showed that a cellulose binding domain was the main factor affecting the ability of hemiellulases to adsorb onto wood fibres.

It has been suggested that action of xylanases may be also be due to removal of xylan from the lignin-carbohydrate (LC) complexes (Kantelinen *et al.,* 1993). It was reported that the treatment of kraft pulp with hemicellulases from the fungus *A. pullulans,* resulted in a specific hydrolysis of remaining hemicelluloses, leading to enhanced leaching of lignin carbohydrate (LC) complexes from the pulp fibres (Yang *et al.,* 1992). Both these hypotheses might mean that the removal of xylan renders the fibre surface more permeable and facilitates subsequent further stages of pulp treatment with bleaching chemicals (Kantelinen *et al.,* 1993). Both softwood and hardwood kraft pulps have been reported to contain LC complexes in which carbohydrates and lignin may be connected to each other, for example by ether or glycosidic linkages. Increased solubilization of xylan-lignin complexes, both from model pulps and from kraft pulps, has been observed in xylanase treatment, indicating that LC complexes may also have a role in xylanase-aided bleaching. The action of xylanase on both reprecipitated and LC xylan in enhancing bleachability suggests that it is probably not only the type but also the location of the xylan that is important in the mechanism of xylan-aided bleaching (Suurnäkki *et al.,* 1997). The

xylanase of *T. reesei* was observed to act relatively uniformly at the surface and in the inner fibres of both pine and birch fibres indicating that the effect of xylanase bleachability is not only an outer surface phenomenon (Suurnäkki *et al.*, 1996). Irrespective of their physical distribution across the fibre wall, xylan substrates may be in one of the four forms (Figure 1.7).

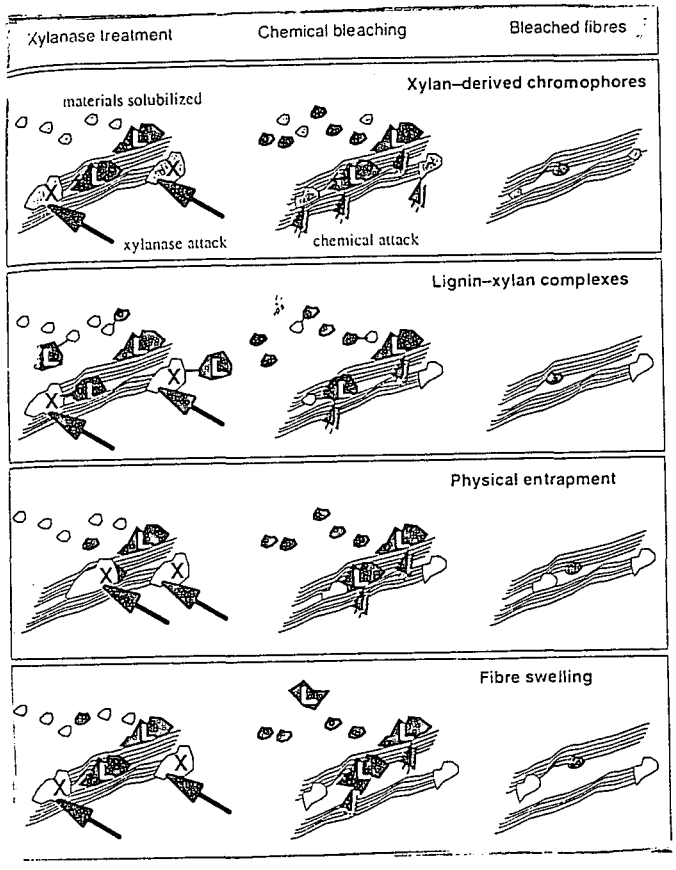

Figure 1.7. Hypothetical target substrates for xylanase during xylanase- aided bleaching xylan-derived chromophores; lignin-xylan complexes; xylan that physically entraps lignin; xylan that modifies fibre swelling. The diagrams illustrate how the attack of these target substrates (L=lignin; X=xylan) on pulp fibres might affect subsequent chemical bleaching (Wong *et al.*, 1997b)

1.7.2 Factors affecting the enzymatic pretreatment of pulp

In order to use enzymes effectively in biobleaching of pulp in mills, several factors must be taken into account. These factors may be related to the different process conditions and mill operations. In practical operating conditions, the various properties of the enzymes, such as substrate specificity, pH and temperature optima, concentration and mode of action, are important. Mill operations may include factors such as the type of raw material used, the pulping process and the bleaching sequence.

1.7.2.1 Operating conditions

The operating conditions, such as pH, temperature, time, enzyme concentration and enzyme dispersion may have a great influence on the action of enzymes in pretreatment of pulp. Due to the alkalinity of the pulp, it would be desirable to use enzymes at pH 9-10. Because most commercial xylanases of the first generation have not met these requirements, adjustment of pH to about 5-7 is necessary (Suurnäkki *et al.*, 1997). Tenkanen and co-workers (1997) were able to purify an acid-tolerant xylanase from *A. kawachii,* with a pH optimum of pH 2.0 – 2.5, and demonstrated good bleaching properties.

The pulp has high temperature and therefore enzymes which can operate at 80-90°C are needed, or the cooling of pulp to 50 – 60°C would be necessary (Suurnäkki *et al.*, 1997). Recent studies have reported that some thermophilic fungi are capable of producing xylanases which are active at temperatures as high as 70°C (Alam *et al.*, 1994; Singh *et al.*, 1995; Haarhoff *et al.*, 1999) and 100°C (Chen *et al.*, 1997).

The degree of hydrolysis necessary for an optimal result can be adjusted by choosing the reaction time and enzyme dosage. A minimum of one to two hours of residence time is required for the enzyme treatment. There is little enzyme action on the pulp beyond four to six hours (Bajpai and Bajpai, 1997).

The amount of enzyme needed is a key parameter with respect to efficiency, enzyme cost and yield loss. The degree of hydrolysis has to be tested on the laboratory scale with each pulp and bleaching sequence used. Generally, it seems that although the degree of hydrolysis (solubilization of carbohydrates) increases as a function of the enzyme dosage used, only minor additional benefit for bleachability can be obtained beyond a certain enzyme dosage (Suurnäkki *et al.,* 1997).

The adequate dispersion of enzyme and acid into the pulp is extremely important to enzyme performance. Studies should be conducted at each new installation to assess the adequacy of the dispersion. In general, the degree of mixing depends on the equipment that is used to add enzyme to the pulp and on the absorbency of the brown stock (Tolan, 1992).

1.7.2.2 Raw material

Different wood species and pulping processes can also play an important role in the action of different hemicelluloses on pulps. Compared to softwood pulp, hardwood kraft pulp contains more xylan, more carboxylic acid groups and surface charge and smaller xylan macromolecules. Relatively more lignin-xylan complexes, where lignin and xylan are covalently linked, seem to occur in hardwood pulps while more lignin-cellulose complexes occur in softwood pulps (Wong *et al.,* 1997a). There is a tendency for hardwood kraft pulp

to show higher benefits with regard to chlorine savings during xylanase aided bleaching (Tolan, 1992).

The action of enzymes is also influenced by the electrochemical interactions between fibres and enzymes. The carboxyl groups within the fibre cell wall are mainly responsible for the swelling properties of pulp in water. The surface charge and swelling of fibres have been reported to affect the action of xylanases (Buchert *et al.,* 1993). The more negative the surface charge, the less was the pulp hydrolyzed. However, the swelling and surface charge may not be the primary factors affecting the hydrolysis. The type of counter-ions and the degree of substitution of the carboxyl groups in the pulp were found to have a profound effect on the action of xylanases in the pulp matrix. Consequently, metal-free pulps were poorly hydrolyzed by hemicellulases. This observed phenomenon of poor hydrolyzability of metal-free pulp is of practical importance in the TCF bleaching sequences, in which the metal removal stage is essential to retain the strength properties of the pulp (Suurnäkki *et al.*, 1997).

1.7.2.3 Pulping process

The optimal mixture of xylanolytic enzymes needed for the complete hydrolysis xylans can be designed according to the chemical composition of the isolated substrate. Other factors such as porosity, specific surface area and charge of the fibres also affect the efficiency of enzymatic treatments when hemicelluloses are bound to the fibre matrix. Other important factors include the distribution of the substrate and its structural organisation within the fibre. In addition to the chemical composition, all these parameters are changed to varying degrees during different types of pulping processes (Viikari *et al.,* 1994).

Xylanase aided bleaching appears most effective on pulp produced by a conventional batch process where there is substantially more redeposited xylan (Kantelinen *et al.,* 1993). Effects of xylanase treatment have been observed in continuous cooked pulps, produced by MCC (modified continuous cook) and EMCC (extended MCC), and in other low Kappa pulps, produced by extended cooking, RDH (rapid displacement of heat), superbatch, kraft-anthraquinone and prehydrolysed kraft processes (Wong *et al.,* 1997a).

1.7.3 Enzyme-aided bleaching using different bleaching sequences

The use of xylanases in biobleaching of different pulps consistently leads to a reduction in a chemical consumption. The enzymes, in order to be effective, depend on the type of pulp, the chemical bleaching sequence used as well as the final target brightness and environment goals of the mill (Table 1.4). Originally, xylanases were applied in order to reduce the consumption of chlorine chemicals, especially chlorine gas. Later, enzymes have been combined with various ECF and TCF bleaching sequences to improve the otherwise lower final brightness value of pulp or to decrease the bleaching costs (Suurnäkki *et al.,* 1997). In chlorine bleaching, an average reduction of 12-40% in chlorine dioxide consumption (Vicuña *et al.,* 1997; Vidal *et al.,* 1997) in prebleaching or a reduction of about 47% in hydrogen peroxide consumption (Vidal *et al.,* 1997) has been reported in both laboratory scale and mill trials. As a result of the reduced use of bleaching chemicals, the AOX load of the bleaching effluent in mill trials has been reported to decrease by 15-20% (Allison *et al.,* 1996).

The reduction in the use of chlorine chemicals has important implications not only for the environment but also for economy as a result of cost savings or increased pulp production capacity. As a result of the replacement of chlorine gas by chlorine dioxide, the requirement

for chlorine dioxide can exceed the production capacities of chlorine dioxide at the mill site. In order to avoid additional investments, xylanase pretreatment allows pulp production with achievement of target brightness with lower chlorine dioxide consumption. Thus, the productivity of the mill is increased in addition to the environmental benefits (Suurnäkki *et al.*, 1997).

Table 1.4. The aims of enzyme-aided bleaching using different bleaching sequences (Viikari *et al.*, 1994)

Bleaching	Sequence	Aim
Conventional	X(C/D) EDED	Reduction of elemental chlorine consumption. AOX reduction.
ECF	XDEDED	Reduction of chlorine consumption. AOX reduction.
	XD(EP)DED	Increase in productivity, when ClO2 is limited.
	OXDEDED	
TCF	OXQPP	Brightness increase.
	OXQPZP	Reduction in chemical consumption.
	OXZP	Retained strength properties.

X=enzyme; C=elemental chlorine; D=chlorine dioxide; P=hydroxide peroxide; O=oxygen delignification; Z=ozone; Q=chelation of metals; E=alkaline washing.

1.7.4 Industrial use of xylanases

During recent years, the main goal in the enzymatic bleaching of kraft (sulphate) pulps has been to reduce the consumption of chlorine chemicals in the bleaching process and cost while maintaining the strength properties of the pulp (Dunlop-Jones and Grönberg, 1995). However, enzymes can also be used successfully for increasing the brightness of pulp, which is of key importance in the development of TCF bleaching sequences (Gübitz *et al.*,

1996; Wong *et al.,* 1997c). Addition of an enzymatic step to any conventional chemical bleaching sequence results in a higher final brightness value of the pulp. The Finnish forest companies were the first in the world to start mill scale trials in 1988 and since 1991 this method has been continuously used on the industrial scale together with other low-chlorine or chlorine-free bleaching methods (Suurnäkki *et al.,* 1997).

In 1992, more than ten mills worldwide were reported to use xylanases continuously for improved bleaching of kraft pulps. Most of the kraft pulp in Europe is produced in Scandanavia, where most of the mill trials have also been performed (Suurnäkki *et al.,* 1997). In Canada, xylanase enzymes were used to treat 750 000 tonnes per year of pulp in six mills in 1994. This represents 8% of Canada's bleached kraft pulp production (Tolan *et al.,* 1995). Cariboo Pulp and Paper mill, in Canada, has used enzymes since February 1992 to enhance the bleaching of oxygen-delignified kraft pulp (Tolan and Thibault, 1997). The Weyerhaeuser Saskatchewan Ltd. Mill in Prince Albert, SK, has used enzymes successfully since October 1992 to enhance the bleaching of kraft pulps (Yee and Tolan, 1997).

Xylanase-aided bleaching has been adopted for continuous use worldwide in several mills and is growing in usage. The mode of action of hemicellulases also points to the applicability and usefulness of these enzymes in combination with new delignifying enzyme systems (Suurnäkki *et al.,* 1997). In South Africa enzyme-aided bleaching biotechnology is still under research and no mill trials have been performed to date. With the environmental regulations becoming strict, it is thus necessary to develop an enzyme-aided bleaching technique that is cost-effective, time saving and one that will be able to reduce or completely eliminate the use of chlorine-based chemicals in pulp bleaching.

CHAPTER 2: CHARACTERISATION OF ENZYMES AND PULP PROPERTIES

2.1 ABSTRACT

A crude and commercial β-xylanase were characterised for their potential application in improving bleachability of paper pulps. Crude β-xylanase was produced under shake-culture at 50°C and pH 6.5 using corn cobs and yeast extract by a thermophilic fungus, *Thermomyces lanuginosus* SSBP. A maximum of 1600 $U.ml^{-1}$ was produced after 7 days. The enzyme displayed pH and temperature optima of 6.5 and 70°C, respectively. Xylanase P, in a concentrated form, exhibited a high activity of 84620 $U.ml^{-1}$ with pH and temperature optima of 5.0 and 60°C, respectively. Three industrial pulps currently used for papermaking in South Africa viz., bagasse, post-oxygen soda and post-oxygen kraft pulps were characterised for Kappa number, brightness and carbohydrate content. Post-oxygen kraft pulp had the highest Kappa number in the range 10.0-10.6 which correlated with its brightness which was between 31.4 and 34.3%. Post-oxygen soda pulp had the lowest Kappa number of 4.6-5.6 and the highest brightness of 66.6-68.1%. Kappa number of bagasse pulp was in the range 8.2-8.7 with an initial brightness of 43.3-45.3%. Carbohydrate analyses indicated that glucose and xylose were the major sugar constituents, in the range 74.7-85.4 and 6.5-23.4%, respectively, in all three pulp types. Mannose was also present in significant amounts (5-7%) in the post-oxygen kraft pulp.

2.2 INTRODUCTION

Pulp consists of wood or other lignocellulosic materials that have been broken down physically and/or chemically such that discreet fibers are liberated and can be dispersed in water and reformed into a web (Biermann, 1993). Wood and pulp contain high amounts of structurally different hemicelluloses, which are polymeric constituents of the plant cell wall together with lignin and cellulose. Hemicelluloses play an important role in fiber morphology and paper physics and also in determining the chemical interactions between fibers, water and the pulping chemical (Viikari *et al.,* 1993). The two main hemicelluloses in wood are xylans and glucomannans with the xylan being the most abundant plant hemicellulose in nature consisting of β-1,4-linked xylose monomers and side-chain substituents. Depending on the wood species, hardwoods contain 15–30% of xylan and 2–5% of glucomannan whereas softwoods contain up to 10% and 20% of xylan and glucomannan, respectively (Sjöström, 1993).

There are four broad categories of pulping processes, chemical, semi-chemical, chemi-mechanical and mechanical pulping, which are in order of increasing mechanical energy required to separate fibers and decreasing reliance on chemical action (Biermann, 1993). Chemical pulping methods may include kraft process, sulphite process and soda process. The major chemical pulping method in the world is the kraft process, which represents more than 70% of the world's annual pulp production of approximately 100 million tons (Bajpai and Bajpai, 1997) and uses alkaline conditions high temperatures to dissolve much of the lignin of wood fibers (Biermann, 1993). Kraft process is useful for any wood species and produces pulp with the highest strength properties but the resultant pulp is strongly coloured and requires large volumes of various chemicals for

bleaching compared to sulfite pulps (Smook, 1992). Sulphite pulping uses acidic conditions and is important in the production of dissolving pulps (Balakrishnan *et al.,* 1998). Sulphite pulps are lighter in colour than kraft pulps and can be bleached easily using less chemicals, but paper sheets produced are weaker (Smook, 1992). Soda process is another minor chemical pulping process which is still used in the papermaking industry in other countries as it has limited use for easily pulped materials like straws and some hardwoods. It uses sodium hydroxide as the cooking chemical and also oxygen for its specificity in delignification relative to other pulping methods (Biermann, 1993).

