APPLICATION OF AMYLASES FOR THE IMPROVEMENT OF WATER DRAINAGE FROM RECYCLED PULP FIBRE

By

Hendrikus Jansen van Vuuren

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In the Faculty of Natural and Agricultural Sciences,
Department of Microbial, Biochemical and Food Biotechnology,
University of the Free State,
Bloemfontein

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Supervisor: Dr J.F. Wolfaardt

The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

Sir William Bragg (1862 - 1942)

Dedicated to my farther, Leon Jansen van Vuuren.

CONTENTS

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PREFACE

Different grades of recycled fibre

Researchers, as early as 1959, became interested in enzymes for application in the production of paper (Kirk and Jeffries, 1996). Currently the demands from the environmental groups and the public in general are that the pulp and paper industry produce more environmentally-friendly products by implementing more benign processes and using sustainable fibre resources (Thies and Kaiser, 2000). Consequently, the use of recycled fibre has increased worldwide and papermaking processes using recycled fibre has improved (Jewitt, 2001).

One of the major challenges when using recycled fibre is the optimisation of the drainage rate. Drainage rate influences machine speeds, production rates, energy demands in the drying section and water consumption of the paper mill (Bhat, 2000). Fines, that are especially abundant in recycled fibre, are one of the major contributing factors to decreased drainage rates of pulp (Egyházi *et al.,* 2001), but residual starch also contributes to the lowered drainage rates (Lascaris *et al.,* 1997a). Starch is used as a binding agent, surface treatment and coating application (Erceg, 1984). The residual starch contained in the recycled fibre is dispersed during repulping and it forms an amorphous surface coating on the paper. This layer can be removed using amylases that hydrolyse starch without affecting the cellulose fibres, thereby increasing the drainage (Lascaris *et al.*, 1997b).

Lascaris *et al.* (1997a) reported improved drainage through the application of amylases. A commercial enzyme was applied at a dosage of 2,25 L/tonne and production

was increased by 19 tonne/day with an increased dry end speed of 22 m/min. The aim of this study was, therefore, to evaluate the effectiveness of different commercial amylases. The amylases were applied to recycled pulp obtained from Sappi Cape Kraft, Milnerton, South Africa, to remove residual starch and thereby improve drainage on the paper machine.

The first step was to evaluate the efficiency of the amylases to hydrolyse secondary starch contained in the pulp. Initially only BAN 480L, Fungamyl 800L, Termamyl 120L and AMG 300L were available. Enzymes SA donated all these enzymes produced by Novozymes (Denmark). Later, during the study, a relatively new enzyme called Duramyl 300L became available and was also included in further work. The activity of these enzymes were determined according to a Novozymes method. Factors that might influence enzymatic activity under mill conditions had to be evaluated and the influences of pulp grade, pulp consistency, shear forces, temperature and pH was evaluated for the enzymes.

Handsheets were made to determine the effect of enzyme treatment on the strength properties of pulp. Handsheets were tested for Bursting Index, Tearing Index, Air Permeance and Handsheets Drainage Time. In an attempt to quantify the drainage improvements on a laboratory scale, Canadian Standard Freeness, Drainage Time, Vacuum Drainage Time and the Water Retention Value were determined, but none of the drainage tests showed significant improvement. It was demonstrated that the enzymes

degraded the secondary starch. The drainage on a pilot scale paper machine was, therefore, evaluated.

K3 and K4 pulp was initially used for the pilot trials, but after the first pilot trial only K3 pulp was used due to the high cost of pilot trials. Pulp was treated at low and high consistency and paper and backwater samples were collected and evaluated for changes in moisture, starch content and Chemical Oxygen Demand (COD). It was evident that pulp treatment with amylase before papermaking could be beneficial for the papermaker, but mill scale evaluation was required to evaluate the enzymes under the specific mill conditions.

Duramyl 300L was chosen for the mill trial at Sappi Cape Kraft, due to it's effective secondary starch breakdown, it's temperature, pH and shear tolerances and efficiency during the pilot trials. Temperature, pH, starch content, COD, Total Dissolved Solids (TDS), and moisture content were measured at a number of sampling points. Furthermore the machine speed, steam consumption in the drying section and moisture and strength properties of the jumbo roll were recorded.

The mill trial was successful to the extent that further mill trials have been approved.

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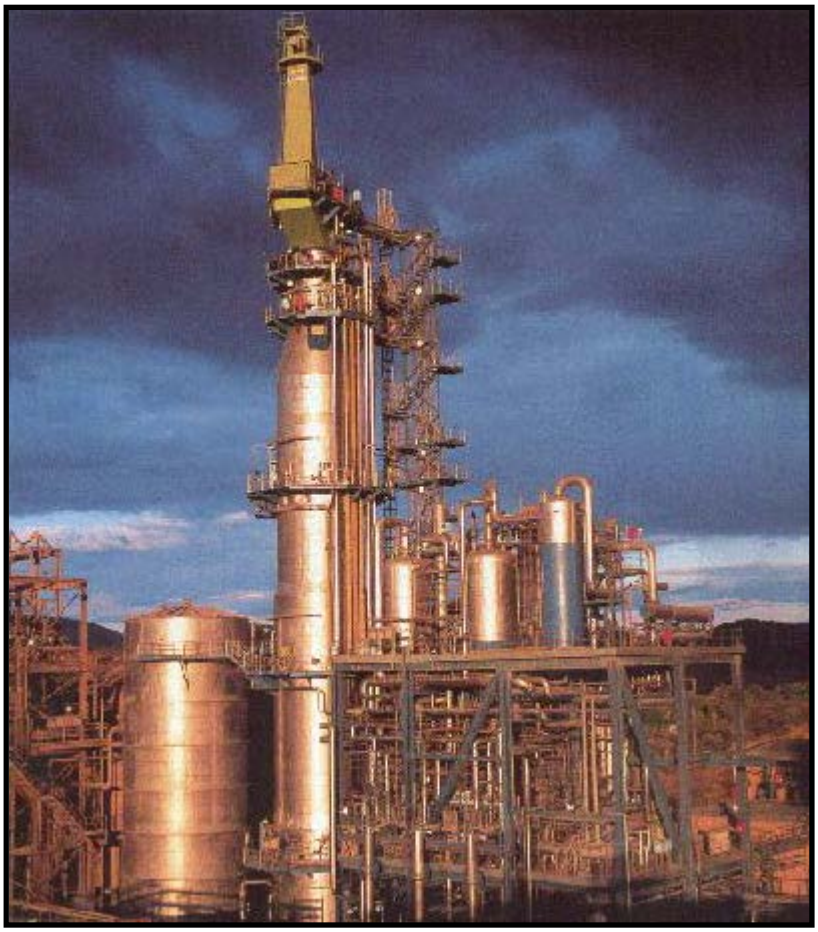
CHAPTER 1

APPLICATIONS OF ENZYMES TO IMPROVE FIBRE FOR PAPERMAKING

A LITERATURE OVERVIEW



Continuous Kraft Digester at Sappi Usutu

ABSTRACT

There is an increased interest in enzymes to improve processes in the pulp and paper industry, due to the specificity of enzymes, the low enzyme dosages required, the suitability of these proteins to different physical conditions and the constant pressure from public and legislation. The focus of this review is on the biotechnological applications of enzymes to improve fibre quality. These applications include biobleaching, depitching, fibre bonding, shive removal, fibrillation, deinking and improvement of drainage. A number of reviews on biobleaching have been written, therefore, this review only touches on the subject. Biobleaching technology is applied on commercial scale and generally makes use of xylanase. Pitch causes a number of problems such as paper breakage, deposits on papers, downtime of paper machines for cleaning and holes in paper, but the pitch can be reduced with the application of lipases. Synthetic adhesives used for the binding of fibreboard can, to some extent, be replaced by lignin-based binding agents produced through application of laccases. These laccases can also be applied directly to improve inter-fibre bonding. Shives lead to reduced strength and breaks during paper production but xylanases have been used effectively for shive reduction during bleaching. Strength of paper depends on inter-fibre bonding and good fibrillation, which will enhance this inter-fibre bonding. Enzymes such as cellulase and xylanase can be used to aid fibrillation to increase the strength of the formed paper. The increase in the use of recycled fibre for papermaking has lead to the successful application of enzymes to improve the deinking processes on an industrial scale. Enzymes used for this process include cellulases that attack surface cellulose fibrils, thereby releasing the ink particle for removal during floatation. Other enzymes used for deinking include xylanases, amylases and lipases. Drainage rate is of paramount importance to the

papermaker because it influences machine speeds, production rates, energy demands of the drying section and water usage of the paper mill. The major contributing factors that reduce drainage rate are fines and secondary starch that are more abundant in recycled paper. Fines can be reduced with cellulases but the process needs to be carefully controlled to prevent strength and yield losses. The secondary starch can be removed with the application of amylases that will not affect the cellulose content of the paper. Challenges that still need to be addressed are to produce enzymes that will function cost effectively and have no adverse effect on the product or production system. Industry should work closely with researchers and be willing to evaluate and apply new enzymatic technologies.

INTRODUCTION

The use of enzymes in the pulp and paper industry is still relatively new when compared to other industries but research and development has increased over the last few decades. The increased focus on enzymes to improve processes in the industry is due to the specificity of enzymes, the low enzyme dosages needed to produce results, the suitability of the proteins to different pH values and temperatures (Takano *et al.,* 1995; Viikari *et al.,* 1994) and the constant pressure from consumers and environmental groups (Sinner and Preselmayr, 1992; Thies and Kaiser, 2000). According to Kirk and Jeffries (1996) the first enzymatic application for pulp and paper was pulp fibrillation by cellulases developed in 1959 by Bolaski and Gallatin (Table 1.1). Many other enzymatic processes such as deinking, bleaching, depitching, drainage improvement and starch modification have been developed since then (Table 1.1).

Table 1.1. Major developments in enzymatic use in the pulp and paper industry. Adapted from Kirk and Jeffries, 1996

Application	**References**
Fibrillation by Cellulase	Bolaski and Gallatin (1959)
Beating with Xylanase	Comtat *et al.* (1984)
Hemicellulose removal with xylanases	Paice and Jurasek (1984)
Prebleaching with xylanases	Viikari *et al.,* (1986)
Improved drainage with cellulase	Fuentes and Robert (1988)
Decrease vessel picking by cellulase	Uchimoto *et al.,* (1988)
Depitching pulp with lipases	Irie *et al.,* (1989)
Deinking with cellulase and xylanase	Kim *et al.,* (1991)
Pulp delignification with laccase	Call and Mucke (1993)
Bleaching with manganese peroxidase	Harazono *et al.,* (1996)

This review will focus on biotechnological applications of enzymes to improve fibre quality. This subject of biobleaching has, however, been thoroughly

reviewed by numerous authors (Tolan *et al.*, 1996; Viikari *et al.*, 1994; Viikari *et al.*, 1993) and discussion of biobleaching applications will, therefore, be limited.

BIOBLEACHING

Some of the unwanted side effects of the bleaching process are unpleasant smelling sulphur compounds and effluent that can enhance eutrophication or contain toxic compounds formed when the lignin reacts with the chlorine during bleaching to form organic chlorine compounds (Sinner and Preselmayr, 1992; Takano *et al.*, 1995; Viikari *et al.*, 1994).

The application of hemicellulases in biobleaching has been commercialised and is applied on many types of pulp and in a variety of bleaching sequences (Tolan *et al.*, 1996). When 42 Canadian pulp mills were evaluated, 18 mills ran xylanase trials and six were regular users of xylanase pre-treatment technology on at least 20 % of the produced pulp. Benefits recorded were an average saving of 11 % in total chemicals across the bleach plant and improved effluent that included decreases of between 12 % and 25 % in AOX and decreases in the effluent colour. Further advantages were an average increase of 1 % in brightness, 5 % increase in tear strength and 10 % increase in pulp throughput (Tolan *et al.*, 1996). Bissoon *et al.* (2002) found that xylanase pre-treatment of bagasse pulp yielded an increase in brightness of up to 2,2 percentage points and with a 30 % lowering in chlorine dosage, a brightness increase of 0,9 percentage points was achieved.

The application of laccase for prebleaching has been expanded by the introduction of laccase-mediator systems, where the mediator extends the substrate range to include non-phenolic compounds (Bourbonnais and Paice, 1996). Increases of up to 6,4 brightness points have been reported but the efficiency of the laccase mediator system in biobleaching is dependant on the choice of mediator and the type of pulp (Kandioller and Christov, 2002).

DEINKING

There is an increase in the use of recycled fibre in the paper-making process (Jewitt, 2001b). In 2001, 36,8 % of the fibre used at paper mills in the United States was recovered while 36,3 % of the fibre used was recycled in 2000 (Anon, 2002). This increase demonstrates the paper industry's continued dependence on recovered fibre as a raw material to manufacture tissue, copy paper, newsprint, boxboard, corrugated containers, and other products. Consequently, there is a growing need for deinking efficiency and the deinking industry is expanding at a tremendous rate. According to Jewitt (2001a), a number of new deinking plants were built since 2000 and many existing plants were expanded. In Asia, China’s Nanpeng paper installed a new deinking line with a capacity of 500 tons per day. In Japan, Oji Paper increased the production of an existing deinking line to 1700 tons per day. In the United States, Alliance Forest Products expanded their deinking line in Alabama to triple their output to 1500 tons per day and in Oregon a new 800 tons per day deinking plant opened recently. Latin America saw an increase in deinking plants and a new deinking plant opened in Mexico with a production facility of up to 250 tons per day.