During pulping more than 90% of the lignin is removed and the residual lignin is responsible for imparting the brownish colour of pulp and the pulp is subsequently bleached in a multi-step chlorine-based chemical sequence (Bajpai and Bajpai, 1997). Strict environmental regulations has forced the pulp and paper industry to try alternative methods to chlorine bleaching which included the use of ozone, hydrogen peroxide and enzymes. Advantages of using enzymes in the biobleaching of pulps would include savings in chemicals for pulping and bleaching, improvement in pulp quality and more acceptable effluent quality (Christov and Prior, 1998). Xylanases have been the most widely studied and tested in the bleaching of pulps. In an industrial setting the temperature of the incoming pulp is around 70°C and the pH is in the alkaline range and this could be overcome by using thermostable xylanases (Zamost *et al.,* 1991). These enzymes should also be completely free of cellulase activity as any activity will have serious economic implications in terms of cellulose loss, degraded pulp quality and increased effluent treatment cost (Bajpai and Bajpai, 1997).

A thermophilic fungus, *Thermomyces lanuginosus* SSBP strain, was isolated from soil in South Africa and produced highest cellulase-free xylanase activity of 9600 U.ml-1 using corn cobs as carbon source (Singh *et al.,* 1995). The enzyme was stable at pH 5.0-9.5 and retained full activity after 24 h incubation at pH 6.5 and 70°C (Singh *et al.,* 1998). This xylanase have been shown to improve bleachability of both bagasse and sulphite pulps. Crude *T. lanuginosus* SSBP xylanase increased brightness of bagasse pulp by 3.4% whereas it was increased by 4.5% by the purified enzyme (Bissoon *et al.,* 1998). When it was used in bleaching of sulphite pulp to dissolving pulp it improved brightness of unbleached sulphite pulp by 2.6 points (Christov and Prior, 1997).

Characteristics of a crude and commercial xylanase were thus analysed for their potential ability to improve the bleaching process of three industrial paper pulps: bagasse, post-oxygen soda and post-oxygen kraft. The pulps were also characterised for Kappa number, brightness and sugar composition prior to any bleaching treatment.

2.3 MATERIALS AND METHODS

2.3.1 Enzyme source

The crude xylanase preparation was produced from a culture of *T. lanuginosus* SSBP, previously isolated from soil at the University of Durban Westville by Singh *et al.* (1995). The culture was routinely subcultured every 2 - 4 weeks on Potato Dextrose agar (PDA) plates (pH 6.5). Xylanase P was supplied in a concentrated form by Iogen Corporation (Ontario, Canada).

2.3.2 Crude enzyme preparation of *T. lanuginosus*

For preparation of the crude enzyme an agar block (1 to 2 cm^2) with an actively growing 5 to 6 day old colony of *T. lanuginosus* SSBP strain was used to inoculate 300 ml Erlenmeyer flasks containing 100 ml of an optimal culture medium (Purkarthofer *et al.,* 1993). This medium contained per litre: 31.2 g coarse corn cobs, 30.2 g yeast extract and 5.0 g KH_2PO_4. Corn cobs were prepared from maize (corn) which had all of its kernels removed, dried and then ground to get particles of 2 to 7 mm and these were then autoclaved at 121°C for 15 min and stored at 4°C before use. The cultures were incubated at 50°C with shaking at 150 rpm for 7 days. Crude enzyme preparation comprised culture supernatants harvested by first filtering through the No.1 Whatman filter paper and then centrifuged at 10 000 *g* for 10 min at 4°C. Supernatants were then analysed for xylanase activity as described in Section 2.3.3, below.

2.3.3 Enzyme assay

Xylanase activities of both *T. lanuginosus* SSBP xylanase and Xylanase P were estimated by determining the release of reducing sugars according to Bailey *et al.* (1992) using a 1% (w/v) solution of oat spelt xylan (Sigma, St Louis, USA) incubated with the appropriately diluted enzyme in 0.05 M citrate buffer, pH 6.5 and 0.05 M citrate-phosphate buffer, pH 5.0, respectively, for 5 min at 50°C, and 60°C, respectively. Released reducing sugars were assayed spectrophotometrically at 540 nm after adding 3,5-dinitrosalicylic acid (DNS) reagent to the reaction mixture, boiling for 5 min and cooling. One unit (IU) of xylanase was defined as the enzyme amount that catalyses the release of 1 μmol of xylose equivalents per minute of reaction.

2.3.4 Characterisation of enzymes

Activities of crude and commercial enzymes were characterised for optimum pH and temperature. Xylanase activity in culture supernatants was studied in the pH range of 3.0 to 12.0 (50 $mmol^{-1}$ citrate buffer, pH 3.0 to 6.5; 50 $mmol^{-1}$ Tris-HCl buffer, pH 7.0 to 9.5; 50 $mmol^{-1}$ glycine-NaOH buffer, pH 10.0) at 50°C and temperatures between 30 and 100°C and at the optimum pH, according to a modified method used by Singh *et al.* (1998). Citrate-phosphate buffer (pH 4.0, 5.0 and 6.0), phosphate buffer (pH 7.0 and 8.0) and carbonate-bicarbonate buffer (pH 9.0) were used in determining optimum pH of Xylanase P. The enzyme was diluted 2000-fold and P together with the 1% xylan substrate was incubated in a waterbath at 50°C for

10 min. Thereafter the reaction was stopped and the reducing sugars, released from the substrate, determined spectrophotometrically at 500 nm. The optimum temperature of Xylanase P was determined at pH 5.0 by incubating the substrate (1% xylan) and the diluted (2000-fold) enzyme in a waterbath at various temperatures (from 30 to 80°C) for 10 min. Thereafter the reaction was stopped and the reducing sugars, released from the substrate, determined spectrophotometrically at 500 nm.

2.3.5 Preparation and characterisation of pulps

Three industrial pulp types, used for papermaking, were used in this study: unbleached bagasse pulp (UBP), post-oxygen soda pulp (POSP) and post-oxygen kraft pulp (POKP). They were obtained, each in two different batches, from three papermaking mills of Sappi (Pty) Ltd, South Africa: Sappi Stanger, Sappi Enstra and Sappi Ngodwana, respectively. Prior to use the pulps were thoroughly washed with tap water until their pH values were close to neutrality and then given a final rinse with distilled water. Pulp suspensions were filtered on a Büchner funnel and then analysed for the initial Kappa number, brightness and sugar composition.

The standard TAPPI test method T 236 cm-85 was used to determine Kappa number of pulps. For brightness determination, pulp sheets were prepared using a Büchner funnel and then dried at 50°C and brightness was measured using a Zeiss Elrepho 85365 photometer. Carbohydrate content of pulps was analysed using High Performance Liquid Chromatography (HPLC) installed with a CarboPac PA10 anion exchange column (Rockland Technologies, Inc., CA, USA), operated

at 31°C with millipore water and 18 mM NaOH as the mobile phase. Pulp samples were hydrolysed with 72% H_2SO_4 at 30°C for 60 min, thereafter autoclaved at 121°C for 60 min, followed by neutralisation with NaOH. were used in this study. The rest of the filtered pulp suspension was placed in polyethylene bags for further enzyme and chemical bleachings, as described in Chapters 3 and 4.

2.4 RESULTS

2.4.1 Enzyme properties

Properties of the two xylanase preparations are summarised in Table 2.1. Xylanase activity of the crude *T. lanuginosus* SSBP xylanase preparation, using oat spelts xylan as the substrate, was determined to be 1600 $IU.ml^{-1}$ (Table 2.1). The highest xylanase acivity level was observed at pH 6.5 (Figure 2.1A) and the optimum temperature for xylanase activity was 70°C (Figure 2.1B), as earlier reported by Singh *et al.* (1998). The activity of Xylanase P was determined to be 84 620 $IU.ml^{-1}$ (Table 2.1). Xylanase P was supplied in a concentrated form which explains its high activity. The optimum pH and temperature for Xylanase P were determined to be 5.0 (Figure 2.2A) and 60°C (Figure 2.2B), respectively.

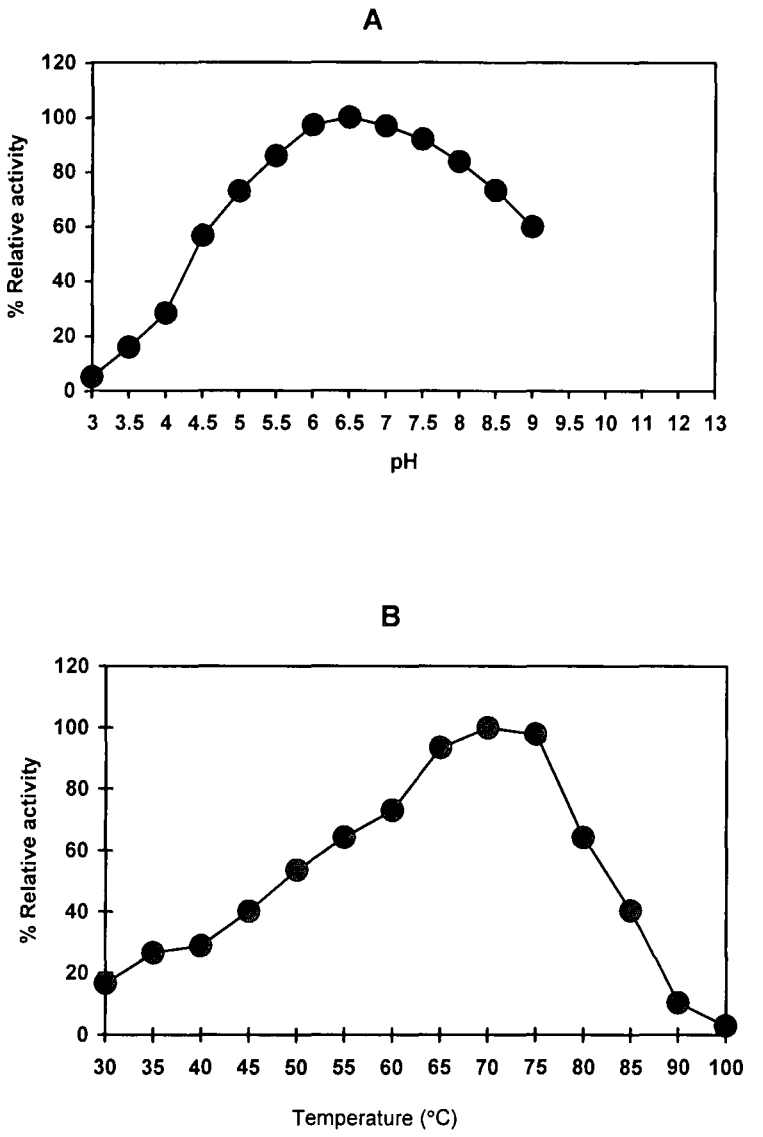

Figure 2.1. Effect of pH (A) and temperature (B) on the activity of *T. lanuginosus* SSBP xylanase

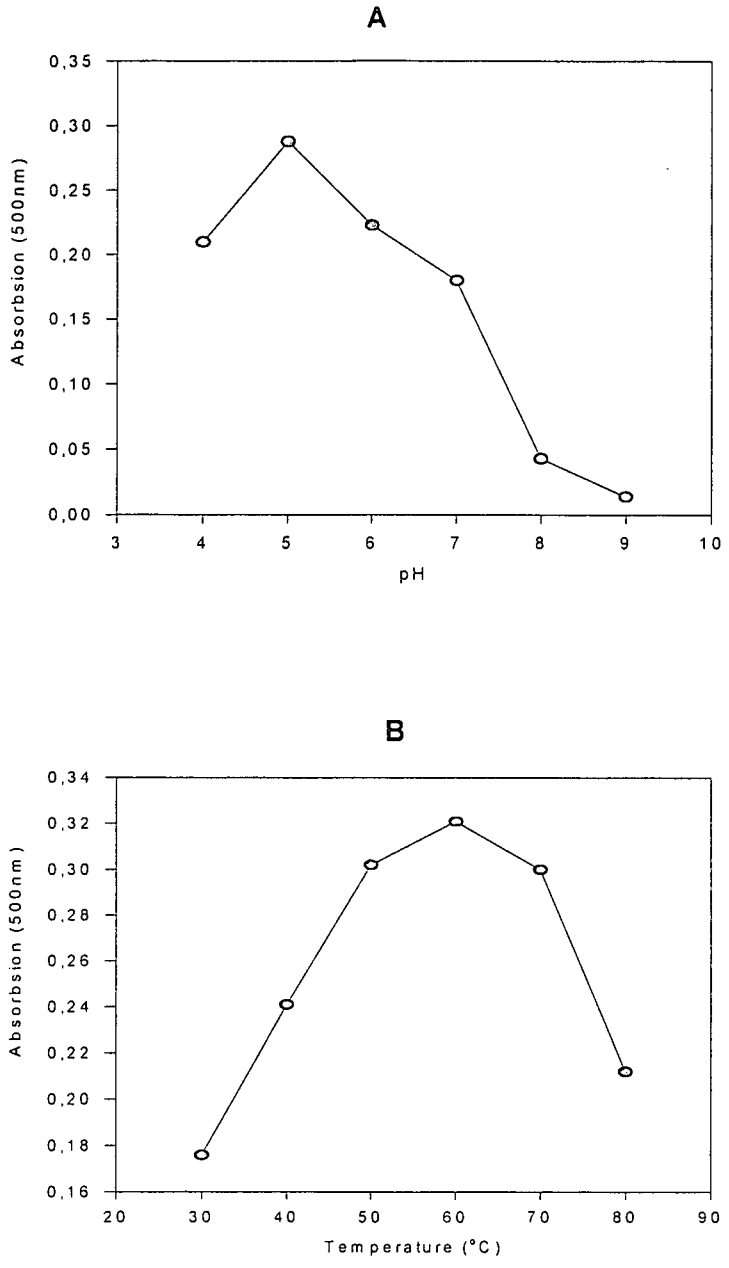

Figure 2.2. Effect of pH (A) and temperature (B) on the activity of Xylanase P

Table 2.1. Properties of crude and commercial enzyme preparations

Enzyme	**Temperature (°C)**	**pH**	**Activity ($IU.ml^{-1}$)**
***T. lanuginosus* SSBP xylanase**	70	6.5	1 600
Xylanase P	60	5.0	84 620

2.4.2 Characteristics of pulp properties

The initial properties of the three pulps used in this study are summarised in Tables 2.2 and 2.3. In both batches, Batch 1 (Table 2.2) and Batch 2 Table 2.3), post-oxygen soda pulp exhibited the lowest Kappa number and the highest brightness than the other two pulps suggesting the existence of a correlation between Kappa number of pulp and its brightness. Post-oxygen kraft pulp was observed to have the highest Kappa number and the lowest brightness that were twice as high and twice as low than that of post-oxygen soda pulp, respectively. Unbleached bagasse pulp also had Kappa number that was much higher than that of post-oxygen soda pulp but lower than that of post-oxygen kraft pulp. Its brightness was a little bit higher than that of post-oxygen kraft pulp and lower than that of post-oxygen soda pulp. The significant difference between properties of each pulp type from one batch to another could be due to the pulping and washing processes of pulps prior to their characterisation. Chemical analyses of pulps revealed that glucose and xylose were the major sugar constituents in unbleached bagasse pulp and post-oxygen soda pulp Tables 2.2 and 2.3). In post-oxygen kraft

pulp mannose was also present in significant concentrations in addition to glucose and xylose.

Table 2.2. Physical and chemical properties of unbleached bagasse, post-oxygen soda and post-oxygen kraft pulps (Batch 1)

Properties	Unbleached bagasse pulp	Post-oxygen soda pulp	Post-oxygen kraft pulp
Kappa number[a]	8.7	5.6	10.6
Brightness (%)[b]	43.3	66.6	31.4
Sugars (%)[c]			
Arabinose	1.8	ND[d]	1.0
Galactose	0.1	ND	0.3
Glucose	74.7	79.1	84.0
Xylose	23.4	21.0	8.9
Mannose	ND	ND	5.8

[a,b,c]Data are averages of two independent determinations
[d]Not detected

Table 2.3. Physical and chemical properties of unbleached bagasse, post-oxygen soda and post-oxygen kraft pulps (Batch 2)

Properties	Unbleached bagasse pulp	Post-oxygen soda pulp	Post-oxygen kraft pulp
Kappa number[a]	8.2	4.6	10.0
Brightness (%)[b]	45.3	68.1	34.3
Sugars (%)[c]			
Arabinose	1.4	ND[d]	0.7
Galactose	ND	ND	0.3
Glucose	76.8	77.7	85.4
Xylose	21.8	22.3	6.5
Mannose	ND	ND	7.1

[a,b,c]Data are averages of two independent determinations
[d]Not detected

2.5 DISCUSSION

Both enzyme preparations were characterised and shown to have the ability of improving bleachability of paper pulps. Crude xylanase was characterised to have its pH and temperature optima of 6.5 and 70°C, respectively, which will make it suitable for industrial application. These results correlated with an earlier report on the characteristics of the *T. lanuginosus* SSBP xylanase by Singh *et al.* (1998) who also reported that this enzyme was at 70°C for 30 min and retained 100% activity at 60°C for 24 h. When *T. lanuginosus* SSBP was cultivated in shake-flask cultures using corn cobs and yeast extract as carbon and nitrogen source, respectively, for 7 days at its optimum pH and temperature it produced β-xylanase activity of 1600 $U.ml^{-1}$. In a recent study by Bissoon *et al.* (1998), this fungal strain was reported to produce xylanase activity of 1565 $U.ml^{-1}$ under the same conditions used in this used. Xylanase P was determined to have pH and temperature optima of 5.0 and 60°C, respectively. Its xylanase activity determined to be 84 620 $U.ml^{-1}$ which was very high compared to that of the crude enzyme. The reason for this high xylanase activity could be that it was obtained from the suppliers in a concentrated form.