Deinking is carried out by repulping the fibre and diluting the pulp to a consistency of approximately 1 %. After dilution the pulp is aerated and flocculants, surfactants and ink solvents are added. The ink particles float to the surface to be gathered and removed (Tolan, 1996). Unfortunately, very little new technology has entered into the deinking process during the last ten years (Jewitt, 2001a) and the development of enzymes to enhance the deinking process has been relatively slow. Consequently, very few mill-trial/application results have been published (Grant, 1998).

The use of Mixed Office Waste (MOW) as a source for secondary fibre has been increasing and, therefore, it has become necessary to develop new technologies to make this pulp grade more acceptable for the manufacturers of high brightness printing paper (Lopez *et al.,* 2001). Laser and xerographic toners are difficult to remove by conventional deinking and a proposed alternative technology would be enzyme assisted deinking. The pH is one of the major factors to take into account when working with enzymes (Elegir *et al.,* 2000). Deinking is mostly done under alkaline conditions due to the presence of calcium carbonate in many of the recycled papers and an enzyme with optimal activity in the alkaline pH range must, therefore, be selected (Jobbins and Franks, 1997).

Cellulases attack the fine surface cellulose fibrils, thereby releasing the ink particle so that it can be removed during floatation while lipases assist to hydrolyse soy-based ink carriers (Bhat, 2000). Viesturs *et al.* (2001) evaluated the effect of cellulase in alkaline deinking and found an increase of 6,6 ISO brightness units when compared to a control sample. Mixed office waste was also treated with lipase and

yielded similar results. Jobbins and Franks (1997) studied deinking on laboratory and pilot scale at neutral pH and used a combination of surfactants and cellulases. The benefits of this treatment included improved efficiency of dirt removal, increased brightness, reduced Chemical Oxygen Demand (COD), enhanced physical properties, increased ash removal, reduced chemical costs and the use of lower grade recycled paper. Elegir *et al.* (2000) used a cellulase-amylase mixture for improved deinking and found that by adding small amounts of amylase to the cellulase mixture the removal of small ink particles improved. The cellulase and amylase also seemed to have a synergistic effect.

Marques *et al.* (2001) compared deinking with xylanase and cellulase assisted deinking and observed an 8 % increase in deinking efficiency by xylanase, while the cellulase treatment gave an increase of 24 %. In another study, where only amylase was used, it was observed that the disintegration process was more efficient and that the deinking was more effective (Lopez *et al.*, 2001). To enhance their deinking process Zollner and Schroeder (1998) also used an amylase treatment to increase particle removal by between 20 and 35 % and it also lowered the Biological Oxygen Demand (BOD) in the wastewater stream.

The breakthrough technology that industry needs could very well be enzyme assisted deinking. There is, however, still a great need for more research into the subject and especially deinking-plant trials should be done to evaluate the efficiency on an industrial scale. Unfortunately, plant trials are very rare due to the slow acceptance of new technology. The education of deinking-plant management should therefore be a priority (Jewitt, 2001a).

PITCH CONTROL

Pitch causes a large number of problems for the papermaker; including paper breakage, deposits on papers, downtime of paper machines due to cleaning and holes in paper (Kirk, 1996). Pitch consists of wood derived hydrophobic compounds and are usually triglycerides that can be hydrolysed by lipases to form glycerol and free fatty acids (Figure 1.1) (Chen *et al.*, 2001; Mathews and Van Holde, 1990; Kirk, 1996). Traditional control of pitch is done by careful selection of raw materials, ageing of logs, proper water clarification, effective log cleaning practices, polymer-based pitch control programs and the addition of alum and talc (Chen *et al.*, 2001; Fitzhenry *et al.*, 2000).

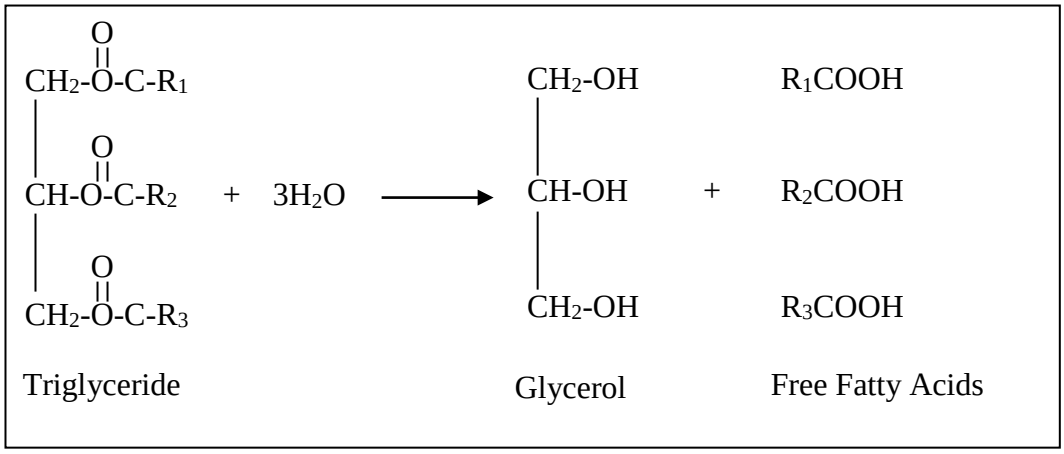


Figure 1.1. Enzymatic hydrolysis of triglycerides by Lipase

Enzymatic pitch control was developed in Japan by the Jujo Paper Co. in 1989 and today it is commercially implemented in Japan (Chen *et al.*, 2001) as well as some mills in North America (Kirk, 1996; Kirk and Jeffries, 1996). A recent success story of enzymatic pitch control is that of the Nanpeng Paper Mill in Southeast China. In December 1999 the mill started the world's fastest newsprint paper machine of the

time with a design speed of 1800 m/min or 180 000 tons per year. They experienced serious problems with pitch and the mill had to shut down on almost a daily basis for up to two hours for cleaning. Traditional pitch control methods were not able to neutralise as much as 3 % pitch per weight of pulp. Ageing of the logs for up to three months not only increased costs, but also decreased brightness, lowered paper strength, decreased pulp yield and produced an odour problem. The mill relied on adding 50 to 57 kg/ton alum and talc to the stock preparation stage in an attempt to control the pitch (Chen *et al.,* 2001).

A commercial enzymatic pitch control system was tested and produced very good results. Within only a few days the pitch deposits decreased dramatically, shutdown frequency went from 7 to 10 times per week to once a week and the machine speed was increased from 1100 m/min to 1350 m/min. After six months the machine shutdown frequency was down to once every ten days, machine speed was up to 1500 m/min and the pulp brightness increased with between 3 to 5 % ISO because the mill could use fresh logs without ageing them first (Chen *et al.,* 2001).

This example of a successful enzymatic pitch control system could greatly assist other mills that experience similar problems. Mill trials should be done to evaluate the success of different enzyme programs for the different mills to suit their individual needs.

DRAINAGE

Drainage rate is of paramount importance to the papermaker because it influences machine speeds, production rates, energy demands of the drying section and water usage of the paper mill. The amount of fines is one of the major contributing factors to decreased drainage rates of pulp (Egyházi *et al.*, 2001). The concentration of fines is especially high in recycled fibre, which is used more frequently throughout the world for paper and paperboard production (Egyházi *et al.*, 2001; Menghua *et al.*, 2001; Pommier *et al.*, 1990; Rutledge-Cropsey *et al.*, 1998; Sarkar, 1997). Fines contribute to the mechanical strength of paper, but high concentrations of fines lead to a decreased drainage rate and a decreased capacity of the paper machine (Egyházi *et al.*, 2001).

In an attempt to increase drainage with enzymes, a lot of research has gone into the use of cellulase, because fines consist mostly of cellulose. The use of cellulases and hemicellulases to degrade fines improves drainability and runnability of paper mills and increases the drainage rate (Bhat, 2000; Viikari *et al.*, 1993). A commercially available blend of hemicellulase and cellulase (Pergalase A40) has been designed to improve the drainage and beatability of paper pulps. Scartazzini (1995) reported an improvement in freeness from 125 ml to 156 ml after pulp treatment with this blend while Sarkar (1997) reported freeness improvements from 305 ml to 425 ml resulting in a production increase of 10 % in mill trials. In a different mill trial production increases of up to 19,4 ton/day were observed due to drainage improvements after Pergalase A40 treatment (Sarkar, 1997). The addition of a higher percentage of recycled fibre to the paper making process had no negative effect on

drainage rate when enzyme was used (Sarkar, 1997). Rutledge-Cropsey *et al.* (1998) also used commercial cellulase to treat pulp and found that the enzyme enhanced the drainage and decreased the vacuum requirements.

There are, however, contrasting theories in literature about the influence of cellulase treatment on the strength properties of the paper. Some authors such as Kim *et al.* (2001), Rutledge-Cropsey *et al.* (1998) and Scartazzini, (1995) reported increased strength properties of the paper, while others such as Jackson *et al.* (1993) report little or no change. Still others reported a decrease in the strength properties when higher enzyme dosages were used (Pala *et al.,* 2001; Sarkar, 1997). The use of cellulases could easily have a negative influence on the paper strength properties when the enzymatic hydrolysis of cellulose is not under very strict control. It can be expected that, when the enzymes have a prolonged exposure to the pulp, the enzymes will cause excessive fibrillation thus decreasing drainage rate and affecting strength (Bhardwaj, *et al.,* 1995). This problem could be especially severe in a mill with a closed water system (Bhardwaj, *et al.,* 1995).

Starch is another factor contributing to lowered drainage rates. Starch is used in the paper and board making process as a binding agent, surface treatment and coating application (Erceg, 1984). The secondary starch contained in the recycled fibre is dispersed during repulping and forms an amorphous surface coating on the fibre that can be removed using amylases (Lascaris *et al* 1997b). Lascaris *et al.* (1997a) reported that drainage could be improved by using amylases. The enzyme hydrolyses starch without affecting the cellulose fibres, thereby increasing pulp drainage (Lascaris *et al.,* 1997b). Lascaris *et al*. (1997b) used a

commercially available enzyme at a dosage of 2,25 L/ton and the results showed an increase in production of 19 ton/day, decreased Shopper-Riegler drainage values of top and bottom headboxes by 20 to 30 units, and an increased dry-end speed of 22 m/min.

Further advantages of amylases are deinking of some starch-based inks, and decreasing the dispersing power of the starch present in wastewater (Erceg, 1984). By decreasing the dispersion power, increased solids flocculation was achieved in the clarifier water, thereby increasing suspended solids removal and reducing the turbidity of the clarifier effluent (Erceg, 1984).

In my opinion, amylases are especially suited for the pulp and paper industry due to their specificity to starch with no cellulytic activity and wide pH and temperature-tolerance ranges (Anon, 1988; 1991; 1999; 2001). This means that the application of the enzyme could be done in various stages of the production line without negative effects on the process. Many other industries use amylase to modify starch, and this makes the enzyme easily available, “industry ready” and relatively inexpensive. This biotechnology could be the preferred way to increase drainage of recycled fibre without affecting the strength properties of the product.

FIBRE ADHESION

The conventional method of board making entails the use of some type of synthetic adhesive such as urea-formaldehyde and phenol-formaldehyde in combination with hot pressing (Felby *et al.,* 1997). Due to constant pressure from customers and growing concerns of environmental impact, the ideal would be to produce fibreboard with good mechanical properties without the use of synthetic adhesives (Felby *et al.,* 1997). When conditions such as temperature, pressure and moisture are properly selected to produce an ideal situation, wood fibres will bond and this process is described as auto-adhesion or self-bonding (Felby *et al.,* 1997).

Enzymatic biotechnology can also be used to increase fibre adhesion during paper and board production. Lund and Felby (2001) proposed that laccase-oxidised lignin that underwent polymerisation may act as a wet strength agent in paper by encapsulation of the fibres in the sheet. Lignin-rich beech extractives were added to kraft pulp and this treatment increased the wet tensile strength when the mixture was treated with laccase. The documented improvements in the wet tensile strength after laccase treatment can in part be attributed to the coupling of phenoxy radicals on lignin associated to adjacent fibre surfaces. This causes cross-linking of the fibres and enhanced water resistance of the formed inter-fibre bonding (Lund and Felby, 2001). Even when conditions were carefully controlled, adhesion did not result in high strength properties without the addition of synthetic adhesives (Felby *et al.,* 1997). The auto-adhesion of wood fibres in fibreboard after laccase treatment was higher due to the laccase-catalysed oxidation of the wood fibres. The adhesive effect was not due to the organic matter content of the enzyme solution, but was only due to the catalytic

effect of the laccase and this increased the strength properties of the board (Felby *et al.,* 1997).