There was a correlation between Kappa number and brightness of all three pulps as it was observed in those pulps with low Kappa number having highest brightness and vice versa, especially in the case of post-oxygen soda pulp and post-oxygen kraft pulp. High Kappa number indicates the presence of high concentrations of lignin and post-oxygen kraft pulp will be difficult to bleach and would require more chemicals for its bleaching compared to the other pulps, as

earlier reported by Smook (1992). Glucose and xylose were the major sugar constituents in all three pulps but mannose was also present in significant amounts in the post-oxygen kraft pulp and this was expected as kraft pulp is produced from softwood which usually has more glucomannan concentrations than xylan (Sjöström, 1993). This could suggest that mannose-degrading enzymes could also be used together with xylanases in biobleaching of post-oxygen kraft pulp. The effect of xylanase pretreatment in biobleaching of paper pulps was investigated in the following two chapters, Chapter 2 and Chapter 3.

CHAPTER 3: BIOBLEACHING EFFECTS OF *T. lanuginosus* SSBP XYLANASE ON PAPER PULPS.

3.1 ABSTRACT

Crude xylanase preparation of *T. lanuginosus* SSBP xylanase was used to treat three different industrial pulps used for papermaking: bagasse, post-oxygen soda and post-oxygen kraft. The enzyme was applied on pulp prior to bleaching at doses of 1, 5 and 10 $U.g^{-1}$ dry pulp for up to 3 h at 60°C and pH 6.5. Effectiveness of xylanase pretreatment was found to be increasing with the increase in enzyme doses used. This resulted in the hydrolysis of pulp hemicellulose which was indicated by the release of carbohydrates and reducing sugars from the pulps. Reduction in Kappa number was used as an indicator for the removal of residual lignin from the pulps. No correlation was observed between the reduction in Kappa number and brightness increase following enzyme and chemical treatments. Bleached bagasse and post-oxygen kraft pulps, pretreated with xylanase at 10 $U.g^{-1}$ for 3 h, gained 4 brightness points over controls while brightness of post-oxygen soda pulp increased by 1 brightness point under the same treatment conditions. On the other hand, viscosity of pulps was slightly modified in comparison with controls. Carbohydrate analyses showed an increase in glucose content and a decrease in xylose content of the xylanase-pretreated pulp samples. The crude xylanase preparation of *T. lanuginosus* SSBP, in combination with the sequence D_1ED_2, led to a 17% saving of chlorine dioxide, without affecting the target brightness.

3.2 INTRODUCTION

Hemicelluloses consists mainly xylans which are complex heteropolysaccharides consisting of β-1,4-linked D-xylopyranose that are highly substituted (Sjöström, 1993). During the kraft pulping process xylan is modified and relocated in the fibres in a reprecipitated form. Xylanases act on these reprecipitated xylans partially hydrolysing them to facilitate extraction of lignin in higher amounts from the fibres (Buchert *et al.,* 1993). Crude xylanase preparations are important in reducing the cost when used in the biobleaching process but have to be free of cellulase for selective removal of hemicellulose without affecting the strength of the cellulosic fibre itself (Hoq and Deckwer, 1995).

Several strains of the thermophilic fungus, *T. lanuginosus,* have been shown to be able to produce cellulase-free xylanases which were active and stable at high temperatures and at pH values closer to neutrality (Alam *et al.,* 1994; Hoq and Deckwer, 1995; Singh *et al.,* 1995; Haarhoff *et al.,* 1999). Xylanases produced by this fungus have shown the ability to improve bleachability of different pulp types. In the biobleaching of dissolving pulp crude xylanase preparation of *T. lanuginosus* solubilised up to 26% of the xylan present in the original bleached pulp (Gübitz *et al.*, 1997b). Another strain of *T. lanuginosus,* MED2D, resulted in a 10.5% reduction in Kappa number of Eucalyptus kraft pulp (Haarhoff *et al.*,1999). The *T. lanuginosus* SSBP strain has also been tested in biobleaching of sulphite and bagasse pulps. In the bleaching of sulphite pulps to produce dissolving pulps crude xylanase produced by this strain increased the brightness of unbleached sulphite pulp by 2.6 points whereas on bagasse pulp brightness was increased by 3.4% compared to 4.5%

increase obtained with a purified enzyme (Christov and Prior, 1997; Bissoon *et al.*, 1998).

The aim of this study was to evaluate the effectiveness of crude *T. lanuginosus* SSBP xylanase in biobleaching of bagasse pulp in comparison to two other industrial pulps also used in papermaking: post-oxygen soda and post-oxygen kraft pulps. The main objective was to investigate the potential of this enzyme in reducing consumption of chlorine dioxide used in pulp bleaching.

3.3 MATERIALS AND METHODS

3.3.1 Enzyme preparation

Crude *T. lanuginosus* SSBP enzyme was prepared and assayed for xylanase activity as described in Section 2.3.2 and Section 2.3.3 of Chapter 2, respectively.

3.3.2 Enzymatic hydrolysis of pulp

Three industrial pulps (Batch 1): unbleached bagasse pulp, oxygen-bleached soda pulp and oxygen-bleached kraft pulp were prepared as described in Section 2.3.5, and Table 2.2. Pulp samples (7 g dry weight) were treated with enzyme doses of 1, 5 and 10 $U.g^{-1}$ oven dry pulp in sealed polyethylene bags with intermittent kneading at consistency of 10% in 0.05 M citrate buffer, pH 6.5 at 60°C for 3 h. Control samples were treated with citrate buffer without any enzyme. After incubation, the slurry was filtered and bleach filtrates were collected for analysed as described in Section 3.3.3, below.

3.3.3 Analyses of hydrolysis products

Enzyme-mediated release of chromophore material was measured in pulp filtrates spectrophotometrically at 280 and 465 nm at 0, 1, 2 and 3 h, respectively. Reducing sugars released were also measured over the same incubation period according to the DNS method of Bailey *et al.* (1992).

3.3.4 Chemical bleaching of pulps

After enzyme pretreatment, pulp samples were washed thoroughly with distilled water and then bleached in a multistage bleaching process using a D_1ED_2 sequence. Control samples treated with citrate buffer only were simultaneously bleached with the same sequence. The first chlorine dioxide treatment (D_1) was applied in sealed polyethylene with intermittent kneading at 10% pulp consistency, incubated at 67°C for 113 minutes with a charge of 2.63% active chlorine. This was followed by alkaline extraction at a charge of 0.7% of 10% NaOH solution performed at 67°C for 67 min. The second chlorine dioxide treatment (D_2) was applied as the first one but with a charge of 1.315% active chlorine at 67°C for 180 min. After each treatment step the pulp samples were washed thoroughly with tap water followed by a final rinse of distilled water. The chemical bleaching conditions of the DED-bleach sequence are summarised in Table 3.1, below.

Table 3.1 Chemical bleaching conditions in D_1, E and D_2 stages

Bleaching conditions	**D_1**	**E**	**D_2**
Consistency (%)	10	10	10
Temperature (°C)	67	67	67
Reaction time (min)	113	67	180
Active chlorine (%)	2.630	-	1.315
NaOH, 10% (%)	-	0.7	-

3.3.5 Analyses of pulps

After enzyme treatment, pulps were analysed for Kappa number and brightness using the standard TAPPI test method T 236 cm-85 and the Elrepho photometer, as described in Section 2.3.4. After DED-bleaching pulps were analysed for brightness and carbohydrate content, as described in Section 2.3.5, and viscosity of fully bleached pulp samples was also determined according to the SCAN-CM 15:88 test method using cupri-ethylenediamine (CED) solution.

3.3.6 Evaluation of enzyme pretreatment effect on chlorine dioxide consumption

Pulp samples were treated with xylanase doses of 10 $U.g^{-1}$ for 3 h to assess the influence of enzyme pretreatment on chlorine dioxide reduction. The control pulp sample was treated with a citrate buffer solution without any enzyme. These samples were then subjected to chemical bleaching using the D_1ED_2 sequence, as described in Section 3.3.4 but at different chlorine charges, as indicated in Table 3.2. Increase in the final brightness was used as an indicator for evaluating the effect of enzyme pretreatment on chlorine dioxide consumption, and therefore fully bleached pulps were only analysed for brightness using the Elrepho photometer, as described in Section 2.3.5.

Table 3.2. Chemical bleaching conditions of pulps used for evaluating the influence of enzyme pretreatment on chlorine dioxide consumption

Enzyme charge (U/g)	D_1 (%)[a]	Reduction at D_1 (%)	D_2 (%)[a]	Reduction at D_2 (%)	Total reduction of ClO_2	
					%	Kg/t pulp
0.0	2.6300	0	1.3150	0	0.0	0.0
10.0	2.6300	0	1.3150	0	0.0	0.0
10.0	2.4985	5	1.3150	0	3.3	0.5
10.0	2.3670	10	1.3150	0	6.7	1.0
10.0	2.3670	10	1.1835	10	10.0	1.5
10.0	2.2355	15	1.1835	10	13.3	2.0
10.0	2.2355	15	1.0520	20	16.7	2.5
10.0	2.1040	20	1.0520	20	20.0	3.0
10.0	2.1040	20	0.9205	30	23.3	3.5
10.0	1.9725	25	0.9205	30	26.7	4.0
10.0	1.8410	30	0.9205	30	30.0	4.5
10.0	1.7095	35	0.9205	30	33.3	5.0

[a]Active chlorine charges

3.4 RESULTS

3.4.1 Release of chromophore material

Release of chromophoric material at wavelengths of 280 and 465 nm from the three pulps pretreated with crude *T. lanuginosus* SSBP xylanase is shown in Figures 3.1 and 3.2, respectively. At both wavelengths there was a general increase in the release of chromophores with an increase in enzyme charge and treatment time. In xylanase-pretreated bagasse pulp a slight decrease in chromophore release at 280 nm was observed between 1 and 2 h, which was followed by a slight increase in the last treatment hour (Figure 3.1A). A same pattern of chromophore release from bagasse pulp was also observed at 465 nm (Figure 3.2A). From post-oxygen soda pulp, more chromophores from pulps pretreatred at a charge of 5 $U.g^{-1}$ dry pulp were released in the first 2 h, at 280 nm, after 3 h chromophores were observed to be released more from pulps treated at 10 $U.g^{-1}$ (Figure 3.1B). At 465 nm chromophore release from post-oxygen soda pulp was not much significant and was at almost the same rate when the two higher enzyme doses were used (Figure 3.2B). In post-oxygen kraft pulp chromophore release at 280 nm increased with the increase in enzyme dose and treatment time (Figure 3.1C). At 465 nm an increase in chromophore release was observed for the first 2 h of treatment and the slightly decreased after 2 h at doses of 5 and 10 $U.g^{-1}$ whereas it was slightly increased at 1 $U.g^{-1}$ (Figure 3.2C). In general, more chromophoric material was observed to be released at the wavelength of 280 nm from all three pulps tested.

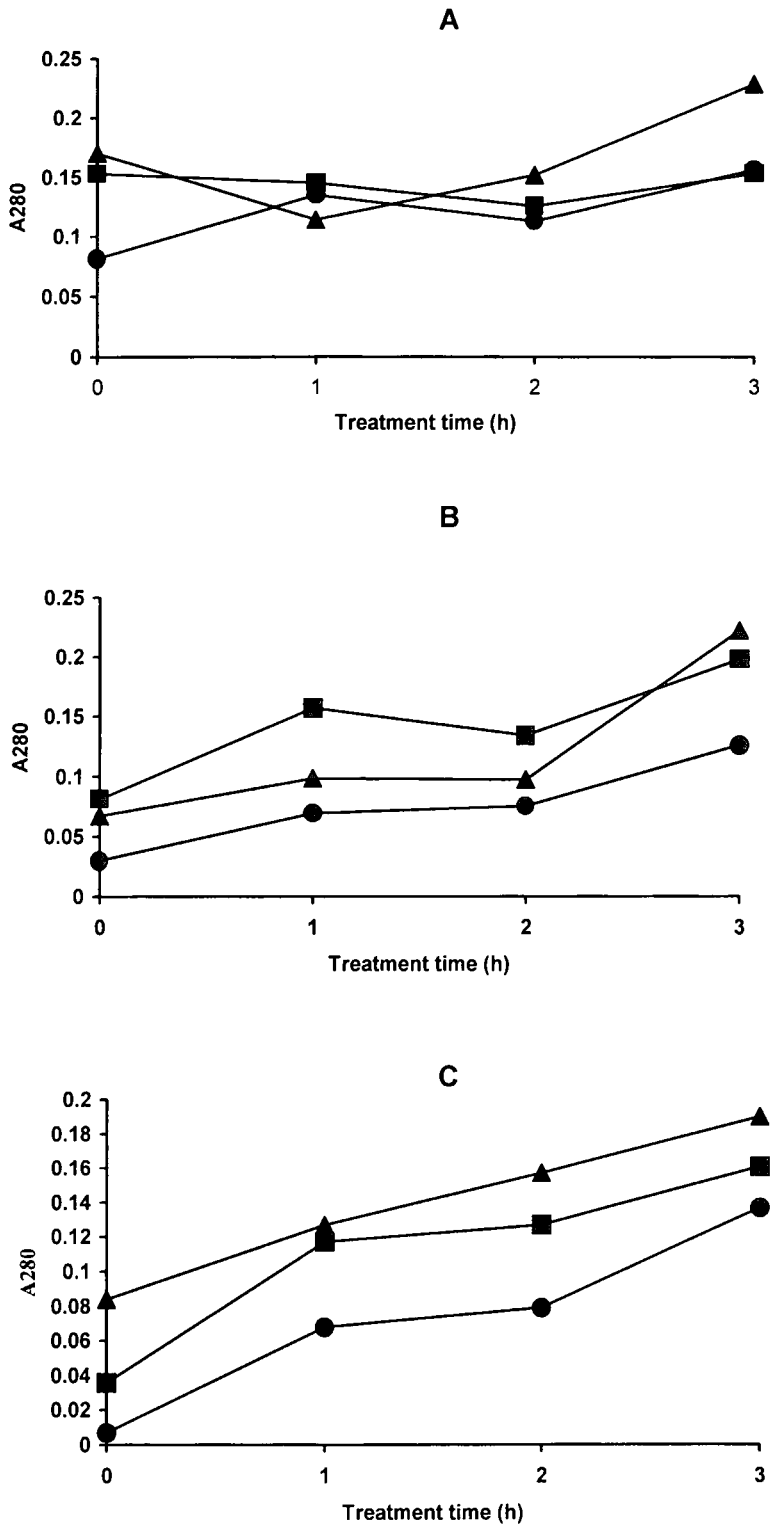

Figure 3.1. Release of chromophore material at 280 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5

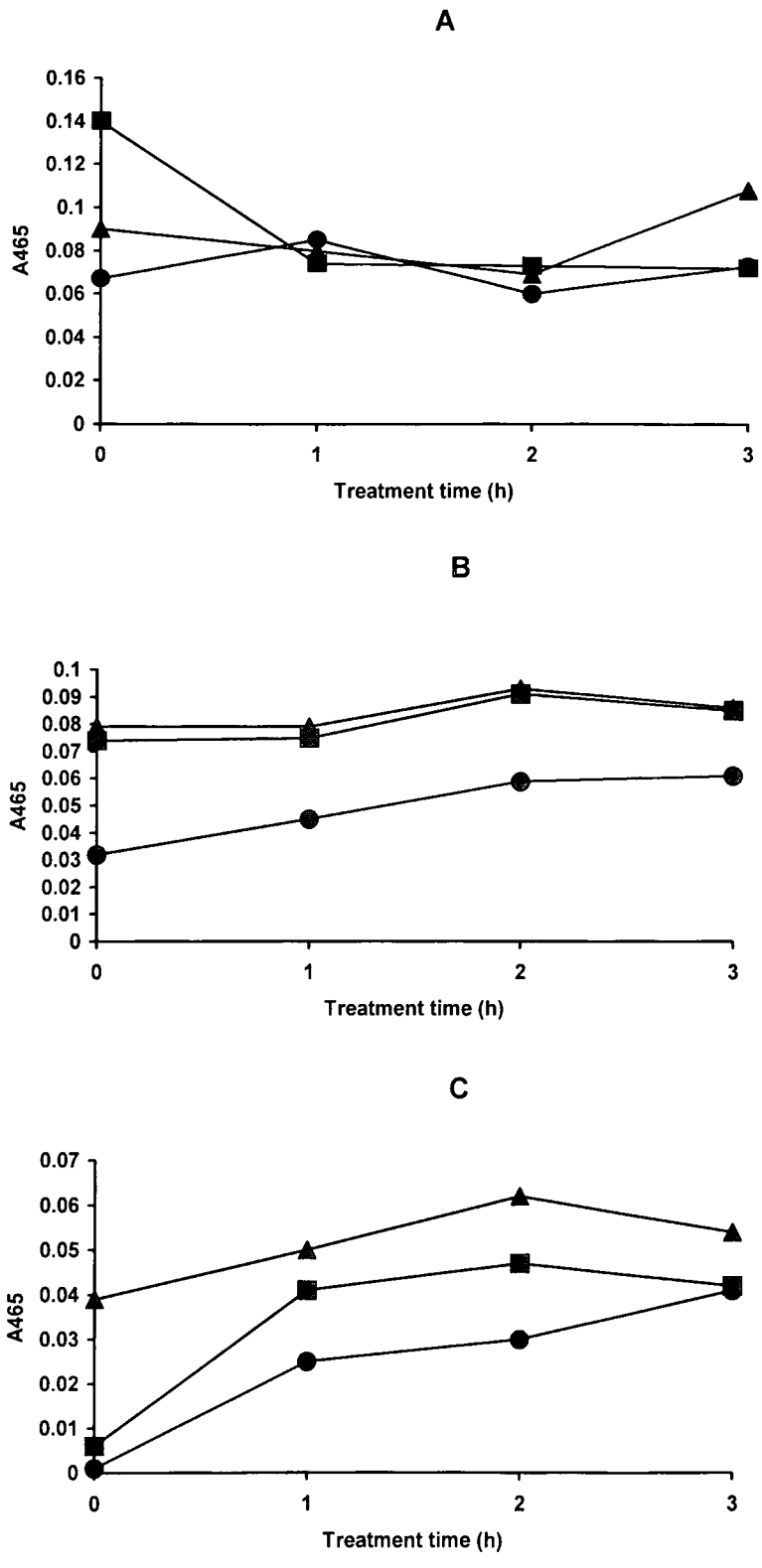

Figure 3.2. Release of chromophore material at 465 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp, and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5