While working with kraft pulp, Wong *et al.* (1999) found that treatment with the laccase mediator system (Laccase/1-Hydroxybenzotriazole (HBT)) increased the density of handsheets and for some pulps the system increased the tensile strength at a given handsheet density. An alternative method to increase fibre adhesion is by modifying lignin with laccase to produce a natural adhesive for the manufacture of particleboard (Hüttermann *et al.,* 2001). The enzyme can also be used to activate the middle-lamella lignin of wood fibres for the production of wood composites (Hüttermann *et al.,* 2001). In both cases the produced fibreboard met the German standard for medium density fibreboards without using any synthetic adhesives. The fibres were bound in a similar way to that of naturally growing wood (Hüttermann *et al.,* 2001).

REDUCTION OF SHIVES

One of the most important quality criteria for bleached kraft pulp is the shive content (Gregersen *et al.,* 1999). Shives appear as splinters that are darker than the bleached pulp and consist of bundles of fibres that have not been separated during the pulping process and this could lead to reduced strength and breaks during paper production (Bajpai, 1999; Gregersen *et al.,* 1999). Shives have thick cell walls and cause a very high local basis weight that results in a total compression during calendaring. This compression will then produce local deformation that reduces

strength of the paper web around the shive with similar characteristics to a small cut in the paper (Gregersen *et al.*, 1999).

A novel commercial enzyme formulation based on xylanase (Shivex), can be used to increase the efficiency of shive reduction during bleaching (Bajpai and Bajpai, 2001). The amount of shives after bleaching was reduced by up to 55 % when the xylanase treatment was done on brownstock prior to bleaching. The enzyme treatment increased the bleaching efficiency and this could mean a possible reduction in chemical and energy consumption with a decrease in the shive content (Bajpai and Bajpai, 2001).

STRENGTH ENHANCEMENT

Strength of paper depends on inter-fibre bonding and good fibrillation will enhance this inter-fibre bonding (Kirk and Jeffries, 1996). Enzymes such as cellulase can be used to aid fibrillation to increase the strength of the formed paper, but the danger exists of the cellulase decreasing the viscosity of the pulp by cleaving the cellulose chains and lowering the degree of cellulose polymerisation (Kirk and Jeffries, 1996).

Rutledge-Cropsey *et al.* (1998) reported improved machine runnability due to increased wet-web strength after treatment with commercial cellulase. Higher tensile strength properties and compression strength values were found in paperboard made by Signal *et al.* (2001) after cellulase treatment. Cellulase treatment further produced

remarkable improvements in tensile, tear and burst when kraft pulp fibres were treated with cellulase before beating (Kim *et al.,* 2001).

To reduce the possibility of decreasing the viscosity of the pulp by cellulase enzymes, some papermakers used other cellulase free enzymes to increase the strength of the formed product. Tolan *et al.* (1996) reported two mills that have commercially implemented xylanase treatments in their papermaking programmes and they found increases in tear strength properties of up to 5 %.

Kondo *et al.* (1996) evaluated totally chlorine free bleaching (TCF) with the introduction of manganese peroxidase (MnP). They found that the physical properties were enhanced, when oxygen bleached kraft pulp was treated with a four-stage bleaching process consisting of sequential MnP treatment, alkaline extraction, MnP treatment and a hydrogen peroxide stage. The results showed higher values for the Burst Index and for the breaking length compared to chlorine bleached pulp. A further addition of polyacrylamide resulted in large improvements of the strength properties of the pulp (Kondo *et al.,* 1996).

CONCLUSIONS

The amount of research going into biotechnology for the pulp and paper industry using enzymes is increasing annually (Thies and Kaiser, 2000). The drive behind the research is not only due to environmental aspects and pressure from consumers, but the use of biotechnology is also driven by the possibility of increasing profits using these applications (Bajpai and Bajpai, 1999; Thies and Kaiser, 2000). Enzymes may sometimes be more expensive, when compared with conventional chemicals, but they are highly specific and, therefore, very small volumes are needed to perform the same tasks as chemicals (Viikari *et al*., 1994).

The academic research currently looks very promising, but mill implementations of enzymatic applications have been limited. Reasons for this could be the unwillingness of mills to become the guinea pigs for new technology. Arguments are often that a proven commercial application will be accepted for trials, but novel technologies could have some adverse effects on the operation of the whole paper machine and plant (Tolan *et al.,* 1996).

Some of the challenges that still need to be addressed by researchers are to produce enzymes that will act cost effectively and not have any adverse effect on the product properties or production systems. Challenges for the industry is to work closely with researchers, to accept and apply the new enzymatic technologies presented and to be willing to assist researchers with development and mill trials.

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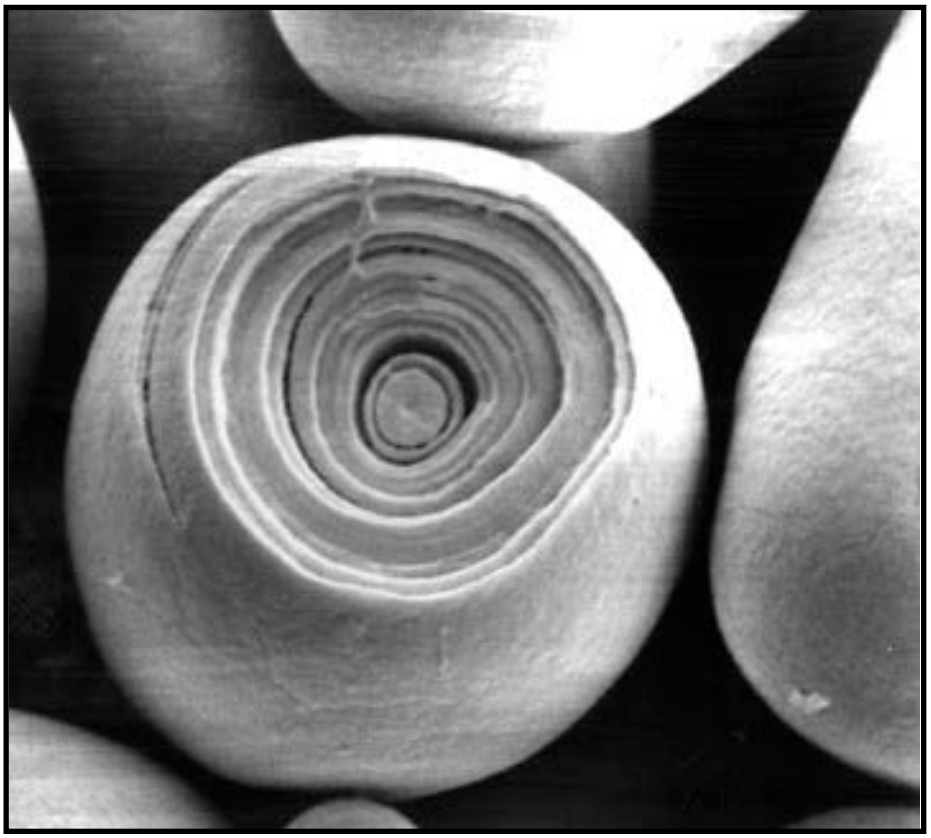
CHAPTER 2

EVALUATION OF COMMERCIAL AMYLASES FOR STARCH DEGRADTION IN RECYCLED PULP



Scanning electron micrograph showing starch globules
(http://mse.iastate.edu/images/microscopy)

ABSTRACT

Starch comprises two high molecular weight polysaccharides, amylose and amylopectin. The hydrolysis of starch is most efficiently accomplished by amylases that hydrolyse α-1,4-glycosidic bonds in amylose and amylopectin. The products formed are glucose, maltose, maltobiose, dextrins of different chain lengths and oligosaccarides. Starch contained in fibres from recycled boxboard cause lower drainage rates and reduced machine speed. The residual starch does not contribute to strength properties and can potentially be degraded by amylases to improve drainage. The aim of the study was to determine the influence of physical parameters on the ability of selected commercial enzymes to degrade starch on recycled pulp. The relative activity of BAN 480L, Duramyl 300L, Fungamyl 800L and Termamyl 120L on pulp at 40 °C, was determined with and without the addition of $CaCl_2$ and it was found that all except Termamyl 120L were sufficiently active. BAN 480L and Duramyl 300L displayed activity over a wide temperature range, while Duramyl 300L had activity over a wide pH range. BAN 480L, Duramyl 300L and Fungamyl 800L all showed good shear tolerance. It was concluded that Duramyl 300L was most suitable for commercial application.

INTRODUCTION

Starch is one of the most abundant plant polysaccharides and natural starch is insoluble in cold water. Starch is usually present as globules that may be lens-shaped or egg shaped and has a distinctive layered structure (Figure 2.1). Starch in its raw state varies in length from 1 to 100 μm and consists of alpha-D-glucose residues linked to form large macromolecules (Nigam and Singh, 1995). This carbohydrate comprises two high molecular weight polysaccharides namely, amylose and amylopectin (Figure 2.2). The amylose fraction comprising *ca.* 30 % of natural starch, consists of long unbranched chains of D-glucose units linked by α-1,4-glycosidic bonds. The amylopectin fraction comprises the remaining 70 % and is highly branched by linking through α-1,6-glycosidic bonds (Figure 2.3).

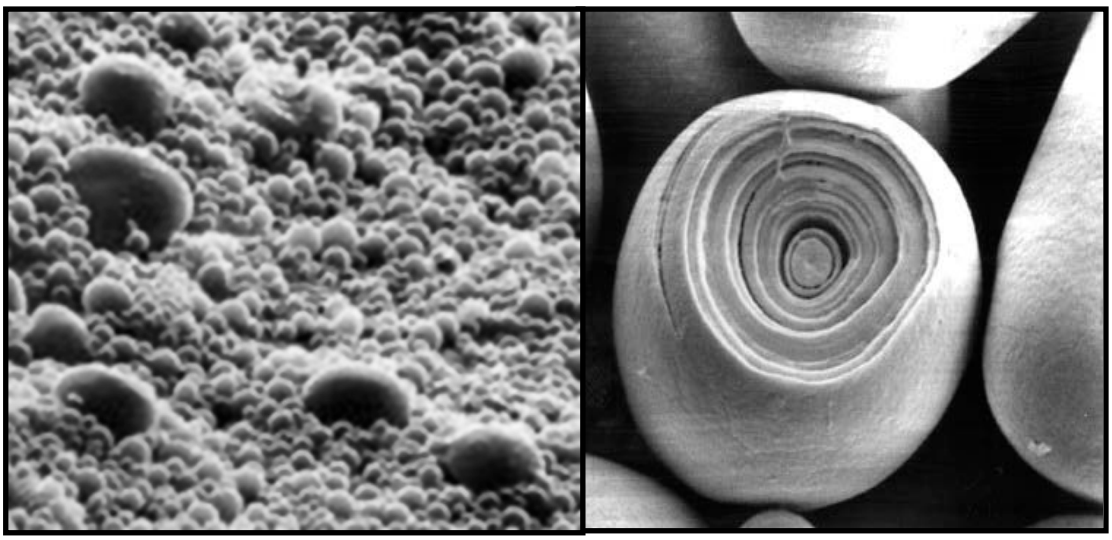
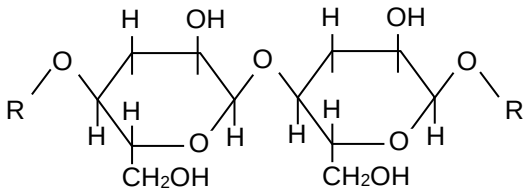


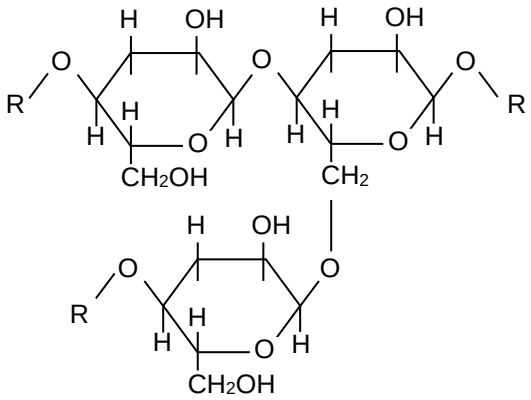
Figure 2.1. Scanning electron micrographs showing starch globules (http://www.fhsu.edu/biology and http://mse.iastate.edu/images/microscopy).

Amylose is a linear molecule consisting of 200 to 500 glucose units per chain that is soluble in hot water. When it is suspended in hot water a helix forms that produces a blue colour when it reacts with iodine, because the iodine halide occupies a position in the interior of the coil. The highly branched amylopectin reacts with iodine to form a violet-to-brown colour. Amylopectin is a poly-1,4-α-D-glucose, but, it is branched in the 1,6-position at approximately every 25th glucose moiety and has a molecular weight greater than 1 x 10^{8} g/mol, making it the largest molecule in nature. Starches from different sources differ considerably in their branching, number of units per chain and other properties (Nigam and Singh, 1995).



Amylose

Figure 2.2. The chemical structure of amylose. Adapted from Mathews & van Holde (1990).



Amylopectin

Figure 2.3. The chemical structure of amylopectin. Adapted from Mathews & van Holde (1990).

The hydrolysis of starch is accomplished in animals, plants and microorganisms by amylases that attack the starch molecule extracellularly. The

"endo"-amylase 1,4-α-D-glucan-hydrolases hydrolyse α-1,4-glycosidic bonds in amylose and amylopectin including those bonds at the centre of the molecule to produce water-soluble products (Figure 2.4). The "exo"-amylase 1,4-α-D-glucan-glucohydrolase removes a single glucose molecule at a time by hydrolysing the α-1,4 and α-1,6-glycosidic bonds in amylose and amylopectin from the non-reducing end of the polysaccharide and it has limited debranching activity. Due to the rapid hydrolysis of the macromolecular structure, the viscosity of the solution and its ability to react with iodine declines sharply. The products formed are glucose, maltose, maltobiose, oligomers (3 to 7 glucose subunits), dextrins (of different chain lengths) and oligosaccarides (Figure 2.5).

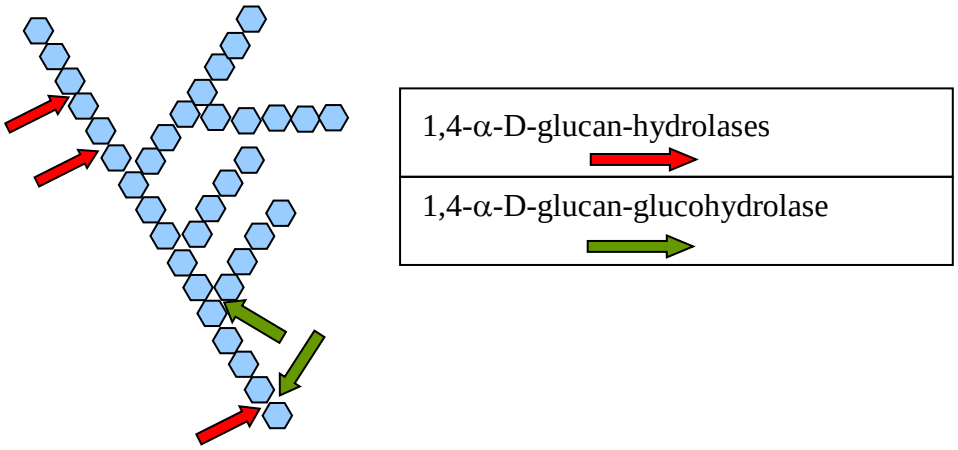


Figure 2.4. A diagrammatic representation of starch and the sites of action of different amylases

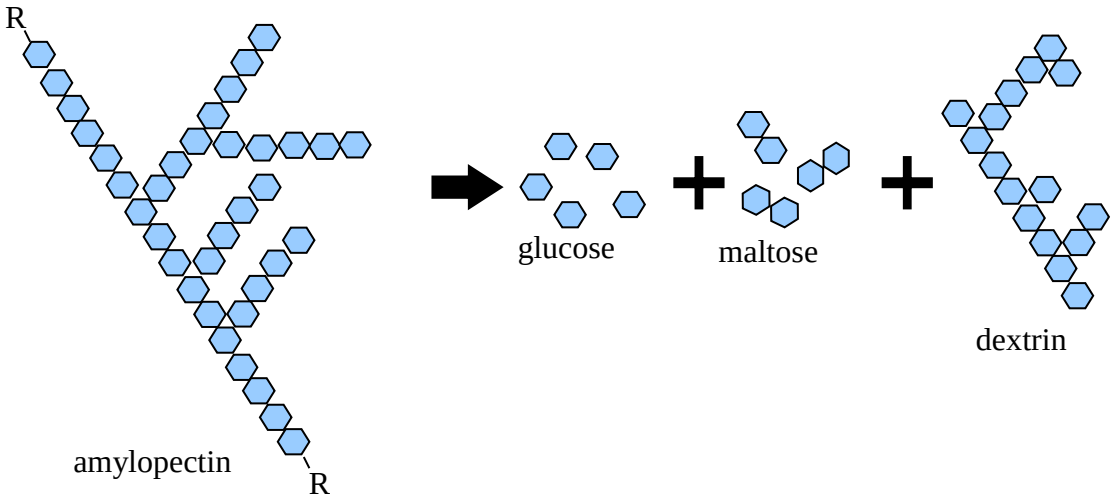


Figure 2.5. A diagrammatic representation of different hydrolysis products from amylopectin.

Starch is added to the fibreboard during production to increase strength and act as a sizing agent (Erceg, 1984). Fibres from recycled boxboard contains residual starch that cause lower drainage rates and reduced machine speed (Lascaris *et al.*, 1997). The residual starch does not contribute to desired board characteristics and can potentially be degraded by amylases to improve drainage. An increase in paper production of up to 6,8 % after amylase treatment was reported (Lascaris *et al.*, 1997). The aim of the study was, therefore, to evaluate the potential of selected commercial enzymes to degrade starch on two grades of recycled pulp under different physical parameters.

MATERIALS AND METHODS

1. Pulp

K3 and K4 pulp were provided by Sappi Cape Kraft. K3 pulp is made from new corrugated container off-cuts and do not contain more that 1 % contaminants such as plastic, cloth and metal and it is used for the production of linerboard. K4 pulp is made of recycled corrugated containers and kraft wrapping and it is used for the production of fluting. The pulp was supplied in noodle form at a consistency of between 24 and 30 %. One hundred grams of each type of pulp was air dried at 105 ± 2 °C overnight. The water content was then calculated gravimetrically on a wet basis. Only freshly repulped fibre was used and the consistency of the pulp was adjusted to low-consistency (1 to 5 %) or high-consistency (20 %) for enzymatic treatment. The mass of pulp used in the experiments, refers to the equivalent mass of bone-dry fibre.

2. Enzymes

Different commercial enzymes from Novozymes (Bagsvaerd, Denmark) were supplied by Enzymes SA (Johannesburg, South Africa). The following 1,4-α-D-glucan-hydrolases (EC 3.2.1.1) were selected based on their specificity towards starch, low cost, commercial availability and wide ranging applications in other industries: BAN 480L, Fungamyl 800L, Termamyl 120L Type S and Duramyl 300L.

BAN 480L is produced from *Bacillus amyloliquefaciens* (Anon., 1985) and Fungamyl 800L from *Aspergillus oryzae* (Anon., 1994). Duramyl 300L is a protein-

engineered amylase, from a genetically modified *Bacillus* sp. (Anon., 2001) while Termamyl 120L is produced from *Bacillus stearothermophilus* (Anon., 1999) and AMG 300L from *Aspergillus niger* (Anon., 1997).

3. Enzyme activity

The activities of BAN 480L, Duramyl 300L, Fungamyl 800L and Termamyl 120L were determined using the Novo Nordisk standard analytical method (Anon., 1978). The reaction temperature for the first three enzymes was 37 °C and for the latter 75 °C. The experiment was done for 20 min at a pH of approximately 6. The method is based on the hydrolysis of starch and the inability of iodine to form a coloured starch-iodine complex with the products of enzymatic hydrolysis. The decrease in the blue-to-purple complex formation was measured spectrophotometrically (Phoenix-2000 UV-VIS Spectrophotometer, Biotech Engineering Management Co., Ltd Nicosia, Cyprus) at 660 nm. The activities of all these enzymes were also determined at 40 °C without buffers in order to approximate industrial conditions. Enzyme activity was calculated as follows:

$$A = \frac{F \times C}{v \times t} \qquad (2.1)$$

where A = Activity (Kilo Novo Units), F = Starch factor (g), C = Concentration of the enzyme stock solution (μl/L), t = Reaction time and v = Volume of enzyme stock solution in reaction mixture.

4. Starch content of pulp

According to the Tappi Test Method T419 (Starch in paper), hot water (100 ml) was added to 1,0 g of pulp (dry equivalent) and disintegrated with a blunted

electric blender (Kenwood Chef 750 W KM300, Kenwood LTD Havant, UK) after which a hot water extraction (94 °C for 15 min) was done. This step also served to deactivate any enzyme that might be present in the pulp. The sample was then vacuum filtered through Whatman 541 filtration paper (Whatman International Ltd, Maidstone, Kent UK), followed by HCl extraction (25 ml 6N HCl for 3 min repeated twice and then 25 ml of concentrated HCl for 20 sec). The residue was washed with approximately 200 ml hot water, made up to 500 ml and then 150 ml was centrifuged for 10 min at 9820 *g* on a Beckman J2-MC centrifuge using a JA14 rotor (Beckman-Coulter, Fullerton, California, USA). The supernatant (25 ml) was mixed with 2,5 ml iodine solution (7,5 g KI and 5,0 g I_2 per litre) and made up to 50 ml with water to form a blue colour complex in the presence of starch. This colour intensity was measured spectrophotometrically (Phoenix-2000 UV-VIS Spectrophotometer, Biotech Engineering Management Co., Ltd Nicosia, Cyprus) at an absorbance of 580 nm. The spectrophotometer was zeroed using a mixture containing 5 % HCl (25 ml) and iodine solution (2,5 ml) made up to 50 ml with water.

To determine the amount of residual starch in the pulp sample, a standard curve was used (Figure 2.6) that was prepared by taking equal amounts of Sigma potato starch (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), BDH soluble starch (Merck, Darmstadt, Germany) and Saarchem soluble starch (Merck, Darmstadt, Germany) and mixing these dry starches thoroughly. The dry starch mixture (0,1 g) was dissolved in 100 ml of water and kept at 94 °C for 15 minutes. The fluid was decanted through a Whatman 541 membrane and then extracted with HCl as above. After extraction the filtrate was made up to 500 ml with demineralised H_2O, centrifuged and aliquots were taken to prepare the calibration curve.

5. Enzymatic activity on pulp

The relative activity of BAN 480L, Duramyl 300L and Fungamyl 800L on pulp, was determined by treating pulp with the enzymes and determining the starch content of the treated samples. The starch content after treatment was expressed as a factor of the starch content of pulp samples prior to enzyme treatment to reflect relative activity.

Different enzyme dosages, ranging between 0 U/g (control) and 2000 U/g, were evaluated to optimise enzyme dosages on pulp. One litre of stock solution containing 70 000 U/L of amylase with 10 ml $CaCl_2$-Tris buffer (63,22 g/L $CaCl_2$ and 1,10 g/L Tris(hydroxymethyl)aminomathane) was made up. The pulp was treated with different volumes of stock solution based on the enzyme activities.

6. Influence of $CaCl_2$ on enzymatic activity

Different α-amylase enzymes differ in their dependency on calcium ions for activity and stability (Sheppard, 1986). Initially experiments were, therefore, done with demineralised H_2O and $CaCl_2$-Tris buffer. Due to cost implications for commercial application this water and buffer solution was replaced with municipal water and pulp treatment was done with BAN 480L to evaluate starch hydrolysis under these conditions.

7. Influence of pulp consistency on enzymatic activity

The efficiency of enzyme treatment on low and high-consistency pulps was evaluated to optimise its application. For low-consistency (5 %) treatment, 1,0 g of

pulp was treated and the reaction mixtures incubated in conical flasks at 40 °C for 30 min on a rotary shaker with different enzyme dosages. The high-consistency (20 %) treatment was done by mixing each treatment with an electric mixer (Kenwood Chef 750 W KM300, Kenwood LTD Havant, UK) for 90 sec at room temperature. The reaction mixtures were incubated for 30 min in sealed plastic bags in a water bath at 40 °C. The starch content of the pulp was determined as described previously and the relative efficiency of the enzymes expressed in terms of the control treatments.

8. Influence of pH on enzymes

The efficiency of BAN 480L, Duramyl 300L and Fungamyl 800L to degrade starch in pulp when incubated at different pH values was determined to evaluate their suitability for industrial application. The experiment was replicated three times for each pH value and enzyme. Thirty grams of K4 pulp were suspended in 3 L of 0,1 M Britton Robinson Buffer. The buffer contained 0,1 M Boric acid, 0,1 M Acetic acid and 0,1 M Phosphoric acid (Xu, 1996) and the pH of the buffer was adjusted with 0,5 M NaOH to pH 4, 5, 6, 7, 8 and 9. The dosages and sampling times that allowed sufficient time for reactions to produce a trend were determined empirically in preliminary experiments. The enzyme dosages for BAN 480L was 140 U/g pulp, for Duramyl 300L was 7 U/g pulp and for Fungamyl 800L it was 400 U/g pulp. The treatments were incubated in a glass beaker at 40 °C in a water bath and pulp samples (100 ml), including a control without enzyme, were taken after 1, 2, 4 and 8 min. The samples were placed in a boiling water bath and incubated for 30 minutes at ± 94 °C to deactivate the enzyme and stop the reactions. The starch was then extracted using the HCl method described previously.

9. Influence of temperature on enzymes

The efficiency of BAN 480L, Duramyl 300L and Fungamyl 800L to degrade starch in pulp when incubated at different temperatures was determined in a replicated (three times) experiment. A suspension of 30 g K4 pulp in 3 L of municipal water was prepared and 0,5 M NaOH was used adjust the pH to 6,0. Treatments were incubated at 25, 35, 45, 55, 65, 75, 85 and 95 °C. The dosages and sampling times that allowed sufficient time for reactions to produce a trend were determined empirically in preliminary experiments. The enzyme charges for BAN 480L, Duramyl 300L and Fungamyl 800L were 800, 70 and 800 U/g pulp, respectively. The treatments, including a control without enzyme, were incubated in glass beakers in a water bath at each temperature and pulp samples (100 ml) were taken after the predetermined incubation time. Treatments with Duramyl 300L were incubated for 5 min, BAN 480L for 16 min and Fungamyl 800L for 16 min. The enzymes were deactivated by lowering the pH to below 1 with HCl. The starch was then extracted using the hot water and HCl method as described previously.