3.4.2 Release of reducing sugars

Xylanase pretreatment was more effective in releasing reducing sugars from bagasse pulp, even at the lowest enzyme dose used of 1 $U.g^{-1}$, compared to oxygen-delignified pulps (Figure 3.3). Reducing sugar release in bagasse pulp was observed to be increasing with an increase in enzyme dose and treatment time (Figure 3.3A). In post-oxygen soda pulp the release of reducing sugars increased with an increase in enzyme dose for the first 2 h and then decreased slightly from 2 to 3 h when an enzyme dose of 10 $U.g^{-1}$ was used (Figure 3.3B). A sharp increase in the release of reducing sugars was observed in post-oxygen kraft pulp treated with the enzyme at 5 $U.g^{-1}$ after 1 h which was followed by a sharp decrease in the next hour at the same enzyme charge (Figure 3.3C). The increase in the release of reducing sugars and chromophoric material could indicate that xylanase treatment of pulps with the crude enzyme of *T. lanuginosus* SSBP was effective in hydrolysing pulp hemicellulose.

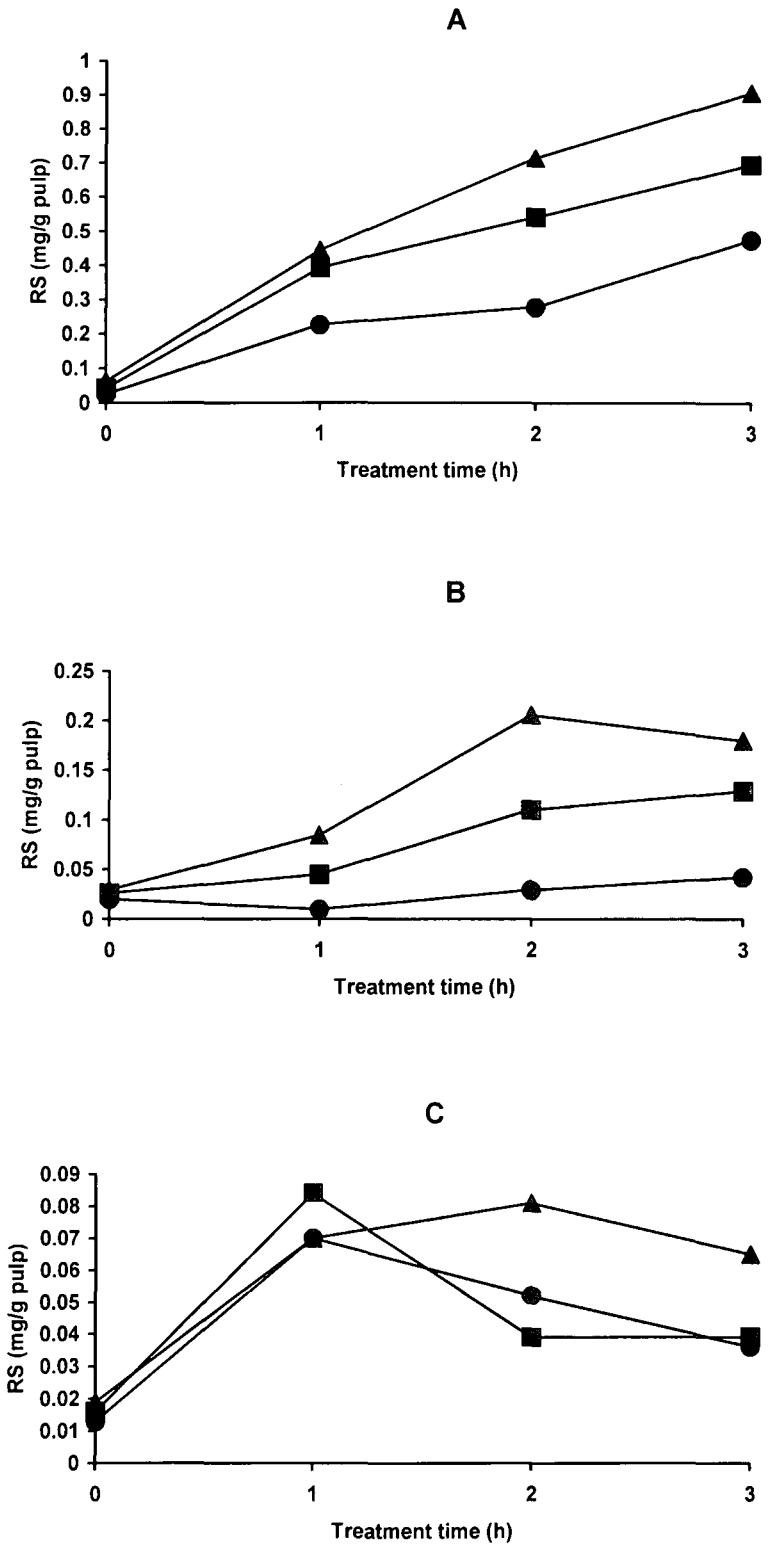

Figure 3.3. Release of reducing sugars (RS) from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5

3.4.3 Effect of enzyme pretreatment on Kappa number reduction

After enzyme treatment, without alkaline extraction, some of the residual lignin was removed from all three pulps. This was indicated by the reduction in Kappa number which was used as an indicator for the removal of residual lignin from the pulps (Table 3.3). Oxygen-delignified pulps, soda nad kraft, were the most affected by the enzyme treatment. Kappa number of post-oxygen soda pulp was reduced by a maximum of 17.9% after 2 h of treatment and that of post-oxygen kraft pulp was reduced by a maximum of 13.2% after 3 h (Table 3.3). In bagasse pulp, maximum reduction in Kappa number was observed after 1 h treatment with enzyme doses of 5 and 10 $U.g^{-1}$ dry pulp and after 1 h reduction achieved was less than 5% with all enzyme doses tested (Table 3.3).

Table 3.3. Kappa number of bagasse, post-oxygen soda and post-oxygen kraft pulps pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5

Treatment time (h)	Enzyme charge (U/g)	Kappa number[a]		
		Bagasse pulp	Post-oxygen soda pulp	Post-oxygen kraft pulp
1	1	8.5 (2.3)[b]	5.5 (1.8)	10.4 (1.9)
	5	8.0 (8.0)	4.8 (14.3)	10.3 (2.8)
	10	8.0 (8.0)	4.9 (12.5)	9.6 (9.4)
2	1	8.4 (3.5)	4.9 (12.5)	9.7 (8.5)
	5	8.4 (3.5)	5.2 (7.1)	10.1 (4.7)
	10	8.3 (4.6)	4.6 (17.9)	9.7 (8.5)
3	1	8.3 (3.5)	5.3 (5.4)	10.3 (2.8)
	5	8.4 (3.5)	4.6 (17.9)	9.7 (8.5)
	10	8.3 (4.6)	4.8 (14.3)	9.2 (13.2)
Control	0	8.7 (0.0)	5.6 (0.0)	10.6 (0.0)

[a]Data are averages of two independent runs
[b]Values in parentheses represent %reduction

3.4.4 Effect of enzyme pretreatment on pulp brightness

Brightness of all xylanase pretreated pulps, before chemical bleaching improved by more than 1 point compared to their respective controls (Tables 3.4, 3.5 and 3.6). A maximum brightness increase of 3.1, 2.4 and 2.3 points was achieved with bagasse pulp pretreated with enzyme dose of 10 $U.g^{-1}$ pulp after 1, 2 and 3 h, respectively (Table 3.4). In post-oxygen soda pulp, a maximum brightness increase of 2.3 points was observed after 3 h with an enzyme charge of 5 $U.g^{-1}$ (Table 3.5) whereas in post-oxygen kraft pulp only a maximum of 1.4 brightness points was achieved with the same enzyme charge after 2 h (Table 3.6). There was no correlation was observed between Kappa number reduction and brightness increase after enzyme and chemical treatments. Kappa number of post-oxygen soda pulp was the most affected by enzyme treatment, followed by that of post-oxygen kraft pulp (Table 3.3), whereas both pulps had gained less brightness increase after enzyme treatment (Tables 3.5 and 3.6, respectively) compared to that gained by bagasse pulp (Table 3.4) which had Kappa number that was less affected by enzyme treatment (Table 3.3).

After chemical bleaching, using the DED sequence, the final brightness of all three pulps increased with an increase in enzyme dose used at each treatment time (Tables, 3.4, 3.5 and 3.6). Brightness of bagasse pulp pretreated with an enzyme dose of 10 $U.g^{-1}$ for 1 and 3 h achieved a maximum increase of 4 and 4.3 points, respectively (Table 3.4). A maximum increase in brightness of 4.5, 4.1 and 4.3 points was achieved with post-oxygen kraft pulp pretreated with the same enzyme charge for 1, 2 and 3 h, respectively (Table 3.6). The same enzyme charge achieved only 1 brightness point after 3 h with post-oxygen soda pulp (Table 3.5).

Table 3.4. Brightness of bagasse pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5

Treatment time (h)	Enzyme charge (U/g)	Brightness[a] after X		Brightness[a] after XDED	
		(%)	(points)	(%)	(points)
1	1	45.4	2.1	78.5	1.9
	5	45.8	2.5	79.2	2.6
	10	46.4	3.1	80.6	4.0
2	1	45.3	2.0	77.9	1.3
	5	45.2	1.9	78.4	1.8
	10	45.7	2.4	78.6	2.0
3	1	44.8	1.5	79.5	2.9
	5	45.1	1.8	79.9	3.3
	10	45.6	2.3	80.9	4.3
Control	0	43.3	0.0	76.6	0.0

[a]Data are averages of two independent runs.

Table 3.5. Brightness of post-oxygen soda pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5

Treatment time (h)	Enzyme charge (U/g)	Brightness[a] after X		Brightness[a] after XDED	
		(%)	(points)	(%)	(points)
1	1	67.9	1.3	91.2	0.4
	5	68.2	1.6	91.2	0.4
	10	68.3	1.7	91.2	0.4
2	1	67.9	1.3	91.4	0.6
	5	68.3	1.7	91.4	0.6
	10	67.8	1.2	91.5	0.7
3	1	68.4	1.8	91.6	0.8
	5	68.9	2.3	91.8	1.0
	10	68.1	1.5	91.8	1.0
Control	0	66.6	0.0	90.8	0.0

[a]Data are averages of two independent runs.

Table 3.6. Brightness of post-oxygen kraft pulp pretreated with *T. lanuginosus* SSBP xylanase (X) at 60°C and pH 6.5

Treatment time (h)	Enzyme charge (U/g)	Brightness[a] after X		Brightness[a] after XDED	
		(%)	(points)	(%)	(points)
1	**1**	32.4	1.0	63.3	3.4
	5	32.7	1.3	63.7	3.8
	10	32.1	0.7	64.4	4.5
2	**1**	32.1	0.7	62.9	3.0
	5	32.8	1.4	62.9	3.0
	10	32.4	1.0	64.0	4.1
3	**1**	32.7	1.3	62.8	2.9
	5	32.4	1.0	63.8	3.9
	10	32.4	1.0	64.2	4.3
Control	**0**	31.4	0.0	59.9	0.0

[a]Data are averages of two independent runs.

3.4.5 Effect of enzyme pretreatment on pulp viscosity

Viscosity of xylanase-pretreated pulps was slightly modified in comparison to their respective controls (Figure 3.4). Xylanase pretreatment reduced the bagasse pulp viscosity by a maximum of 10% at low enzyme concentrations of 1 and 5 $U.g^{-1}$ after 1 h treatment and then improving it slightly after 2 h whereas at 10 $U.g^{-1}$ pulp viscosity was decreasing with an increase in treatment time (Figure 3.4A). Post-oxygen soda pulp viscosity was slightly affected by low enzyme concentration of 1 $U.g^{-1}$ pulp only after 2 h (Figure 3.4B). Viscosity of post-oxygen soda pulp and post-oxygen kraft pulp were reduced by a maximum of 9% at 10 $U.g^{-1}$ pulp after 1 h and became stable afterwards (Figures 3.4B and 3.4C, respectively).

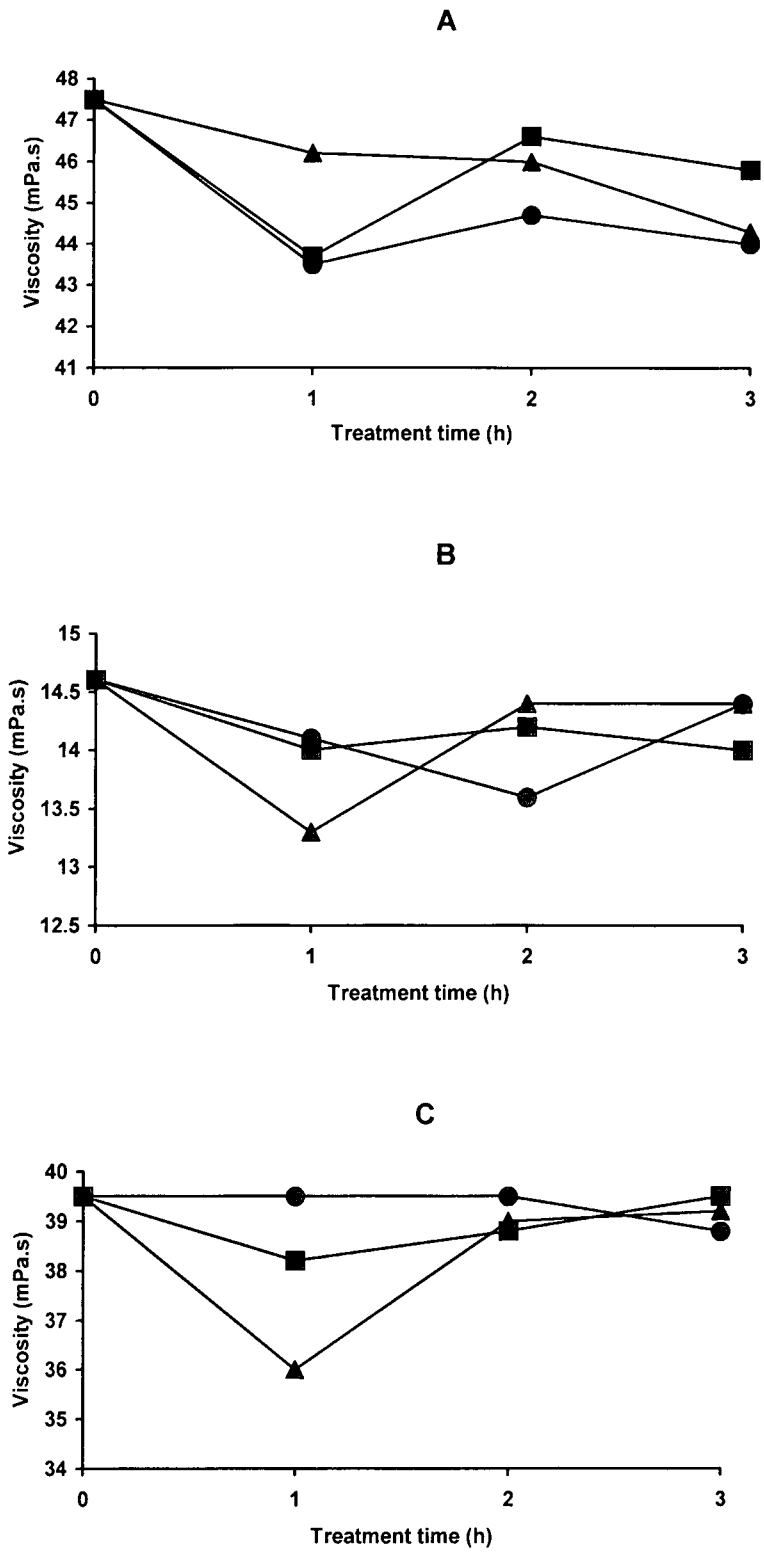

Figure 3.4. Viscosity of **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp, pretreated with crude *T. lanuginosus* SSBP xylanase at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 6.5, after DED-bleaching

3.4.6 Effect of enzyme pretreatment on sugar composition

Chemical analyses of bagasse, post-oxygen soda and post-oxygen kraft pulps, pretreated with crude xylanase, after DED-bleaching showed an increase and a decrease in glucose and xylose concentrations, respectively (Tables 3.7 – 3.12). A maximum increase of 0.8% in glucose concentration was achieved in bagasse pulp treated with an enzyme dose of 10 $U.g^{-1}$ for 1 h with a subsequent decrease of 0.7% in xylose content (Table 3.8). Glucose and xylose concentrations of post-oxygen soda pulp increased and decreased by 1.4% each after 3 h at the same treatment charge (Table 3.10). In post-oxygen kraft pulp a maximum of 0.5% in glucose content was achieved after 3 h whereas xylose content decreased by a a maximum 0.6% after 1 h (Table 3.12). The major sugar constituents of fully bleached bagasse pulp were glucose in the region of 75.7 to 76.9% and xylose in the region of 22 to 23.6% (Table 3.7). In the post-oxygen soda pulp the major sugar constituents were glucose in the region of 80.2 to 81.2% and xylose was in the region of 18.8 to 20.4% (Table 3.9). Post-oxygen kraft pulp had 84.6 to 87.3% of glucose, 7.3 to 8.4% xylose content and mannose in the region of 4.2 to 6.0% as the major sugar constituents (Table 3.11).