10. Influence of shear on enzymes

Different concentrations of tested enzymes were made up to 200 ml in $CaCl_2$ buffer. The concentrations of BAN 480L, Duramyl 300L and Fungamyl 800L were 2000, 1400, and 2000 U/ml, respectively. The enzyme solutions were exposed to homogenisation using a Heidolph Diax 600 (Heidolph Instruments GmbH & Co, Schwabach, Germany) at 24 000 rpm. The enzymes underwent shear treatment for a total of 60 minutes and samples (1,0 ml) were taken after 0, 1, 2, 5, 10, 15, 30 and 60 min.

The sheared enzyme samples were added to 10 ml starch solution (6,95 g/L) for a final enzyme dosage of 200, 140 and 200 U/ml for BAN 480L, Duramyl 300L and Fungamyl 800L, respectively. The concentration of the starch remaining after enzyme digestion for 60 min at 40 °C was determined as described earlier.

11. Experimental design and statistical analysis

Where possible and unless otherwise indicated, completely randomised experimental designs were used for all trials and the data subjected to one-way analysis of variance. Means of different treatments were tested for significant differences with Tukeys test (Winer, 1971) at 95 % confidence.

RESULTS AND DISCUSSION

1. Enzyme activity

The activities of the enzymes as determined in the present study differed from those specified by the supplier (Table 2.1). These differences can probably be attributed to different types of starch that were used in the assay. All enzyme dosages used in further experiments were based on the activity as determined in this section.

Table 2.1. Activities of enzymes as provided by the supplier and as determined at 40 °C and at the optimal temperature.

	Activity (KU/ml)		
	Supplier	40 °C	Optimal Temperature
BAN 480L	480	303	292
Duramyl 300L	300	239	256
Fungamyl 800L	800	793	792
Termamyl 120L	120	40	135

The reduced activity of Termamyl 120L observed at 40 °C is due to the enzyme's high temperature range. Even though Duramyl 300L has a very high optimal temperature (±70 °C) according to the suppliers, the enzyme is still active at 40 °C. It was decided to exclude Termamyl 120L from further experiments since these were done at 40 °C.

2. Starch content of pulp

The standard curve (Figure 2.6) showed the following relationship:

$$Y = 42{,}779X + 0{,}2722 \qquad (2.2)$$

where Y = Starch concentration (mg/L) and X = Absorbance (nm) ($R^2 = 0{,}9976$). This equation was used to calculate all starch concentrations when spectrophotometric determinations were used.

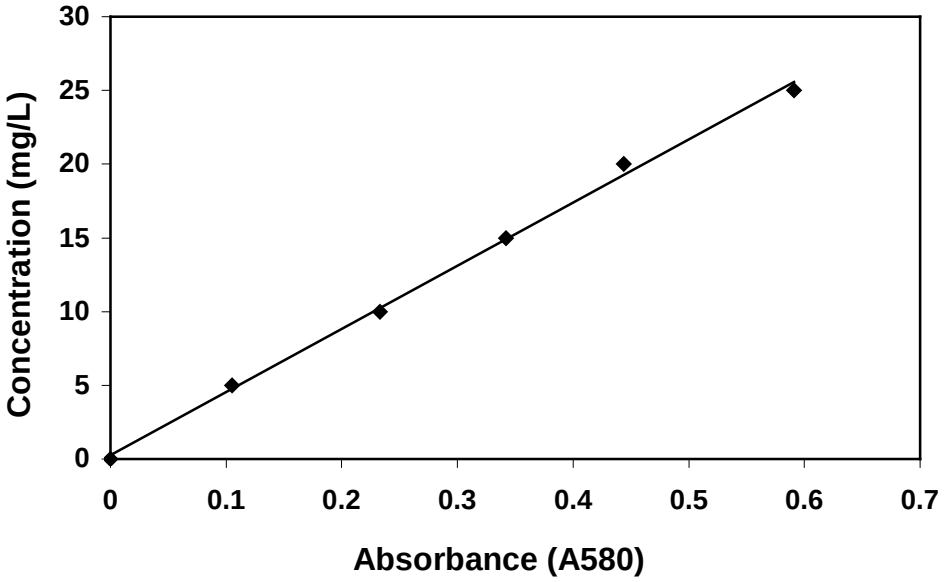


Figure 2.6. Standard curve for the relationship between starch concentration and absorbance at 580 nm

3. Influence of $CaCl_2$ on enzymatic activity

$CaCl_2$ in the form of $CaCl_2$-Tris buffer did not have a significant influence on enzymatic activity on pulp when compared to results obtained when tap water without any $CaCl_2$ addition was used (Figure 2.7). It appears, therefore, that the pulp and the tap water contained enough calcium to provide stability for the enzyme.

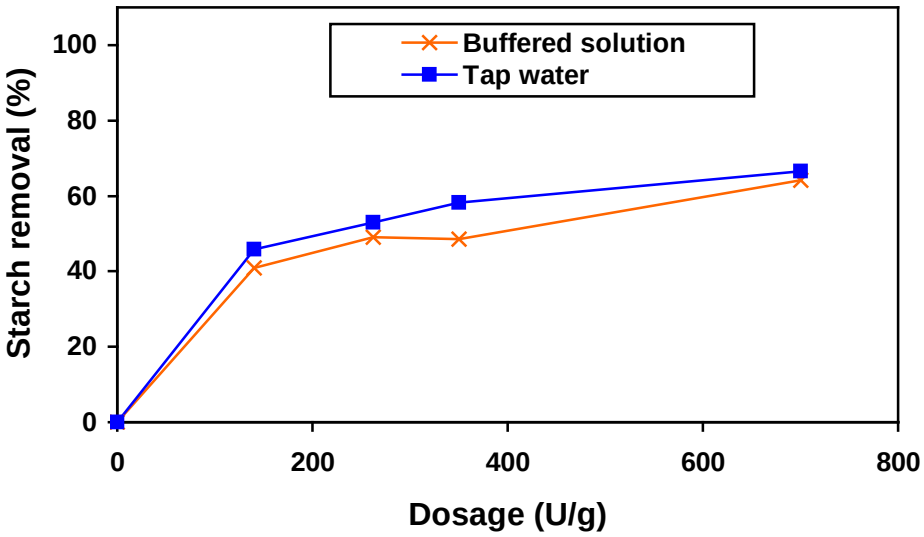


Figure 2.7. Starch removal from pulp after treatment with BAN 480L in $CaCl_2$-Tris buffered solution and in municipal water

4. Influence of pulp consistency on enzymatic activity

All the enzymes successfully degraded the starch in recycled K4 pulp using low (5 %) and high (20 %) consistencies (Figures 2.8 and 2.9). Over the range of enzyme dosages, the high-consistency treatments with Duramyl 300L removed up to 76 % of the starch, BAN 480L 44 % of the starch and Fungamyl 800L only 26 % (Figure 2.8). Duramyl 300L was the most effective at low-consistency on K4 pulp with more than 67 % removal of the residual starch, followed by Fungamyl 800L with 39 % and BAN 480L with 32 % removal (Figure 2.9). These results (Figure 2.8 and 2.9) were used to determine a suitable enzyme dosage for use in further experiments. These dosages for BAN 480L, Duramyl 300L and Fungamyl 800L on high-consistency pulp treatment were 400, 140 and 400 U/g pulp, respectively. For low-consistency treatments, the dosage for BAN 480L was 140 U/g pulp, for Duramyl 300L it was 140 U/g pulp and for Fungamyl 800L it was 175 U/g pulp.

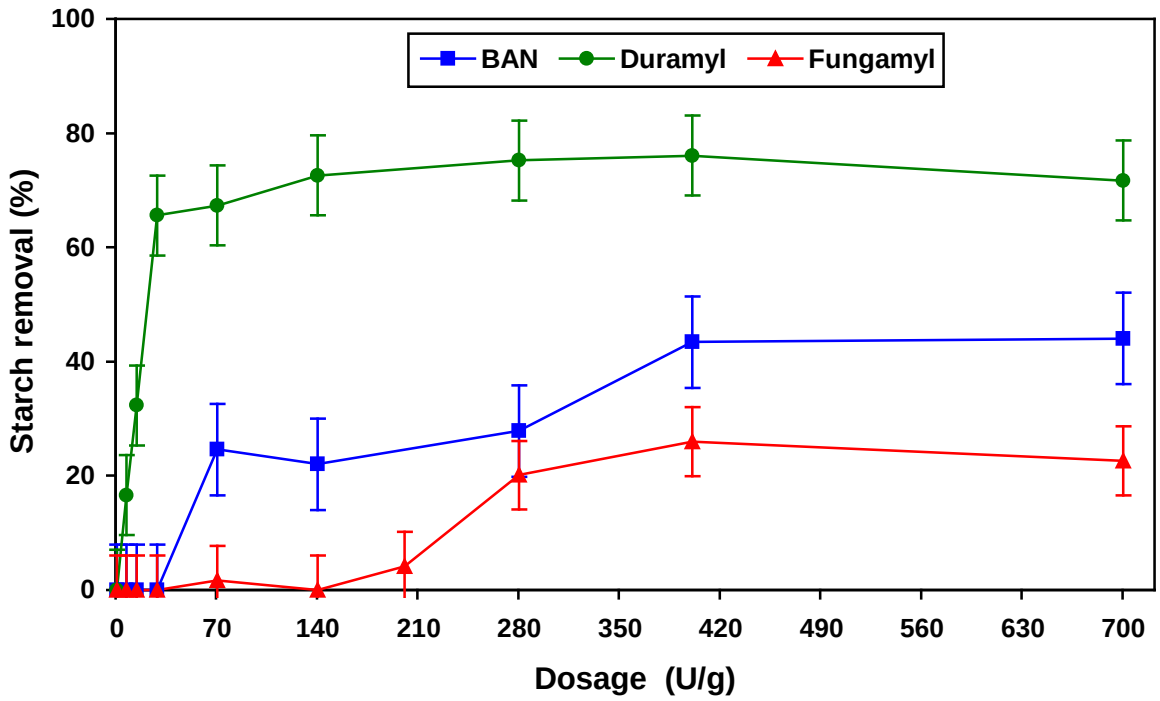


Figure 2.8. Starch removal by different amylases at high-consistency (20 %) on K4 pulp after incubation for 30 min at 40 °C. Error bars = Q values at $p \leq 0{,}05$

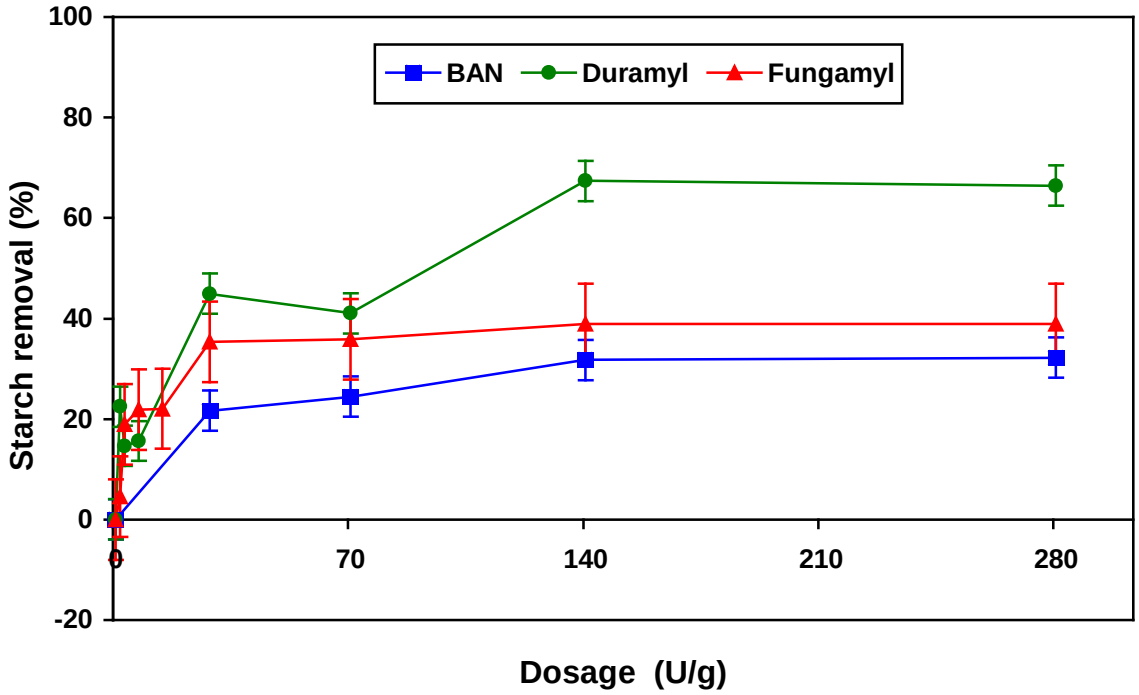


Figure 2.9. Starch removal by different amylases at low-consistency (5 %) on K4 pulp after incubation for 30 min at 40 °C. Error bars = Q values at $p \leq 0,05$

5. Influence of pH on enzymes

The pH range where the different enzymes were most active was similar for all the recorded incubation times (Appendix A). The results after incubation for 4 min is used for illustration (Figure 2.10, 2.11 and 2.12). Duramyl 300L displayed activity over the widest pH range, being significantly most active in the range of pH 6 to 8 (Figure 2.10). BAN 480L and Fungamyl 800L were most active at pH 6 and significantly less active at the other pH values (Figure 2.11 and Figure 2.12). These results indicate that Duramyl 300L would be most suitable for industrial applications especially where the pH is in the alkaline range or pH is variable.