Table 3.7. Sugar composition of bagasse pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	1.3	0.04	75.7	23.1	ND[b]
1	1	1.2	0.04	76.6	22.2	ND
	5	1.1	0.04	76.9	22.0	ND
	10	1.1	0.04	76.5	22.4	ND
2	1	1.1	0.04	76.1	22.9	ND
	5	1.1	0.04	76.4	22.5	ND
	10	1.1	ND	76.3	22.6	ND
3	1	1.1	0.04	75.3	23.6	ND
	5	1.1	0.04	76.7	22.2	ND
	10	1.0	ND	76.4	22.6	ND

[a]Data are averages of two independent runs
[b]Not detected

Table 3.8. Changes in glucose and xylose concentrations of bagasse pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Glucose increase (%)	Xylose decrease (%)
1	0.8	0.7
2	0.6	0.5
3	0.7	0.5

Table 3.9. Sugar composition of post-oxygen soda pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	ND[b]	ND	79.8	20.2	ND
1	1	ND	ND	80.3	19.7	ND
	5	ND	ND	81.2	18.9	ND
	10	ND	ND	80.2	19.8	ND
2	1	ND	ND	80.4	19.6	ND
	5	ND	ND	79.6	20.4	ND
	10	ND	ND	80.7	19.4	ND
3	1	ND	ND	80.6	19.4	ND
	5	ND	ND	80.8	19.2	ND
	10	ND	ND	81.2	18.8	ND

[a]Data are averages of two independent runs
[b]Not detected

Table 3.10. Changes in glucose and xylose concentrations of post-oxygen soda pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Glucose increase (%)	Xylose decrease (%)
1	0.4	0.4
2	0.9	0.8
3	1.4	1.4

Table 3.11. Sugar composition of post-oxygen kraft pulp, pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	0.7	0.3	84.6	8.5	5.9
1	1	0.8	0.3	84.7	8.3	5.9
	5	0.8	0.5	84.8	8.0	5.9
	10	0.9	0.5	84.8	7.9	5.9
2	1	0.8	0.4	84.9	8.0	5.9
	5	0.9	0.3	87.3	7.3	4.2
	10	1.0	0.5	84.8	8.0	5.8
3	1	0.8	0.5	84.6	8.2	5.9
	5	0.8	0.2	85.1	8.4	5.5
	10	0.7	0.2	85.1	8.0	6.0

[a]Data are averages of two independent runs.

Table 3.12. Changes in glucose and xylose concentrations of post-oxygen kraft pulp, pretreated with *T. lanuginosus* SSBP xylanase at 10 $U.g^{-1}$ pulp at 60°C and pH 6.5, after DED-bleaching

Treatment time (h)	Glucose increase (%)	Xylose decrease (%)
1	0.2	0.6
2	0.2	0.5
3	0.5	0.5

3.4.7 Effect of enzyme pretreatment on chlorine dioxide consumption

Increase in final brightness of pulps, which is one of the primary goals of enzymatic pretreatment, was used as an indicator to evaluate the influence of xylanase pretreatment of pulps on reducing chlorine dioxide consumption. Xylanase treatment had the potential of reducing chlorine dioxide consumption in bleaching of all three pulps tested without affecting their final target brightness. In both bagasse pulp and post-oxygen soda pulp target brightness was reached with a total reduction in chlorine dioxide consumption of 16.7% in xylanase pretreated samples (Table 3.13 and Table 3.14, respectively). This suggested that about 2.5 kg of chlorine dioxide could be saved in bleaching a ton of pulp. The total savings in chlorine dioxide achieved in bleaching post-oxygen kraft pulp, due to xylanase pretreatment, was 10% or 1.5 kg per ton of pulp, as shown in Table 3.15.

Table 3.13. Reduction of chlorine dioxide charges in bleaching of bagasse pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	76.5
10.0	0.0	0.0	79.8
10.0	3.3	0.5	79.0
10.0	6.7	1.0	78.8
10.0	10.0	1.5	77.4
10.0	13.3	2.0	76.7
10.0	16.7	2.5	76.5
10.0	20.0	3.0	76.3
10.0	23.3	3.5	73.9
10.0	26.7	4.0	73.4
10.0	30.0	4.5	73.3
10.0	33.3	5.0	67.5

[a]Data are averages of two independent runs.

Table 3.14. Reduction of chlorine dioxide charges in bleaching of post-oxygen soda pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	90.6
10.0	0.0	0.0	91.2
10.0	3.3	0.5	90.8
10.0	6.7	1.0	90.8
10.0	10.0	1.5	90.7
10.0	13.3	2.0	90.6
10.0	16.7	2.5	90.6
10.0	20.0	3.0	90.4
10.0	23.3	3.5	90.1
10.0	26.7	4.0	89.9
10.0	30.0	4.5	89.3
10.0	33.3	5.0	89.2

[a]Data are averages of two independent runs.

Table 3.15. Reduction of chlorine dioxide charges in bleaching of post-oxygen kraft pulp pretreated with *T. lanuginosus* SSBP xylanase at 60°C and pH 6.5

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	59.2
10.0	0.0	0.0	61.6
10.0	3.3	0.5	60.8
10.0	6.7	1.0	59.6
10.0	10.0	1.5	59.5
10.0	13.3	2.0	58.7
10.0	16.7	2.5	58.6
10.0	20.0	3.0	57.8
10.0	23.3	3.5	57.5
10.0	26.7	4.0	56.3
10.0	30.0	4.5	55.8
10.0	33.3	5.0	52.6

[a]Data are averages of two independent runs.

3.5 DISCUSSION

Crude xylanase from *T. lanuginosus* SSBP has been studied and shown to be effective in improving brightness of bagasse pulp as well as in releasing chromophoric material and reducing sugars (Bissoon *et al.,* 1998). The main objective of this study was thus to compare the *T. lanuginosus* xylanase effect on bagasse pulp to other two pulps which are also used in papermaking, namely post-oxygen soda pulp and post-oxygen kraft pulp and also to evaluate its influence on chlorine dioxide consumption in bleaching of these three pulps.

Pulp hemicellulose of all three pulps was solubilised facilitating increased delignification of pulps which was indicated by the release of chromophoric material and reducing sugars. This release of hydrolysis products appeared to be increasing with an increase in enzyme dose and an increase in treatment time. Reducing sugars and chromophore material were released at high concentrations from xylanase-pretreated bagasse pulp samples compared to both oxygen-delignified soda and kraft pulps tested under the same treatment conditions suggesting that the crude enzyme was more effective in hydrolysing the hemicellulose of bagasse pulp. Although the best release of both hydrolysis products on all three pulps tested was observed at an enzyme concentration of 10 $U.g^{-1}$ dry pulp after 3 h, there was also a significant increase in the release of hydrolysis products when lower enzyme doses of 1 and 5 $U.g^{-1}$ were applied also for 3 h. This may be an indication that enzyme doses of 1 and 5 $U.g^{-1}$ dry weight pulp may also be used efficient removal of xylan from pulp. Bissoon *et al.* (1998) observed a higher release of chromophoric material from bagasse pulp with the purified enzyme compared to the crude enzyme. *T.*

lanuginosus xylanase was able to solubilise 20 – 26% xylan in biobleaching of dissolving pulps (Gübitz *et al.,* 1997b). According to a study done in 1997 by Davis and co-workers (as cited by Haarhoff *et al.,* 1999), release of chromophores from the pulp indicated the degradation of lignin-hemicellulose linkages. The increase in the release of reducing sugars and chromophoric material from pulps pretreated with crude *T. lanuginosus* SSBP xylanase was supported by the reduction in Kappa number which was also observed after enzyme treatment prior to alkaline extraction. This suggested that lignin was made more extractable by the enzyme treatment. There was, however, no correlation between the release of hydrolytsis products and the reduction in Kappa number. While most release of chromophores and reducing sugars was observed in bagasse pulp, Kappa number of bagasse pulp was reduced to a lesser extent compared to that of post-oxygen soda pulp and post-oxygen kraft pulp.

There was also no correlation between Kappa number reduction and brightness increase after enzyme and chemical treatments. Kappa number of post-oxygen soda pulp and post oxygen kraft pulp were the most affected by enzyme treatment but their brightness gain after enzyme treatment was less than that of bagasse pulp which Kappa number was less affected by enzyme treatment. Post-oxygen soda pulp which had the highest reduction in Kappa number of 17.9% gained less brightness points than bagasse pulp and post-oxygen kraft pulp which had their brightness improved by more than 4 points after chemical bleaching. The increase in brightness of post-oxygen kraft pulp after enzyme treatment was less than that of bagasse pulp and post-oxygen soda pulp but after chemical bleaching post-oxygen kraft pulp achieved more brightness points than the other two pulps. This could suggest crude xylanase

was not effective enough in hydrolysing post-oxygen kraft pulp and it was more dependent on chemicals for its bleaching. Purified and crude xylanase of *T. lanuginosus* SSBP at high enzyme doses of 150 $U.g^{-1}$ pulp increased brightness of bagasse pulp by 4.5% and 3.4%, respectively, and a lower enzyme dose of 5 $U.g^{-1}$ increased bagasse pulp brightness by more than 3% (Bissoon *et al.,* 1998). When the crude enzyme preparation was used in biobleaching of sulphite pulps for production of dissolving pulps brightness increase of 2.6 points was achieved in the unbleached sulphite pulps (Christov and Prior, 1997).

Viscosity of all xylanase pretreated pulps was slightly modified compared to that of control samples. It was affected in the first hour of treatment being reduced by a maximum of 10% but became stable as the treatment time progressed. Although *T. lanuginosus* SSBP was characterised and shown to produce high cellulase-free xylanase (Singh *et al.,* 1995) this could suggest the importance of determining cellulase activity in the crude enzyme before application in pulp bleaching. Christov and Prior (1997) detected 0.1 $IU.ml^{-1}$ of cellulase activity in a culture of *T. lanuginosus* SSBP they used in their study.

Carbohydrate analyses of fully-beached pulps indicated that there was a general increase in glucose concentrations and a decrease in xylose concentrations as enzyme dose is increased and treatment time is prolonged. The glucose concentration was only increased significantly in the post-oxygen kraft pulp compared to the other two pulp types under the same enzyme treatment and bleaching conditions. The significant amount of mannose present in post-oxygen kraft pulp suggested that this

pulp type was a product of softwood which had significant amount of glucomannan (Sjöström, 1993). This could be reason why the xylanase pretreatment alone was not effective enough in hydrolysing its hemicellulose and could suggest the use of mannanases for further hydrolysis of this pulp type. This could also suggest that accessory enzymes present in the crude enzyme preparation of *T. lanuginosus* SSBP do not contribute significantly to the removal of xylan from pulp, as earlier suggested by Gubitz *et al.* (1997b) and Bissoon *et al.* (1998).

When the DED-sequence was used in chemical bleaching of pulps, enzyme pretreatment with crude *T. lanuginosus* SSBP xylanase led to chlorine dioxide by a maximum of 16.7% suggesting that about 2.5 kg of chlorine dioxide could be saved in bleaching one ton of pulp. According to a study conducted in 1975 by Browning (as cited by Haarhoff, 1999), chlorine dioxide bleaching decreases paper strength properties. This chlorine dioxide reduction in bleaching of pulp achieved with the aid of crude xylanase could suggest that the application of the crude xylanase could not only increase brightness of pulp but could also improve other paper strength properties. This could also lead to the release of effluents with lesser AOX and other organochlorine compounds which are toxic to the environment.

Although enzyme technology in the South African pulp and paper industry is still under intensive research results obtained in this study indicated that the crude *T. lanuginosus* SSBP xylanase has potential for large-scale application. It has shown stability at high temperatures and at an alkaline pH and therefore can adjust well to mill conditions and can also be produced at high levels from coarse corn cobs which are a cheap carbon source.

CHAPTER 4: BIOBLEACHING EFFECTS OF A COMMERCIAL XYLANASE ON PAPER PULPS

4.1 ABSTRACT

A commercially available xylanase preparation was tested in bleaching of three different industrial pulps used for papermaking: bagasse, post-oxygen soda and post-oxygen kraft. Xylanase concentrations of 1, 5 and 10 $U.g^{-1}$ dry weight pulp were applied on pulp prior to bleaching for up to 3 h at the optimum pH and temperature of the enzyme. Release of chromophores and reducing sugars from pulps indicated that pulp hemicellulose was hydrolysed by xylanase pretreatment with an effect found to be increasing with the increase in enzyme doses used. Pulp Kappa number, used as an indicator for the removal of residual lignin from the pulps, was reduced by enzyme treatment without alkaline extraction. Bagasse and post-oxygen kraft pulps, pretreated with xylanase at 10 $U.g^{-1}$ for 3 h gained 5 and 5.7 brightness points, respectively, over controls after D_1ED_2 bleaching while post-oxygen soda pulp brightness increased by 1.7 brightness points under the same treatment conditions. On the other hand, viscosity of pulps was slightly modified in comparison with controls. Carbohydrate analyses showed an increase in glucose content and a decrease in xylose content of the xylanase-pretreated pulp samples. In combination with the sequence D_1ED_2 this commercial xylanase preparation led to savings of chlorine dioxide that were in the range of 10 to 20% without affecting the target brightness.

4.2 INTRODUCTION

The pulp and paper industry has become one of the major polluters of the environment due to one of the stages of its pulp bleaching process, chlorination, which involves addition of chlorine or chlorine dioxide in removing residual lignin in the pulp (Christov and Prior, 1998). Alternative bleaching methods currently being studied include oxygen delignification techniques, replacement of molecular chlorine with chlorine dioxide as well as the use of enzymes for biobleaching, to reduce the production of chlorinated organic substances (Vidal *et al.,* 1997).

Hemicellulases have been the commonly used enzymes in pulp bleaching as they selectively affect the accessible hemicellulose fraction of wood pulps. Xylanases have been the most widely studied of the hemicellulose-degrading enzymes and have been shown to be the most effective (Bajpai and Bajpai, 1997). These enzymes are proposed to be working by either degrading xylan which is in the fibre pores, enhancing the free flow of bleach chemicals into the fibre or by cleaving lignin-carbohydrate bonds (Roncero *et al.,* 1998). According to Zamost *et al.* (1991), several studies have shown that xylanases are used by the pulp and paper industry in the biobleaching processes to enhance the brightness of bleached pulps, to decrease the amount of chlorine used in the bleaching stages, and to increase the freeness of pulps in the recycled paper process.

Several commercially available xylanase preparations, most of which were active at slightly acidic or neutral pH, have been investigated in pulp bleaching. The most

commonly used commercial xylanases include Cartazyme HS/HT, Pulpzyme HA/HB/HC, Irgazyme 40-4X, Ecopulp X-200 and Ecopulp XM which contains both xylanase and mannanase. According to Zamost *et al.* (1991), Pulpzyme HA (Novo Nordisk A/S) which was produced by a strain of *Trichoderma reesei,* was the first commercially available xylanase, to be used in the biobleaching of wood pulps and it reduced Kappa number of oxygen delignified birch kraft pulp by 20%. Kraft pulps pretreated with Cartazyme achieved a higher brightness compared to those pretreated with a crude xylanase preparation of *Streptomyces thermviolaceus* under the same treatment conditions (Garg *et al.,* 1998). In one study, three different commercial xylanases, in combination with the $D_OE_{OP}D_1$ sequence, led to chlorine dioxide reductions of 40% in bleaching of kraft pulps (Vicuña *et al.,* 1997).

This study was thus carried out to compare the effectiveness of Xylanase P, a commercial xylanase, in biobleaching of three different industrial pulps: unbleached bagasse, post-oxygen soda and post-oxygen kraft. Another study was made to evaluate the influence of Xylanase P treatment on the consumption of bleaching chemicals in pulp bleaching using a DED-sequence.

4.3 MATERIALS AND METHODS

4.3.1 Enzyme preparation

Xylanase P was supplied by Iogen Corporation (Ontario, Canada), and its activity determined as described in Section 2.3.3, and characteristics are summarised in Table 2.1.

4.3.2 Enzymatic hydrolysis of pulp

Characteristics of three industrial pulps (Batch 2): unbleached bagasse pulp, oxygen-bleached soda pulp and oxygen-bleached kraft pulp are summarised in Table 2.3. The pulps were prepared and enzymatically hydrolysed as described in Section 2.3.5 and 3.3.2, respectively.

4.3.3 Analyses of hydrolysis products

Release of chromophores and reducing sugars was determined as described in Section 3.3.3.

4.3.4 Chemical bleaching of pulps

After the enzyme treatment, the pulps were bleached in a multistage bleaching process using a D_1ED_2 sequence, as described in Section 3.3.4.

4.3.5 Analyses of pulps

Pulp properties, such as Kappa number, brightness after enzyme treatment and brightness, carbohydrate content and viscosity after DED-bleaching were analysed as described in Section 2.3.5 and 3.3.5, respectively.

4.3.6 Evaluation of enzyme pretreatment on chlorine dioxide consumption

To study the influence of enzyme pretreatment on chlorine dioxide reduction pulp samples were all subjected to the chemical bleaching conditions described in Section 3.3.6 and outlined in Table 3.1.

4.4 RESULTS

4.4.1 Release of chromophore material

Results of enzyme bleach filtrates analyses for chromophore release at wavelength of 280 and 465 nm are presented in Figures 4.1 and 4.2. From all three pulps an increase in chromophore release with an increase in enzyme dose was observed at both wavelengths tested. However, more chromophores were released at 280 nm than at 465 nm, and at longer treatment times. In terms of chromophore and colour release at both wavelengths, Xylanase P treatment was more effective in hydrolysing hemicellulose of bagasse pulp and post-oxygen kraft pulp, as indicated by the high amount of chromophores released (Figures 4.1A, 4.2A, 4.1C and 4.2C). Chromophore release from post-oxygen soda pulp was less compared to that from the other two pulps at both wavelengths (Figures 4.1B and 4.2B). These results could suggest that Xylanase P was effective in hydrolysing the pulp hemicellulose, xylan in particular, especially in bagasse and post-oxygen kraft pulps.

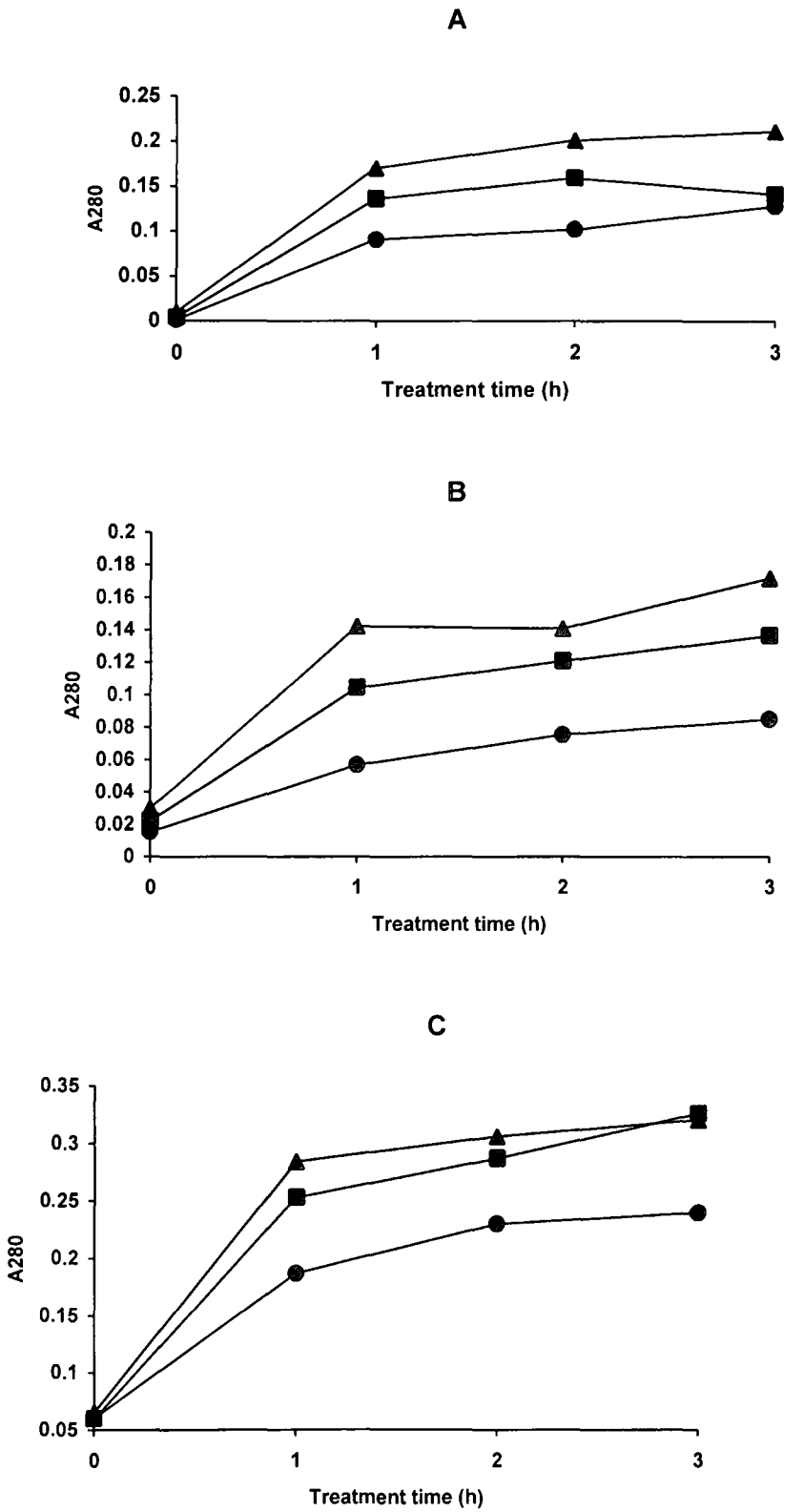

Figure 4.1. Release of chromophore material at 280 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0

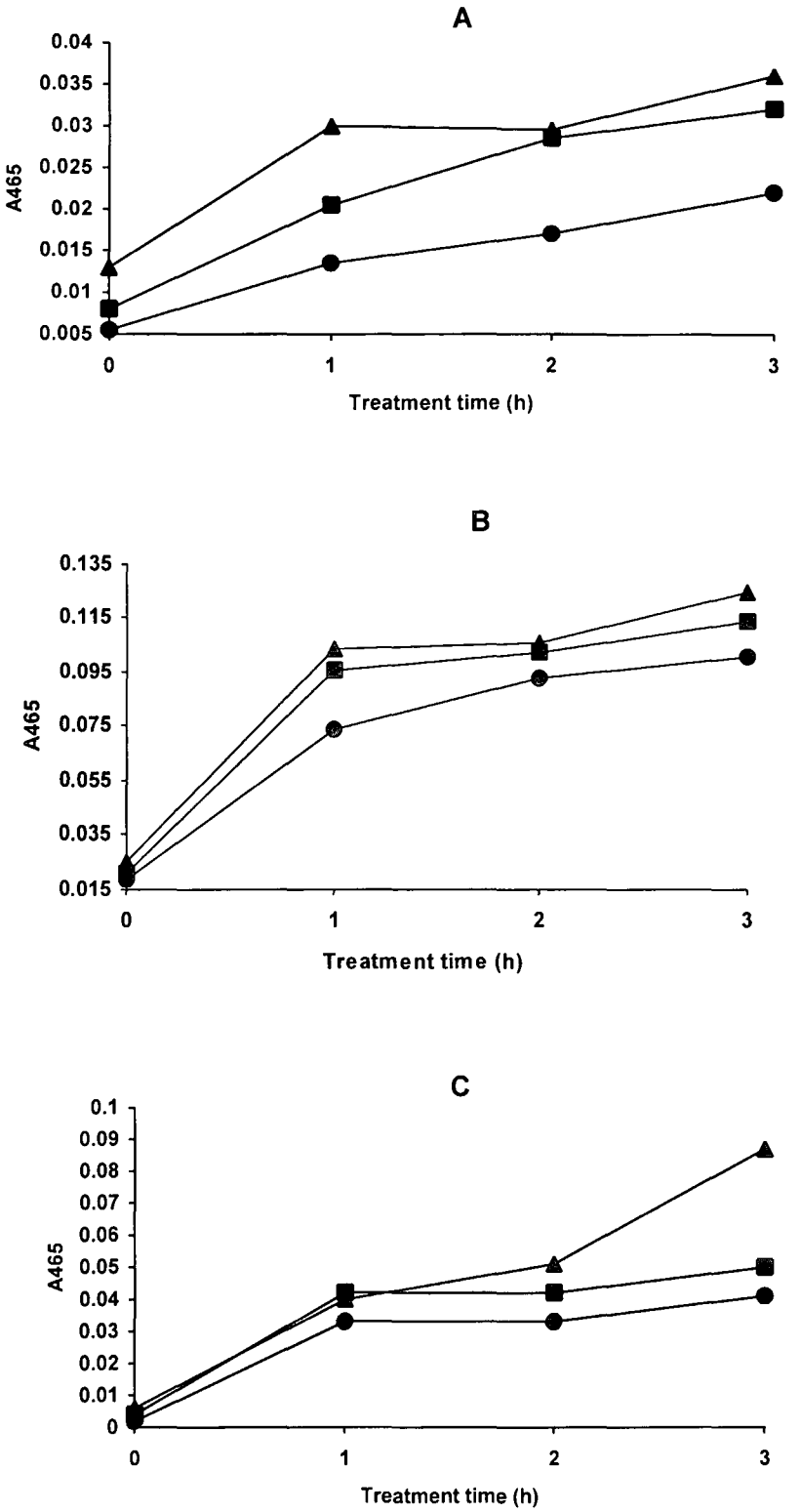

Figure 4.2. Release of chromophore material at 465 nm from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0

4.4.2 Release of reducing sugars

Release of reducing sugars from all three pulps increased with the increase in enzyme dose and treatment time. Reducing sugars were released at a higher rate from bagasse pulp at the highest enzyme dose of 10 $U.g^{-1}$ dry weight pulp even after 2 h treatment (Figure 4.3A). Release of reducing sugars from post-oxygen soda pulp (Figure 4.3B) and post-oxygen kraft pulp (Figure 4.3C) was at a lower rate than that observed in bagasse pulp. All three pulps showed best results when the highest enzyme dose of 10 $U.g^{-1}$ dry weight pulp was used for 3 h. These results confirmed those of chromophore release, discussed above, which indicated that xylanase pretreatment of pulp with Xylanase P was effective in hydrolysing pulp hemicellulose and in that way removing xylan from pulps.

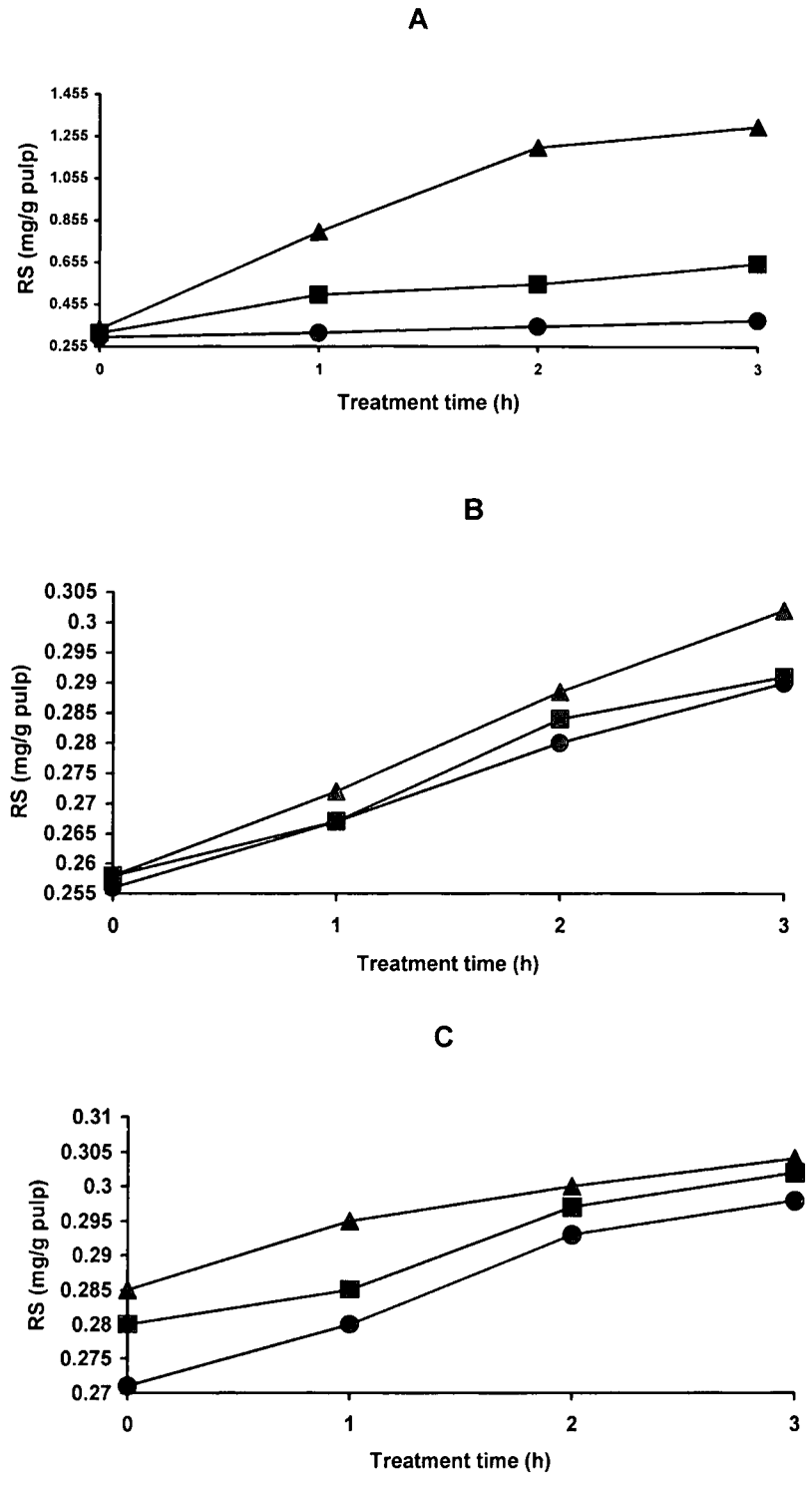

Figure 4.3. Release of reducing sugars (RS) from **(A)** bagasse pulp, **(B)** post-oxygen soda pulp and **(C)** post-oxygen kraft pulp treated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0

4.4.3 Effect of enzyme pretreatment on Kappa number reduction

Table 4.1 presents the results of Kappa number determination of all three pulps after enzyme treatment without alkaline extraction. Results obtained showed a significant reduction of Kappa number on all pulp types, indicating that a certain percentage of lignin was made more extractable by enzyme treatment. Xylanase P was more effective on bagasse pulp reducing its Kappa number by a maximum of 20.7% after 3 h treatment at an enzyme dose of 10 $U.g^{-1}$ dry weight pulp. With the post-oxygen soda pulp a maximum reduction of 13% in Kappa number was obtained after 2 h treatment at enzyme doses of 5 and 10 $U.g^{-1}$ dry weight pulp whereas in post-oxygen kraft pulp Kappa number was reduced by 8% after 3 h also at 10 $U.g^{-1}$ dry weight pulp.