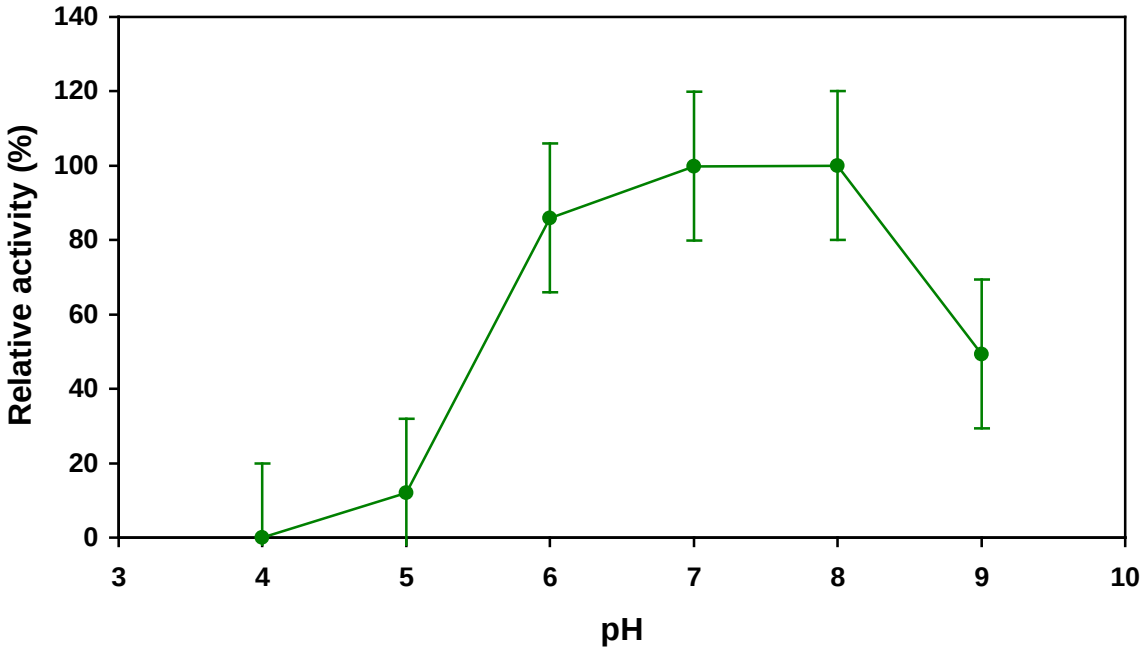


Figure 2.10. The influence of different pH values on the relative activity of Duramyl 300L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

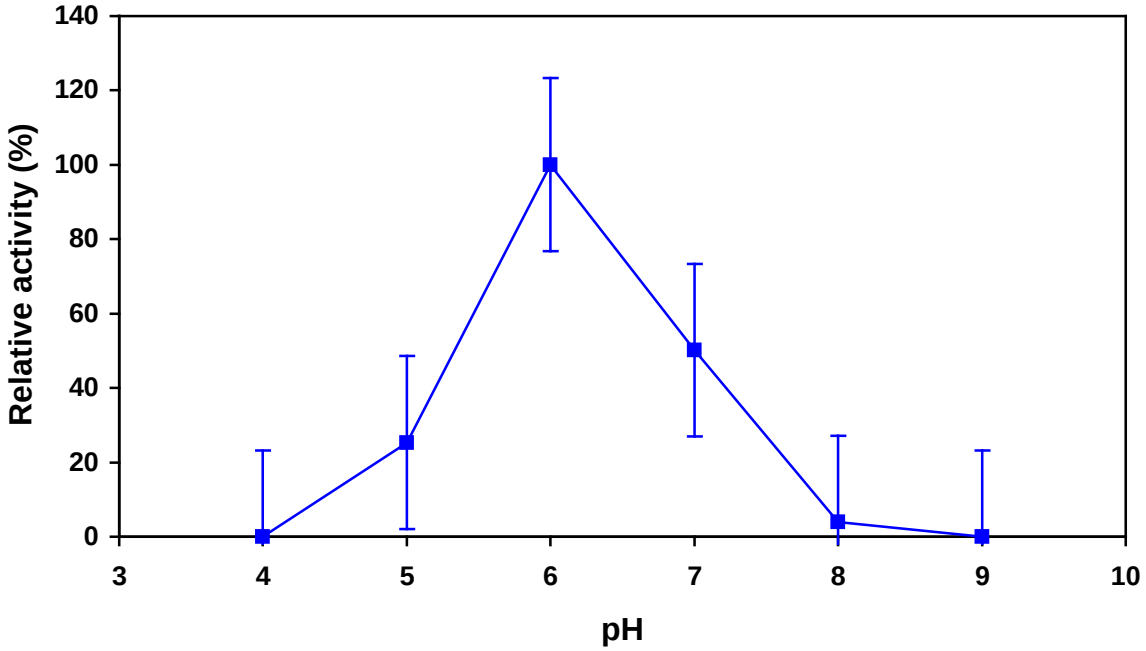


Figure 2.11. The influence of different pH values on the relative activity of BAN 480L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

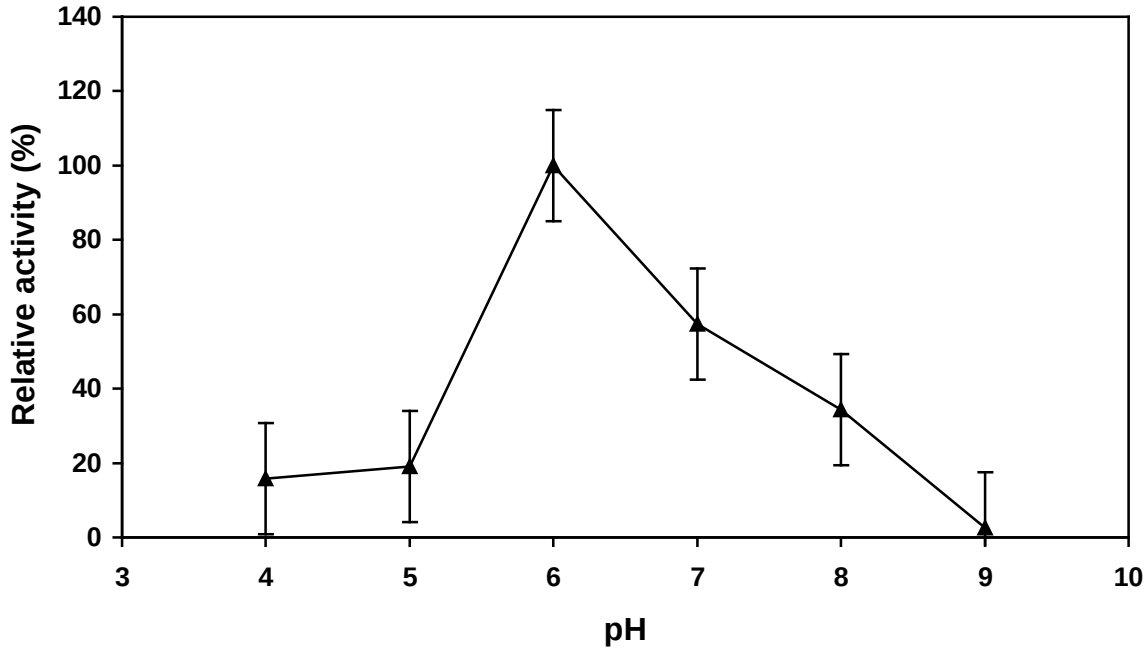


Figure 2.12. The influence of different pH values on the relative activity of Fungamyl 800L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

6. Influence of temperature on enzymes

The relative activities of all the enzymes over a range of different temperatures were similar to the activities provided by the supplier (Anon, 1985; 1994; 2001). The fact that the reactions took place in pulp does not seem to have a serious effect on temperature tolerance. At the lower temperatures the relative activities of the enzymes on pulp were much lower when compared to their respective optimal temperatures. This difference from the activity found on pure starch (compare Table 2.1) can probably be attributed to the fact that the solution was not buffered in the same way as for activity determination or to unknown effects of the pulp. BAN 480L displayed activity over the widest temperature range, being significantly most active between 55 and 85 °C (Figure 2.13).

Duramyl 300L was significantly most active between 65 and 85 °C (Figure 2.14) and Fungamyl 800L was significantly most active between 55 and 65 °C (Figure 2.15). These results indicate that BAN 480L was the most temperature tolerant by displaying activity over the widest range of temperatures. Duramyl 300L was active in the higher temperature range. Depending on the specific process and application, BAN 480L and Duramyl 300L would be suitable for industrial applications.

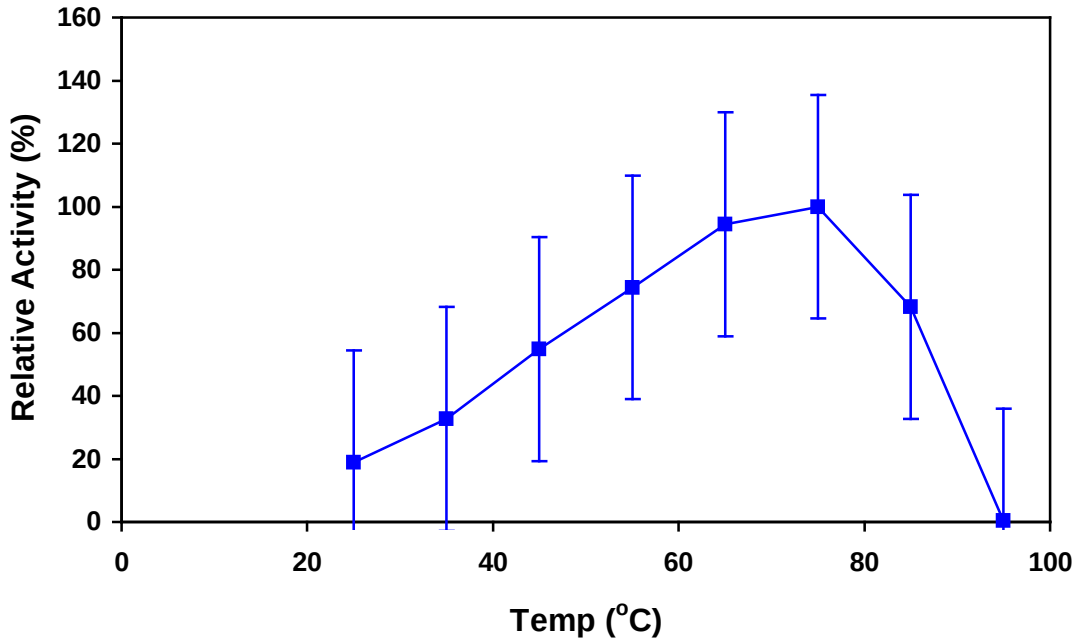


Figure 2.13. The influence of different temperatures on the relative activity of BAN 480L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

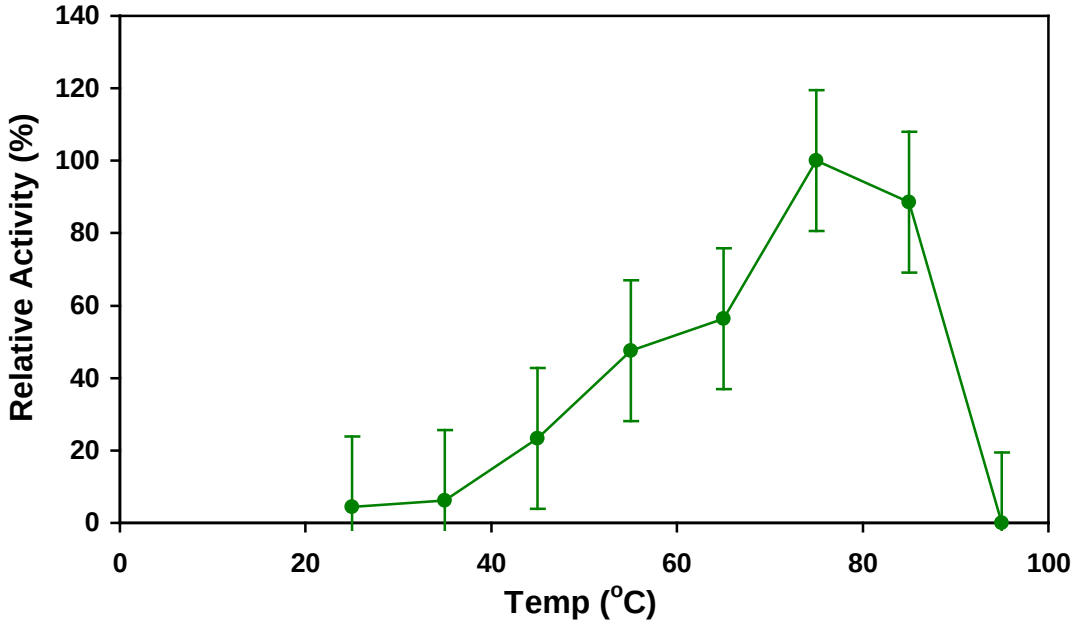


Figure 2.14. The influence of different temperatures on the relative activity of Duramyl 300L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

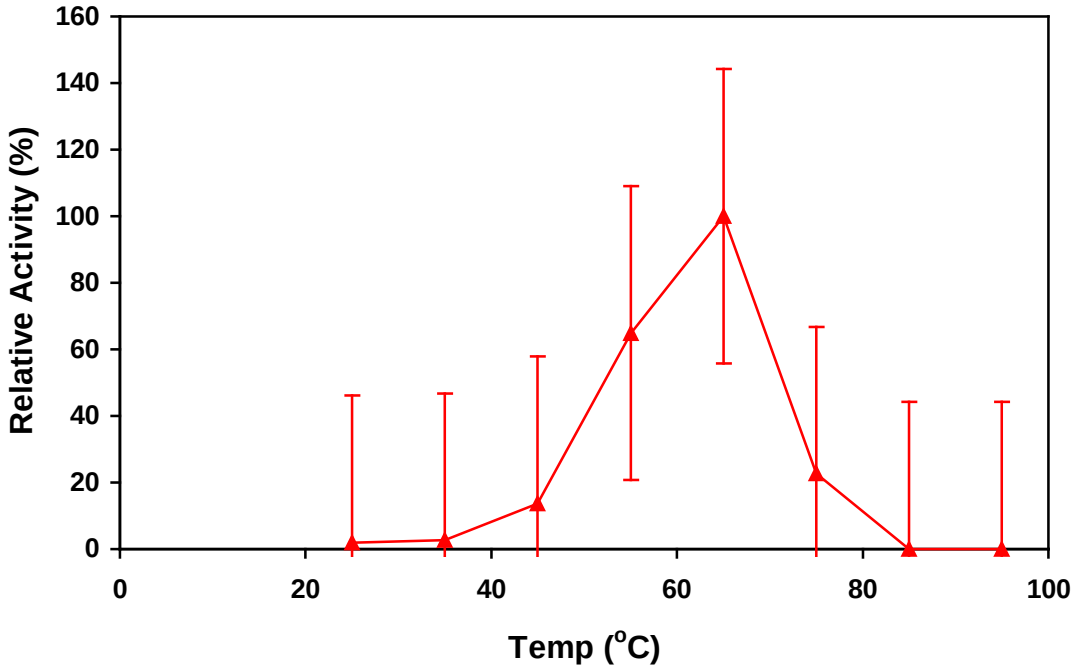


Figure 2.15. The influence of different temperatures on the relative activity of Fungamyl 800L after 4 min incubation. Error bars = Q values at $p \leq 0,05$

7. Influence of shear on enzymes

BAN 480L had a relative activity of 100 % after 10 min exposure to shear forces but at 15 min the activity was significantly decreased to 89,5 % (Figure 2.16). Duramyl 300L was 90,6 % active after 5 min of exposure to the shear forces, but was significantly less active (80,7 %) after 10 min (Figure 2.17). Fungamyl was significantly less active (77,9 %) after only 1 min of exposure to shear forces (Figure 2.18). It appears that BAN 480L or Duramyl 300L could be more suited to industrial application where high shear forces are experienced.

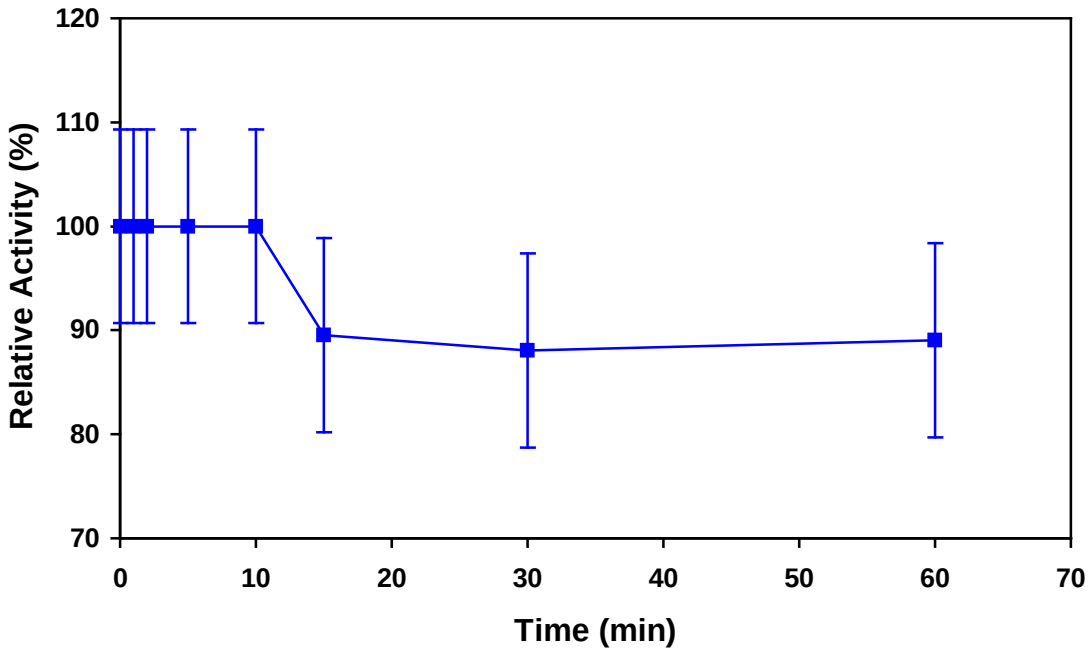


Figure 2.16. The influence of shear forces on the relative activity of BAN 480L. Error bars = Q values at $p \leq 0,05$

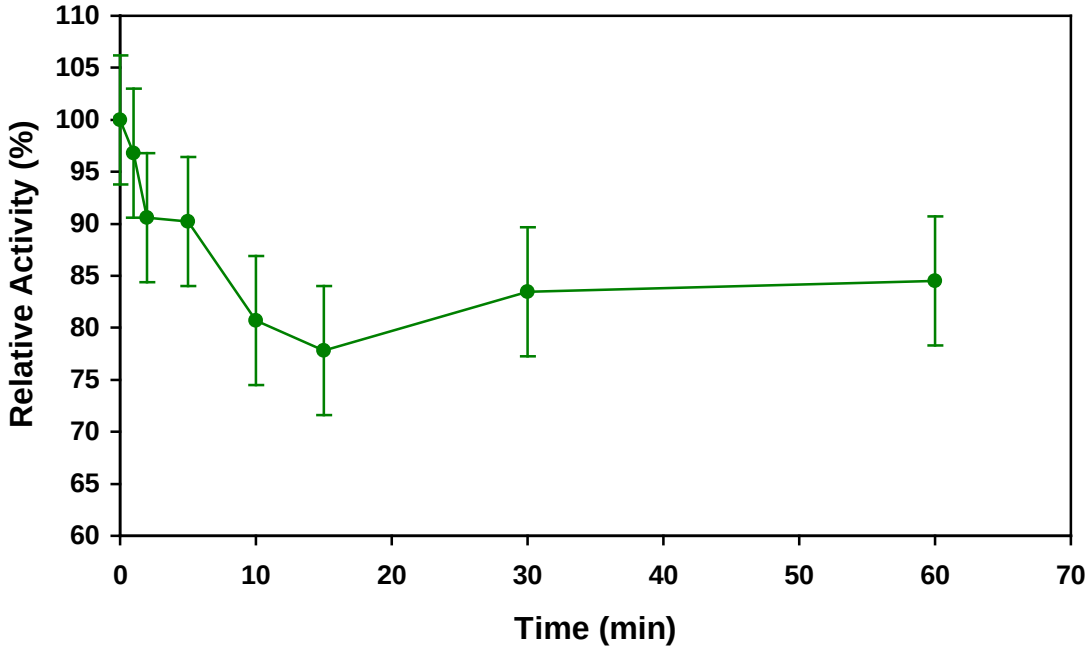


Figure 2.17. The influence of shear forces on the relative activity of Duramyl 300L. Error bars = Q values at $p \leq 0,05$

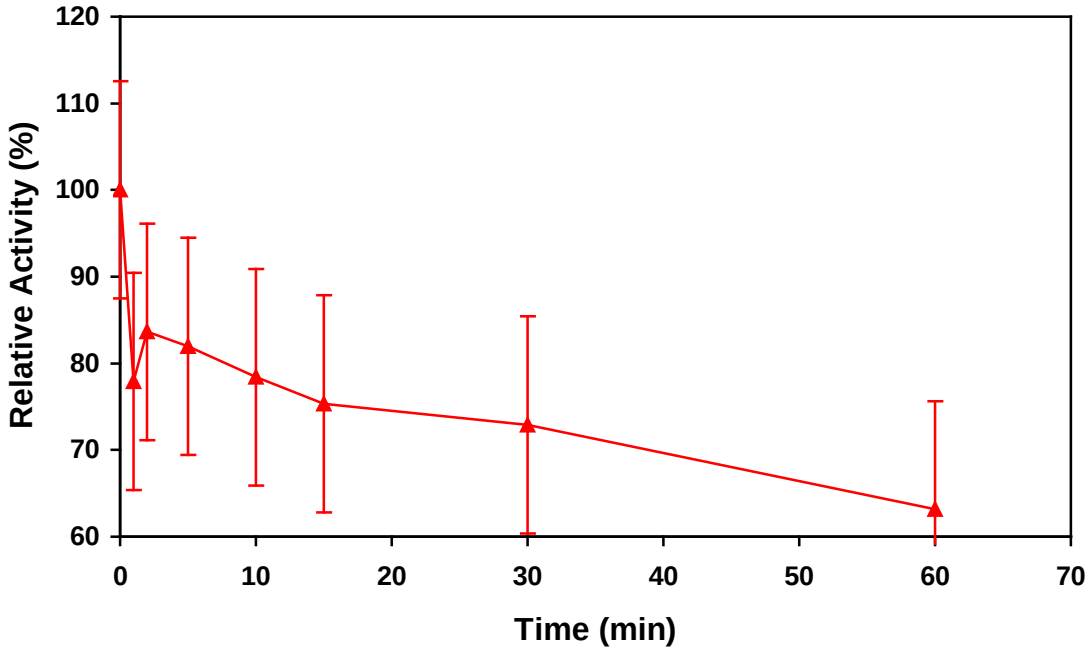


Figure 2.18. The influence of shear forces on the relative activity of Fungamyl 800L. Error bars = Q values at $p \leq 0,05$

CONCLUSIONS

BAN 480L, Duramyl 300L and Fungamyl 800L were able to sufficiently degrade secondary starch in pulp at 40 °C without the addition of $CaCl_2$, but Termamyl 120L was excluded from further studies due to its low activity at 40 °C. BAN 480L, Duramyl 300L and Fungamyl 800L all displayed activity over a range of pH values. Duramyl 300L was more active in the alkaline range while BAN 480L and Fungamyl 800L were most active under slightly acidic conditions. Duramyl 300L would be best suited to an industrial process especially when the pH is not well controlled and does not go lower than pH 5. During the temperature evaluation BAN 480L had the highest average relative activity over the range tested. Duramyl 300L had more relative activity above 40 °C but was still active at the lower temperatures. All the enzymes displayed good tolerance to shear, but BAN 480L was the most tolerant. Duramyl 300L displayed the best results overall and could be best suited for industrial application.

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CHAPTER 3

THE INFLUENCE OF STARCH DEGRADATION ON THE CHARACTERISTICS OF RECYCLED PULP

Fibre yard at Sappi Cape Kraft

ABSTRACT

Starch is added to fibre at the wet end during papermaking to improve dry strength, paper-machine runnability, and printability. When fibre is recycled, it contains secondary starch that decreases drainage rate. Amylases can hydrolyse secondary starch to increase the drainage rate. Handsheets were made with pulp treated with amylases namely: BAN 480L, Duramyl 300L and Fungamyl 800L. These handsheets were tested for strength properties that included Bursting Index, Tearing Index and Air Permeance. Canadian Standard Freeness, Drainage Time, Vacuum Drainage Time and the Water Retention Values were determined to evaluate the influence of amylase treatment on lab scale drainage of pulp. Some of the enzymatic treatments improved pulp strength. None of the drainage tests showed significant improvement when compared with control samples. These results can possibly be ascribed to variability of secondary starch and recycled pulp or that tests were not repeatable enough to quantify drainage improvement. The starch contained in the recycled pulp was effectively degraded, so a pilot trial to evaluate drainage improvements was recommended.