Table 4.1. Kappa number of bagasse, post-oxygen soda and post-oxygen kraft pulps pretreated with Xylanase P at 60°C and pH 5.0

Treatment Time (h)	Enzyme charge (U/g)	Kappa number[a]		
		Bagasse pulp	Post-oxygen soda pulp	Post-oxygen kraft pulp
1	1	7.3 (11.0)[b]	4.2 (8.7)	9.7 (3.0)
	5	7.2 (12.2)	4.3 (6.5)	9.5 (5.0)
	10	6.8 (17.1)	4.2 (8.7)	9.3 (7.0)
2	1	7.2 (12.2)	4.1 (10.9)	9.8 (2.0)
	5	7.1 (13.4)	4.0 (13.0)	9.6 (4.0)
	10	6.9 (15.9)	4.0 (13.0)	9.6 (4.0)
3	1	7.0 (14.6)	4.3 (6.5)	9.9 (1.0)
	5	7.1 (13.4)	4.3 (6.5)	9.5 (5.0)
	10	6.5 (20.7)	4.2 (8.7)	9.2 (8.0)
Control	0	8.2 (0.0)	4.6 (0.0)	10.0 (0.0)

[a]Data are averages of two independent runs
[b]Values in parentheses represent %reduction

4.4.4 Effect of enzyme pretreatment on pulp brightness

Results on pulp brightness after enzyme treatment correlated with the Kappa number reduction results as bagasse pulp gained 2.9 brightness points (Table 4.2), compared to post-oxygen soda pulp and post-oxygen kraft pulp which gained 1.1 and 1.7 brightness points, respectively (Table 4.3 and Table 4.4, respectively).

In combination with the D_1ED_2 bleach sequence xylanase pretreatment, at an enzyme dose of 10U g^{-1} dry weight pulp for 3 h, improved brightness of bagasse and post-oxygen pulps by 5 and 5.7 points over their respective controls, as shown in Table 4.2 and Table 4.4, respectively. Under the same treatment conditions brightness of post-oxygen soda pulp gained 1.7 brightness points (Table 4.3). Results obtained with bagasse and post-oxygen kraft pulps could suggest that bleaching of post-oxygen kraft pulp is more dependent on chemicals as its brightness was less improved by enzyme treatment alone compared to that of bagasse pulp but gained more brightness points after chemical bleaching than did bagasse pulp, as shown in Tables 4.2 and 4.4.

Table 4.2. Brightness of bagasse pulp pretreated with Xylanase P (X) at 60°C and pH 5.0

Treatment time (h)	Enzyme charge (U/g)	Brightness after enzyme (X) [a]		Brightness after XDED[a]	
		(%)	(points)	(%)	(points)
1	**1**	46.7	1.4	76.5	0.9
	5	47.2	1.9	77.0	1.4
	10	47.3	2.0	78.5	2.9
2	**1**	46.7	1.4	76.9	1.3
	5	47.4	2.1	77.6	2.0
	10	47.9	2.6	79.7	4.1
3	**1**	47.5	2.2	77.2	1.6
	5	47.8	2.5	79.4	3.8
	10	48.2	2.9	80.6	5.0
Control	**0**	45.3	0.0	75.6	0.0

[a]Data are averages of two independent runs

Table 4.3. Brightness of post-oxygen soda pulp pretreated with Xylanase P (X) at 60°C and pH 5.0

Treatment time (h)	Enzyme charge (U/g)	Brightness after enzyme (X) [a]		Brightness after XDED[a]	
		(%)	(points)	(%)	(points)
1	**1**	68.2	0.1	90.9	0.7
	5	68.5	0.4	91.3	1.1
	10	69.1	1.0	91.4	1.2
2	**1**	68.7	0.6	91.2	1.0
	5	68.8	0.7	91.5	1.3
	10	69.2	1.1	91.6	1.4
3	**1**	68.7	0.6	91.6	1.4
	5	69.1	1.0	91.7	1.5
	10	69.2	1.1	91.9	1.7
Control	**0**	68.1	0.0	90.2	0.0

[a]Data are averages of two independent runs

Table 4.4. Brightness of post-oxygen kraft pulp pretreated with Xylanase P (X) at 60°C and pH 5.0

Treatment time (h)	Enzyme charge (U/g)	Brightness after enzyme (X) [a]		Brightness after XDED[a]	
		(%)	(points)	(%)	(points)
1	**1**	34.1	0.5	59.9	0.1
	5	35.1	0.8	63.0	3.2
	10	35.1	0.8	63.4	3.6
2	**1**	35.1	0.8	62.1	2.3
	5	35.3	1.0	62.7	2.9
	10	35.7	1.4	62.8	3.0
3	**1**	35.2	0.9	63.3	3.5
	5	35.8	1.5	65.3	5.5
	10	36.0	1.7	65.5	5.7
Control	**0**	34.3	0.0	59.8	0.0

[a]Data are averages of two independent runs.

4.4.5 Effect of enzyme pretreatment on pulp viscosity

Viscosity of all three pulps pretreated with xylanase P was slightly modified in comparison with controls after final chemical bleaching. For the first two hours of enzyme treatment viscosity of bagasse pulp was slightly improved at lower enzyme concentrations of 1 and 5 $U.g^{-1}$ pulp after 1 and 2 h, respectively, but after 3 h viscosity improved much better at 10 $U.g^{-1}$ pulp than at the lower charges (Figure 4.4A). Viscosity of post-oxygen soda pulp was also increased with a notable increase observed after 2 h at an enzyme concentration of 5 $U.g^{-1}$ dry weight pulp (Figure 4.4B). Post-oxygen kraft pulp viscosity was the most stable of the three pulps showing a slight improvement at 5 $U.g^{-1}$ pulp after 2 h and at $10U.g^{-1}$ pulp after 3 h (Figure 4.4C). In general, Xylanase P affected the viscosity of all three pulps in the same manner being more effective at the lowest dose after 1 h treatment and more effective at the highest dose after 3 h (Figures 4.4A, 4.4B and 4.4C).

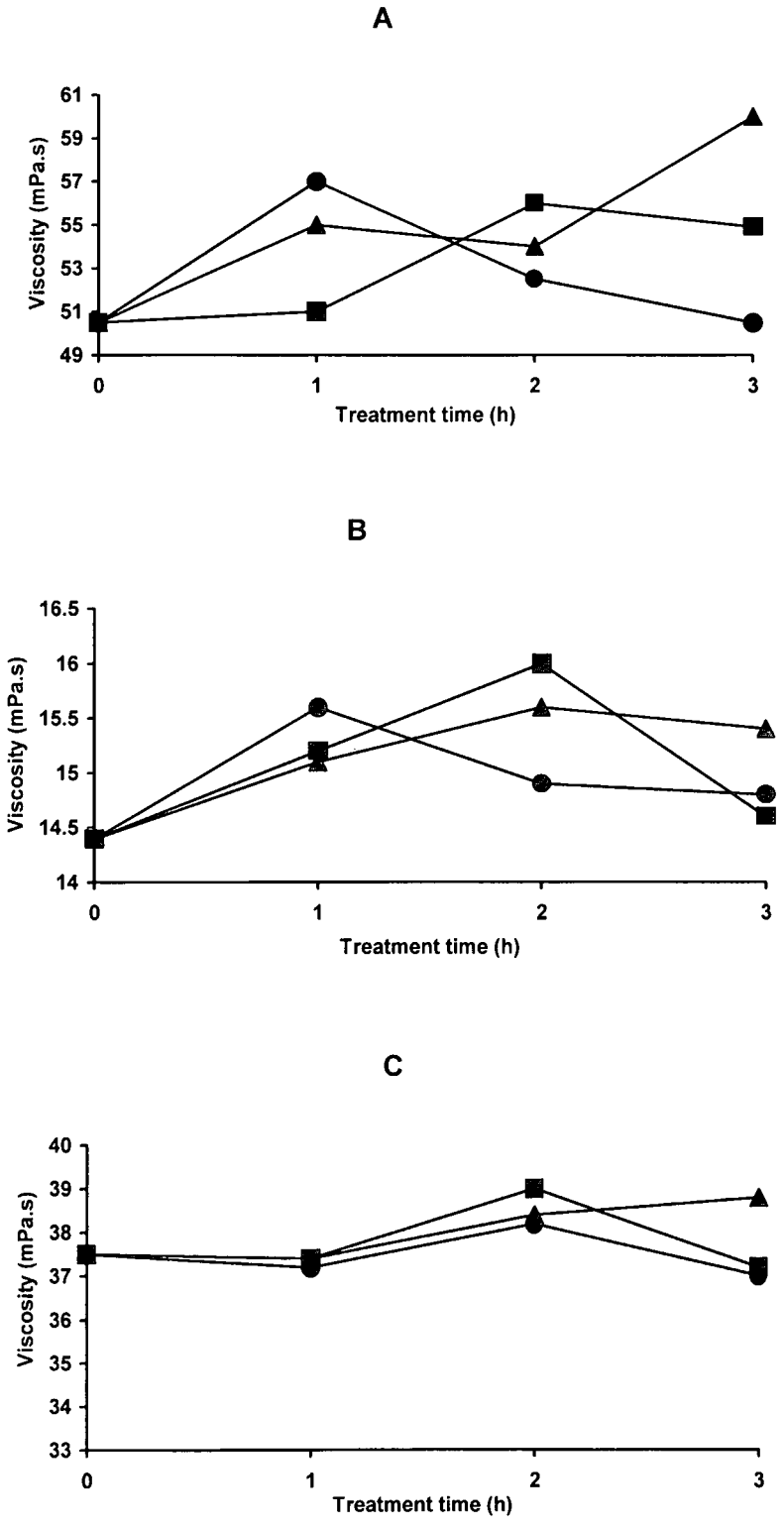

Figure 4.4. Viscosity of **(A)** bagasse pulp, **(B)** post-oxygen soda and **(C)** post-oxygen kraft pulp, pretreated with Xylanase P at 1 IU/g (●), 5 IU/g (■) and 10 IU/g (▲) at 60°C and pH 5.0, after DED-bleaching

4.4.6 Effect of enzyme pretreatment on sugar composition

Carbohydrate analyses of bagasse, post-oxygen soda and post-oxygen kraft pulps, pretreated with xylanase, after DED-bleaching showed a general increase in glucose concentration and a general decrease in xylose content (Tables 4.5, 4.6 and 4.7). Chemical analyses of xylanase pretreated bagasse pulp showed an increase in glucose content from 77 to 80.3% and a decrease in xylose content from 21.5 to 18.5% (Table 4.5). Glucose content of bagasse pulp pretreated at an enzyme dose of 10 $U.g^{-1}$ pulp after 3 h improved by 2.3% compared to the control whereas a loss of 3% in xylose was obtained, as shown in Table 4.6. Glucose content of post-oxygen soda pulp increased by up to 6% with the xylose content decreasing by a maximum of 7% (Tables 4.8). Xylose content of post-oxygen kraft pulp was not significantly affected by the enzyme treatment as it decreased by only 0.4% after 2 h at 10 $U.g^{-1}$ pulp whereas glucose concentrations increased by 4.2% and 3.5% after 1 and 3 h, respectively (Table 4.10). The major sugar constituents of fully bleached bagasse pulp were glucose in the range 77.0% to 80.3% and 18.5% to 21.5% xylose concentration (Table 4.5). In the post-oxygen soda pulp the major sugar constituents were glucose in the range 77.9% to 83.9% and xylose in the range 16.1% to 22.1%, as shown in Table 4.7. In the post-oxygen kraft pulp the major sugar constituents included glucose in the range 86.1% to 89.6%, 5.6% to 6.0% xylose and mannose in the range 3.0% to 4.0%. The significant amount of mannose in post-oxygen kraft pulp suggests the presence of glucomannan in this pulp type and mannanases could be used for further hydrolysis of this pulp.

Table 4.5. Sugar composition of bagasse pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	1.4	0.1	77.0	21.5	ND[b]
1	1	1.3	ND	77.4	21.3	ND
	5	1.5	ND	78.5	20.0	ND
	10	1.2	ND	78.9	19.9	ND
2	1	1.2	ND	78.2	20.6	ND
	5	1.2	ND	78.0	20.8	ND
	10	1.4	ND	79.0	18.7	ND
3	1	1.3	ND	78.7	20.0	ND
	5	1.3	ND	79.5	19.2	ND
	10	1.2	ND	80.3	18.5	ND

[a]Data are averages of two independent runs
[b]Not detected

Table 4.6. Change in glucose and xylose concentrations of bagasse pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching

Tretament time (h)	Glucose increase (%)	Xylose decrease (%)
1	1.9	1.6
2	2.0	2.8
3	2.3	3.0

Table 4.7. Sugar composition of post-oxygen soda pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	ND[b]	ND	77.9	22.1	ND
1	1	ND	ND	79.4	20.6	ND
	5	ND	ND	79.7	20.3	ND
	10	ND	ND	79.8	20.2	ND
2	1	ND	ND	81.3	18.7	ND
	5	ND	ND	82.6	17.4	ND
	10	ND	ND	84.2	15.8	ND
3	1	ND	ND	83.4	16.6	ND
	5	ND	ND	82.2	17.8	ND
	10	ND	ND	83.9	16.1	ND

[a]Data are averages of two independent runs
[b]Not detected

Table 4.8. Change in glucose and xylose concentrations of post-oxygen soda pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching

Treatment time (h)	Glucose change (%)	Xylose change (%)
1	1.9	1.9
2	6.3	6.3
3	6.0	7.0

Table 4.9. Sugar composition of post-oxygen kraft pulp, pretreated with Xylanase P at 60°C and pH 5.0, after DED-bleaching

Treatment time (h)	Enzyme charge (U/g)	Sugars (%)[a]				
		Arabinose	Galactose	Glucose	Xylose	Mannose
Control	0	0.7	0.3	86.1	5.8	7.1
1	1	0.6	0.3	86.2	5.4	7.5
	5	0.6	0.3	89.1	5.8	4.2
	10	0.7	0.4	90.3	5.6	3.0
2	1	0.7	0.6	88.7	5.9	4.0
	5	0.7	0.3	86.6	5.8	6.5
	10	0.7	0.3	88.0	5.4	5.6
3	1	0.7	0.4	89.6	5.5	4.0
	5	0.7	0.4	89.1	5.8	4.0
	10	0.5	0.3	89.6	5.6	4.0

[a]Data are averages of two independent runs

Table 4.10. Change in glucose and xylose concentrations of post-oxygen kraft pulp, pretreated with Xylanase P at 10 $U.g^{-1}$ pulp at 60°C and pH 5.0, after DED-bleaching

Tretament time (h)	Glucose change (%)	Xylose change (%)
1	4.2	0.2
2	1.9	0.4
3	3.5	0.2

4.4.7 Effect of enzyme pretreatment on chlorine dioxide consumption

Brightness increase of pulps was used as an indicator for evaluating the effect of enzyme pretreatment on chlorine dioxide consumption in a DED-bleach sequence. Xylanase pretreatment of bagasse pulp and post-oxygen soda pulp achieved a total reduction in chlorine dioxide consumption to a maximum of 20% without affecting the final brightness attained with respective controls, as illustrated in Table 11 and Table 4.12, respectively, suggesting that about 2.5 kg chlorine dioxide could be saved to bleach a ton of pulp pretreated with Xylanase P. The total savings in chlorine dioxide achieved in bleaching post-oxygen kraft pulp, due to xylanase pretreatment, was 10% or 1.5 kg per ton of pulp (Table 4.13). These results suggested that post-oxygen kraft pulp was more sensitive to chemical bleaching than bagasse and post-oxygen soda pulps.

Table 4.11. Reduction of chlorine dioxide charges in bleaching of bagasse pulp pretreated with Xylanase P at 60°C and pH 5.0

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	76.3
10.0	0.0	0.0	81.5
10.0	3.3	0.5	80.6
10.0	6.7	1.0	80.6
10.0	10.0	1.5	80.4
10.0	13.3	2.0	80.0
10.0	16.7	2.5	78.4
10.0	20.0	3.0	78.2
10.0	23.3	3.5	76.1
10.0	26.7	4.0	75.2
10.0	30.0	4.5	74.3
10.0	33.3	5.0	73.9

[a]Data are averages of two independent runs

Table 4.12. Reduction of chlorine dioxide charges in bleaching of post-oxygen soda pulp pretreated with Xylanase P at 60°C and pH 5.0

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	90.4
10.0	0.0	0.0	91.8
10.0	3.3	0.5	91.6
10.0	6.7	1.0	91.5
10.0	10.0	1.5	91.2
10.0	13.3	2.0	91.1
10.0	16.7	2.5	90.6
10.0	20.0	3.0	90.6
10.0	23.3	3.5	90.2
10.0	26.7	4.0	90.2
10.0	30.0	4.5	90.2
10.0	33.3	5.0	89.6

[a]Data are averages of two independent runs

Table 4.13. Reduction of chlorine dioxide charges in bleaching of post-oxygen kraft pulp pretreated with Xylanase P at 60°C and pH 5.0

Enzyme charge (U/g)	Total reduction of ClO_2		Brightness (%)[a]
	%	Kg/ton pulp	
0.0	0.0	0.0	60.2
10.0	0.0	0.0	65.8
10.0	3.3	0.5	63.2
10.0	6.7	1.0	62.2
10.0	10.0	1.5	60.7
10.0	13.3	2.0	60.0
10.0	16.7	2.5	59.9
10.0	20.0	3.0	59.1
10.0	23.3	3.5	57.6
10.0	26.7	4.0	55.8
10.0	30.0	4.5	55.4
10.0	33.3	5.0	55.3

[a]Data are averages of two independent runs

4.5 DISCUSSION

Xylanase P was effective in hydrolysing pulp hemicellulose and in facilitating bleachability of pulps. Solubilisation of pulp hemicellulose was indicated by the release of chromophores and reducing sugars from all three pulps tested which increased with an increase in enzyme dose and treatment time. Chromophores were released in high concentrations from bagasse pulp and post-oxygen kraft pulp suggesting that xylan of these pulp types was more accessible to enzyme attack than that of post-oxygen soda pulp. From all three pulps the highest concentrations of chromophores were released when the highest dose of 10 $U.g^{-1}$ dry weight pulp was used for 3 h. Significant increase in the release of hydrolysis products which was observed when enzyme doses of 1 and 5 $U.g^{-1}$ were used for 3 h could suggest that lower enzyme doses of this commercial xylanase could also be used for efficient removal of xylan from pulp. According to Ruiz *et al.* (1999a) the new bonds that are formed between the hemicellulose and lignin during the kraft cooking process makes it difficult to achieve complete hydrolysis using enzymes.

According to Yu *et al.* (1994), most lignin loss occurs with alkali extraction with or without enzyme treatment. However, significant reductions in Kappa number of pulp samples pretreated with Xylanase P were observed. This decrease in lignin content, indicated by Kappa number reduction, correlated with the increase of brightness after enzyme treatment and could be attributed to the action of the enzyme permitting the crossing of lignin in solution (Spiridon *et al.*, 1998). Kappa number of bagasse pulp was reduced by a maximum of 20.7%, with a brightness increase of 2.9 points, compared to that of post-oxygen soda pulp and post-oxygen kraft pulp which had

Kappa number reductions of 13% and 8% and gaining 1.1 and 1.7 brightness points, respectively. One suggestion on how xylanases work is that they may act by partial depolymerisation of xylan, releasing lignin-xylan complexes and facilitating lignin removal by subsequent chemical bleaching. (Spiridon *et al.,* 1998). There was, however, no correlation between Kappa number reduction and brightness increase obtained after chemical bleaching.

Brightness results after the DED bleach sequence suggested that post-oxygen kraft pulp was more dependent on chemical for its bleaching than xylanase pretreatment. After chemical bleaching brightness of post-oxygen kraft pulp improved by 5.7 points compared to bagasse pulp which gained 5 brightness points after 3 h of enzyme treatment at 10 $U.g^{-1}$ dry weight pulp. Before chemical bleaching xylanase treatment improved brightness of bagasse pulp more than that of post-oxygen kraft pulp. Post-oxygen soda pulp gained less than 2 brightness points over control under the same treatment conditions. According to Roncero *et al.* (1998), viscosity decreases with decreasing Kappa number but that was not the case with the pulps pretreated with Xylanase P as viscosity of these pulps was slightly increased with reduction in Kappa number. According to Kantelinen *et al.* (1993), some studies have shown that cellulase-free xylanases have the ability to increase viscosity of kraft pulp due to partial hydrolysis of low DP xylan in the pulp. This could suggest that although Xylanase P was not tested for cellulase activity, it might be free of any cellulase and its application as a pretreatment step in pulp bleaching could improve pulp and paper strength properties.

Glucose concentration of bagasse pulp and post-oxygen kraft pulp increased by 2.3% compared to 6.3% and 4.2% increase achieved with the post-oxygen soda pulp and post-oxygen kraft pulp, respectively. Xylose content of bagasse pulp decreased by 3.0% while that of post-oxygen soda pulp and post-oxygen kraft pulp decreased by 7.0% and 0.4%, respectively. Glucose and xylose were the major sugars in bagasse pulp and post-oxygen soda pulp while in post-oxygen pulp mannose was also present in significant concentrations. This could suggest that bagasse pulp and post-oxygen soda pulp are hardwoods containing high xylan content while post-oxygen kraft pulp is a softwood with glucomannan content slightly higher than that of xylan, as suggested by Sjöström (1993). This could also explain the reason why xylanase treatment on post-oxygen kraft was not as effective as in bagasse pulp and post-oxygen soda pulp, as indicated by brightness gained after enzyme treatment prior to chemical bleaching. This could suggest that mannanases could be used together with xylanases to further enhance hydrolysis of post-oxygen kraft pulp. Some studies using commercial enzymes have shown that mannanases, when used with xylanases, resulted in more mannan being extracted from pulp compare to either using xylanase or mannanase alone (Yu *et al.,* 1994). This was also observed in a study involving the use of enzymes in bleaching of dissolving pulps (Gübitz *et al.,* 1997b).

Savings of chlorine dioxide up to a maximum of 40% were found to be higher in oxygen delignified pulps rather than conventional pulps after pretreatment with commercial xylanases (Vicuña *et al.,* 1997). However, in this study Xylanase P led to savings of chlorine dioxide to a maximum of 20% in bagasse pulp and post-oxygen soda pulp but in post-oxygen kraft pulp only 10% reduction in chlorine dioxide was

achieved. In conclusion, Xylanase P treatment of all three pulps tested was effective in improving bleachability in subsequent bleaching stages. With the aid of Xylanase P, it is possible to produce high brightness pulps without using elemental chlorine which could in turn lead to the release of environmentally safe bleach effluents by paper mills.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

5.1 THE RESEARCH IN PERSPECTIVE

The pulp and paper industry, which is one of the largest industries in the world, has become the major polluter of the environment and South Africa being one of the major international producers of pulp and paper products is no exception to this problem. One of the mills of Sappi (South African pulp and paper industries), Sappi Saiccor, the world's largest producer of dissolving pulp (Molony, 1999), had two environmental concerns due to its chemical bleaching processes resulting into effluent discharge into the sea and sulphur dioxide (SO_2) emissions into the atmosphere (Meadows, 1999).

Application of biotechnological methods, such as the use of enzymes in pulp bleaching, has been suggested as the alternative which might lead to the reduction of toxic pollutants generated in the manufacture of pulp and paper to the minimum levels and improve the existing technology in a cost-effective way (Christov and Prior, 1998). These methods would lead to savings in chemicals for pulping and bleaching, improvements in pulp quality and more acceptable effluent quality. According to Suurnäkki *et al.* (1996), in the pulp and paper applications limited enzymatic modifications of the pulp fibres can be achieved with a minimum loss of yield due to the specificity of enzymes.

The initial objective of this study was to compare the effectiveness of a crude and commercial xylanase in biobleaching of three different industrial pulps used in South Africa for paper making, viz., bagasse pulp, post-oxygen soda pulp and post-oxygen kraft pulp. The main objective was to evaluate their potential in reducing chlorine dioxide consumption in bleaching of pulp using a modern bleach sequence. The crude xylanase was produced in shake-culture using corn cobs and yeast extract by a thermophilic fungus, *T. lanuginosus* strain SSBP, isolated from South African soil by Singh *et al.* (1995). This fungal strain had been previously shown to produce high cellulase-free xylanase with high activity of 9 600 $U.ml^{-1}$ (Singh *et al.,* 1995). Xylanase activity determined obtained in this study was much lower at 1 600 $U.ml^{-1}$ using oat spelt xylan as the substrate. This was however, comparable to the activity obtained in a recent study by Bissoon *et al.* (1998) which was 1565 $U.ml^{-1}$ under the same cultivation conditions.

Crude *T. lanuginosus* SSBP xylanase was characterised to have its pH and temperature optima at 6.5 and 70°C which correlated with previous report of Singh *et al.* (1998) who also reported that this enzyme was stable at wide pH range of 5.0 - 9.5 and at temperature of 70°C which gives it great potential to industrial application. Another important characteristic of this enzyme is its freeness in cellulase activity which can have serious economic implications as it can lead to cellulose lose, degraded pulp quality and increased effluent treatment cost (Bajpai and Bajpai, 1997). Although Christov and Prior (1997) and Bissoon *et al.* (1998) detected some cellulase activity in *T. lanuginosus* SSBP culture filtrates these activities, 0.1 and 0.08 $U.ml^{-1}$, were too low to cause any significant loss in pulp viscosity. The commercial

enzyme, Xylanase P, was supplied in a concentrated form by Iogen Corporation (Ontario, Canada) which explains the high activity of 84 620 $U.ml^{-1}$ detected. Optimum pH and temperature for Xylanase P was 5.0 and 60°C, respectively.

Xylanase P has not been applied to paper pulps in South Africa previously whereas *T. lanuginosus* SSBP xylanase had been extensively studied on the bleaching of bagasse pulp (Bissoon *et al.,* 1998). Crude xylanase increased brightness of bagasse pulp by 3.5% compared to 4.5% gained with purified enzyme (Bissoon *et al.,* 1998). When applied on the three pulp types used in this study, both xylanases were able to hydrolyse pulp hemicellulose as indicated by the release of chromophoric material and reducing sugars from pulps which increased with an increase in enzyme dose and treatment time. Enzyme treatment of pulps, using both xylanase preparations, proved to be most effective at the highest enzyme dose used of 10 $U.g^{-1}$ dry weight pulp after 3 h. Unbleached bagasse pulp was found to be the most sensitive to xylanase treatment, as indicated by the high concentrations of chromophores and reducing sugars which were released after enzyme treatment compared to the release of these hydrolysis products from post oxygen soda and post oxygen kraft pulps. This could indicate that the fibre bound xylan in unbleached was more susceptible to enzymatic attack as suggested by Christov *et al.* (1997). Although the enzyme treatment proved to be most effective at an enzyme dose of 10 $U.g^{-1}$ dry weight pulp with both xylanase preparations, significant results were also obtained with the lower enzyme doses of 1 and 5 $U.g^{-1}$ dry weight pulp doses used in this study suggesting that these doses could also be used for the efficient removal xylan from pulp prior to chemical bleaching.

There are many theories on how the enzymes improve bleachability of pulp and one of them is that xylanases may act by partially hydrolysing xylan facilitating extraction of lignin in high amounts with higher molecular weights from the fibres (Buchert *et al.*, 1993). However, for effective solubilisation of xylans in pulp the substrate must be accessible to xylanases. Reduction in Kappa number, which was used as an indicator for the removal of residual lignin from the pulp, without alkaline extraction was observed on all three pulp types tested. The crude xylanase preparation was most effective in reducing Kappa number of post-oxygen soda pulp by up to 18% whereas the commercial xylanase preparation was mostly effective in reducing Kappa number of bagasse pulp by up to 21%. Brightness analyses of pulps after enzyme treatment showed that both xylanase preparations were more effective in biobleaching of bagasse pulp than the oxygen pre-bleached pulps. Significant increases in brightness of bagasse pulp after enzyme treatment, using a crude xylanase preparation of *T. lanuginosus* SSBP, without alkaline extraction were also obtained in a study by Bissoon *et al.* (1998). These reductions in Kappa number could suggest that application of xylanase as a pretreatment step could have a positive effect in improving pulp brightness after subsequent chemical bleaching.

This was confirmed by the brightness increases observed in xylanase-pretreated pulps, after using a conventional bleach sequence, D_1ED_2, compared to brightness of pulp samples that did not undergo enzyme treatment. Brightness of bagasse pulp and post-oxygen kraft pulp, pretreated with the crude xylanase, gained more than 4 brightness points over their respective controls. The commercial xylanase preparation improved brightness of bagasse pulp and post-oxygen kraft pulp by up to

more than 5 brightness points over their respective controls which was up to more than 1 brightness point better than that achieved with the crude xylanase preparation. Brightness points gained by bagasse pulp, after enzyme treatment only with both xylanase preparations, were more than those gained by the post-oxygen kraft pulp. However, after final chemical bleaching, brightness increase was the same as or more than that of bagasse pulp with the crude and the commercial xylanase preparation, respectively, and this could suggest that the post-oxygen kraft pulp is more dependent on chemical bleaching. Brightness of post-oxygen soda pulp was the least affected of the three pulps by the enzyme treatment, with each of the xylanase preparation tested, only achieving less than two brightness point gain after final chemical bleaching. The light colour of post-oxygen soda pulp, compared to that of bagasse and post-oxygen pulps which is more brownish, could be the contributing factor which could suggest that the enzyme did not have enough substrate to act upon. This could be confirmed by the amount of chromophores and reducing sugars released by post-oxygen soda pulp which was less than that released by the other two pulps which could indicate that most of the xylan from this pulp type was probably removed by oxygen delignification.

In combination with the DED-bleach sequence, xylanase pretreatment using crude xylanase reduced viscosity of bagasse pulp by a maximum of 10% with that of the oxygen-delignified pulps also reduced but to a lesser extent than bagasse pulp. Xylanase P. Kantelinen *et al.* (1993) cited some studies of cellulase-free xylanases which increased viscosity of kraft pulps and this could suggest that Xylanase P when used in pulp bleaching has the potential of improving other pulp and paper strength

properties. improved viscosity of all three pulp types, after chemical bleaching. Both enzyme preparations had the same effect on the carbohydrate concentration of all three pulps tested showing a general increase in glucose content and a general decrease in xylose content. The commercial xylanase was, however, more effective than the crude xylanase in increasing and decreasing concentrations of glucose and xylose, respectively. Chemical analyses of pulps showed that glucose and xylose were the main sugar constituents of all fully bleached pulp types, whereas mannose was also present in significant amounts in fully bleached post-oxygen kraft pulp. This indicated that the post-oxygen kraft pulp was produced from softwood in which glucomannan represents major part of the hemicellulose (Sjöström, 1993). This could suggest that mannanases could also be used for efficient hydrolysis of post-oxygen kraft pulp hemicellulose and this could be one reason why the brightness of this pulp type was less affected by xylanase treatment alone but increased significantly after chemical bleaching.

The main goal of enzyme application in pulp bleaching at industrial scale was to reduce the consumption of chlorine chemicals but enzymes have also been used for increasing pulp brightness (Viikari *et al.,* 1994). When *T. lanuginosus* SSBP xylanase and Xylanase P were tested for their potential in reducing chlorine dioxide consumption in a modern DED-bleach sequence, they led to savings of chlorine dioxide to a maximum of 17% and 20%, respectively, without affecting the final brightness. These results suggested that application of crude *T. lanuginosus* SSBP xylanase or Xylanase P as a pretreatment step in pulp bleaching could lead to reduction chlorine dioxide in the range 2.5-3 kg/ton of pulp in a DED-bleach

sequence. According to Ruiz *et al.* (1999b), the approximate price of xylanase in 1996 was US $ 2 per ton of pulp. A calculation of relative economic benefits in an elemental chlorine free process (ECF) sequence reveals that the reduction of 5 kg ClO_2/ton of pulp leads to savings of about US $ 2 per ton of pulp in ClO_2 cost alone.

In conclusion, this study has demonstrated that:

(i) Xylanase application as a pretreatment step in bleaching of pulp could enhance the bleaching process;

(ii) Crude xylanase of *T. lanuginosus* SSBP, although less effective in biobleaching of pulp compared to Xylanase P, also enhanced pulp bleaching significantly as demonstrated by substantial increases in pulp brightness obtained;

(iii) Both xylanase preparations used in this study proved to have the potential of reducing consumption of chlorine dioxide in a conventional bleach sequence which could in turn lead to cost savings of the bleaching process and environmentally safe effluents, or even possible total effluent free (TEF) pulps.

One important characteristic about the *T. lanuginosus* SSBP xylanase is that it is stable over a wide pH range of 5.0 – 9.5 and at 70°C retaining 100% activity for 24 h (Singh *et al.,* 1995). In an industrial setting the temperature of incoming pulp is around 70°C with a pH in the alkaline range. *T. lanuginosus* SSBP xylanase could therefore be able to cope with these conditions and could probably be suitable for industrial application. The major problem with Xylanase P, like most commercial

enzymes, is that it is most active at slightly acidic pH of 5.0 and at lower temperature of 60°C. This will require adjustment of pulp pH and if the pulp temperature is too high it might not be effective at industrial scale as it is at laboratory scale. Depending on the price of the competing bleach chemical and costs involved in enzyme production and bleaching process one could assess if using enzymes in pulp bleaching will be economically feasible.

5.2 FUTURE RESEARCH

The encouraging results obtained in this study led to the proposal of the following aspects for future work in the pulp and paper biotechnology field in order to elucidate better understanding of the role of xylanases in enhancing bleachability of paper pulps and in turn reducing bleaching chemicals and the subsequent production of chlorinated organic compounds leading to environmentally safe effluents:

(i) Compare biobleaching effect of crude *T. lanuginosus* SSBP xylanase, and Xylanase P to that of a purified xylanase preparation;

(ii) Optimise enzyme treatment conditions of pulp and test the other pulp properties such as opacity, burst index and tensile index;

(iii) Investigate any synergistic effect between the xylanase and other hemicellulolytic accessory enzymes to enhance xylanase efficiency;

(iv) Test potential of crude *T. lanuginosus* SSBP xylanase and Xylanase P in reducing bleaching chemicals used in new and developing bleach sequences; and

(v) Develop bleaching technology towards increasing pH and temperature stability of xylanases.

Xylanases have been applied at industrial scale by several mills in countries like Canada and Finland and resulted in the enhancement of bleaching and decreasing bleaching costs in ECF processes (Bajpai and Bajpai, 1997; Tolan and Thibault, 1997; Yee and Tolan, 1997). In South Africa the technology of xylanase application in pulp bleaching is still at laboratory scale with encouraging results, such as the ones obtained in this study and it is hoped that mill scale trials will resume by 2001.

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