INTRODUCTION

Starch is added to fibre at the wet end during papermaking to improve dry strength, paper-machine runnability, and printability. Starch is also used as surface sizing to increase surface strength and reduce dust, thereby improving printing characteristics. Coating starches are used to bind coating colours during printing (Erceg, 1984). When recycled fibre is used in the production of paper and board, the recycled starch can lower drainage rates and reduce paper-machine speed (Lascaris *et al.*, 1997). However, the secondary starch does not contribute to board characteristics and can potentially be degraded by amylases to improve drainage or increase machine speeds (Lascaris *et al.*, 1997).

A paper-mill trial was conducted during the production of fluting and liner from 40 % newsprint, 40 % office waste and 20 % waste paper (Lascaris *et al.*, 1997). Amylase treatment caused a reduction in the Shopper Reigler values of the top and bottom machine chests and headboxes. The Tear Index values were higher, but the Burst Index values were significantly lower (Lascaris *et al.*, 1997). It has been shown that the commercial amylase enzymes tested were effective to degrade secondary starch (Chapter 2), but the influence of enzyme treatment on drainage and strength properties of the pulp used at Cape Kraft is still unknown. The aim of this study was, therefore, to use the commercial enzymes to treat pulp from Sappi Cape Kraft and to evaluate changes in paper and drainage properties.

MATERIALS AND METHODS

1. Influence of amylases on handsheet properties

Handsheets were used for the physical testing of pulp according to the Tappi Test Method T220 (Physical testing of pulp handsheets) (Anon, 1988). These tests included Thickness, Basis Weight, Bursting Strength, and Tearing Resistance. In addition, Air Permeance (Tappi, T460) and Drainage Time of Handsheets (Tappi, T221) were also evaluated. The tests reflect the potential contribution of the pulp to the strength of the product.

K4 pulp that is used in the production of fluting was used during the formation of the handsheets. The pulp consists of recycled corrugated containers and kraft wrapping. The quantities of pulp used in this study refer to the dry weight equivalent of pulp. For the production of the handsheets, a pulp suspension (1 % consistency) was made in a 3-L beaker and then treated with BAN 480L, Duramyl 300L and Fungamyl 800L. The enzyme dosages were 400, 140 and 400 U/g pulp, respectively and a control sample without enzyme was included. The treatments were incubated at 40 °C in a thermostatically controlled water bath for 30 min and stirred continuously with an overhead stirrer.

Handsheets from treated pulp were made according to the Tappi Test Method T205 (Forming handsheets for physical testing). Ten handsheets were made for every replicated treatment. The handsheets were dried according to Tappi Test Method T402 (Standard conditioning and testing atmospheres for paper, board, pulp handsheets, and related products).

Basis Weight (r) was determined on four of the preconditioned handsheets of every treatment according to Tappi Test Method T220 (Physical testing of pulp handsheets). The thickness of a single handsheet (t) was measured according to Tappi Test Method T411 (Thickness (calliper) of paper, paperboard, and combined board) with a Motor-operated micrometer, (Labtech, St Laurent, Quebec, Canada). The thickness was measured twice for each sheet at two non-overlapping areas and the bulk density was calculated.

Bursting Index of treated handsheets was determined by following the prescriptions of Tappi Test Method T403 (Bursting strength of paper). Bursting strength indicates the paper's resistance to rupture and the test is done with a Bursting tester (Labtech, St Laurent, Quebec, Canada). The burst test was repeated 10 times on each set of handsheets. The average bursting strength for each replicated treatment was used to calculate the Burst Index.

The Tear Index of single handsheets was measured according to Tappi Test Method T414 (Internal tearing resistance of paper) with an Elmendorf-type tearing tester (Labtech, St Laurent, Quebec, Canada). For each set of handsheets, eight tearing strength tests were done and the tearing index was calculated to compare with control treatments.

Air Resistance of treated handsheets was determined as described in Tappi Test Method T460 (Air resistance of paper). Air Resistance is defined as the

resistance offered by a sheet of paper (or handsheet) to the passage of air through the sheet. For each enzyme treatment the test was repeated at least eight times and the average air resistance calculated.

Drainage Time of Handsheets was done by following Tappi Test Method T221 (Drainage time of pulp). This method records the time required to drain all free water during formation of the handsheet. The relationship between time and basis weight was used to calculate the drainage time of pulp.

$$d_s = \frac{35d}{r - 25} \tag{3.1}$$

where d_s = drainage time of pulp (sec), d = average time to drain free water (sec) and r = average basis weight of handsheets (g/m^2) for each replicated treatment.

2. Influence of amylases on drainage

2.1. Canadian Standard Freeness

The influence of enzyme treatment on drainage from pulp was evaluated by testing the Canadian Standard Freeness (CSF) as described in the Tappi Test Method T227 (Freeness of pulp). K3 pulp used for the production of liner was used in this experiment. K3 consists of new corrugated container off-cuts and do not contain more that 1 % contaminants such as plastic, cloth and metal. The pulp was made up in a 500 ml glass beaker to a consistency of 1 %, treated with enzyme and then diluted to 0,3 % consistency at 20 °C for the freeness evaluation.

The pulp was treated with BAN 480L at different dosages (0, 62, 135, 140, 215, 536 U/g pulp) at 40 °C for 30 min in a thermostatically controlled water bath. These enzyme concentrations were based on the amount of starch degradation observed previously (Chapter 2) and achieved 0, 25, 40, 50, 60 and 70 % starch degradation respectively.

2.2. Drainage Time

This test is based on the Canadian Standard Freeness test and uses the same apparatus (Figure 3.1), but the bottom orifice is plugged so that water could only flow from the side orifice. The test was initially done eight times with untreated pulp to evaluate the repeatability. Following this, the influence of enzyme on drainage time was evaluated using three treatment methods (Table 3.1). A range of BAN 480L dosages (0, 62, 135, 140, 215 and 536 U/g pulp) were tested using all three the methods. These dosages are based on the amount of starch degradation observed previously (Chapter 2) and achieved 0, 25, 40, 50, 60 and 70 % degradation of secondary starch respectively.

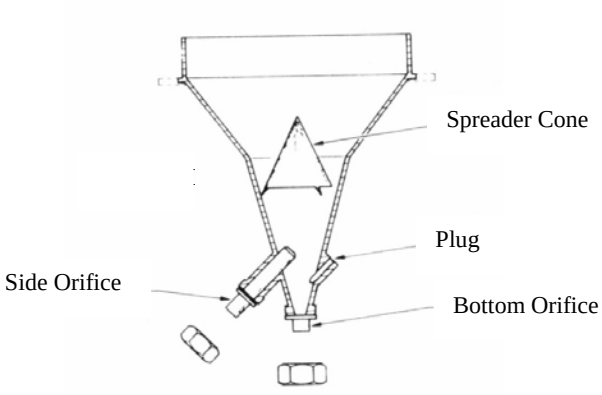

Figure 3.1. The Funnel of the Canadian Standard Freeness Tester (Tappi, T227)

Table 3.1. Different treatment methods used for pulp to determine the influence of amylases on drainage time.

	Method 1	**Method 2**	**Method 3**
Pulp consistency (%)	1	2	2
Disintegration volume (ml)	3000	3000	250
Incubation Volume (ml)	500	250	250
Temperature (°C)	40	40	40
Duration (min)	30	30	30
Process	Bulk	Bulk	Batch

After enzymatic treatment, the samples were further diluted to one litre to give a 0,5 % consistency and placed in the top cylinder of the freeness tester with the bottom lid closed. The airtight lock was established and the bottom lid was opened. Simultaneous to the opening of the top valve, a stopwatch was started and the time was noted for the different volumes of water (400, 500, 600, 650, 700 and 750 ml) to drain. The influence of enzyme treatment on each drainage volume was evaluated in a factorial experiment with two factors (enzyme dosage and treatment method) and each treatment was replicated twice.

2.3. Vacuum Drainage

The vacuum drainage evaluation is based on a confidential method provided by SA Paper Chemicals (Anon., 1997). The method determines the time required for the free water to drain from pulp at a specific consistency under vacuum. The time was recorded from application of the vacuum until the breaking of the vacuum through the web. K3 and K4 pulp were used, and 5 g of pulp was disintegrated in 250 ml water with an overhead stirrer. Different membranes, namely: Whatman 541, Whatman 113 (Whatman International Ltd, Maidstone, Kent UK) and a wire mesh (with an average pore size of 0,5 mm^2) in combination with different enzymes (BAN 480L, Fungamyl 800L, Termamyl 120L and AMG 300L) were used at

different dosages (Table 3.2). AMG 300L, an amyloglucosidase, was used in combination with the other amylases to degrade limited dextrins and other starch degradation products. The enzyme has limited debranching capabilities and removes a single glucose moiety at a time from the non reducing end of the molecule. The pulp was treated with enzyme in glass beakers (500 ml) for 30 min in a thermostatically controlled water bath. The experiments were replicated three times for each treatment.

Table 3.2. Treatments used in different experiments to determine the influence of enzymes on vacuum drainage

	Membrane	**Treatments**	**Dosage (U/g pulp)**	**Temperature (°C)**
Experiment 1	Whatman 541	BAN	0 to 1000	40
		Fungamyl	0 to 1000	40
Experiment 2	Whatman 113	BAN	0 to 550	40
		BAN * + AMG	1020 0 to 550	40
Experiment 3	Wire Mesh	BAN	0 to 700	40
		BAN * + AMG	1020 0 to 550	40
Experiment 4	Wire Mesh	BAN * + AMG	1020 0 to 700	60
		Termamyl * + AMG	1020 0 to 700	60
Experiment 5	Whatman 113	BAN * + AMG	1020 0 to 700	60
		Termamyl * + AMG	1020 0 to 700	60

* Combination of enzymes to evaluate possible synergism

2.4. Water Retention Value

The water retention value was determined according to Tappi Useful Method UM256 (Water retention value) (Anon, 1991). This method measures the amount of water retained by a wet specimen after centrifuging under standard conditions and evaluates the dewatering behaviour of pulp on the paper machine.

Pulp at a consistency of 1 % was made up in a batch of 3 L and the enzymes were applied to samples of 99,0 ml in glass beakers for 30 min at 40 °C in a thermostatically controlled water bath. The sample of 99,0 ml at 1 % consistency produced a pad of 1400 g/m^2 in sintered glass filtering crucibles with a radius of 15 mm after centrifugation. A dosage of 280 U/g pulp of BAN 480L, Duramyl 300L, Fungamyl 800L and AMG 300L were respectively used. Each treatment was replicated three times.

After enzyme treatment the samples at 1 % consistency was filtered through pre-weighed crucibles (30 ml, porosity 2) and then centrifuged at 900 *g* for 30 min at 23 °C. Specimens were immediately weighed after centrifugation and then placed overnight at 105 °C in a drying oven. After drying, and cooling in a desiccator the specimens were again weighed for gravimetric determination of Water Retention values.

3. Experimental design and statistical analysis

Completely randomised experimental designs were used for all experiments and the data subjected to one-way analysis of variance. Means of different treatments were tested for significant differences with Tukeys test (Winer, 1971) at 95 % confidence.

RESULTS AND DISCUSSION

1. Influence of amylases on handsheet properties

No differences were observed between the bulk density of any of the treatments (Table 3.3). The Burst Index of the handsheets made with Fungamyl 800L treated pulp was significantly ($p \leq 0,05$) higher than the control treatments (Table 3.3). The same pattern was observed for Tear Index, when handsheets made after Fungamyl 800L treatment resulted in improved handsheets (Table 3.3). None of the strength properties of handsheets from enzyme treated pulp was lower than the control treatments (Table 3.3).

Table 3.3. Influence of different enzyme treatments on Bulk density, and strength properties of pulp.

Treatment	Bulk Density (cm^3/g)	Burst Index ($kPa.m^2/g$)	Tear Index ($mN.m^2/g$)
Control	2.038 a*	1.627 b*	8.61 b*
Duramyl 300L	2.037 a	1.565 b	9.02 ab
BAN 480L	2.046 a	1.543 b	8.20 b
Fungamyl 800L	2.023 a	1.854 a	9.72 a

* Values in the same column followed by the same letter do not differ significantly ($p \leq 0,05$).

The results showed that the handsheets made with the control treatment had significantly higher Air Permeance than Fungamyl 800L and BAN 480L treated handsheets (Table 3.4). None of the treated handsheets had an average Air Permeance higher than the control (Table 3.4). The Permeability Index of samples treated with BAN 480L was significantly lower than the control sample and the other samples did not differ significantly ($p \leq 0,05$).

Table 3.4. Average air permeability of different handsheets sets formed after enzyme treatment.

	Air Permeability (ml/min)	Permeability Index (ml/min/g)
Control	878.84a*	6.41a*
Duramyl 300L	845.22ab	6.36a
BAN 480L	628.51c	4.47b
Fungamyl 800L	734.53bc	5.37ab

* Values in the same column followed by the same letter do not differ significantly ($p \leq 0,05$).

The average drainage time of each set of handsheets seemed lower than that of the control, but due to the large variance, no significant differences existed between different enzyme treated handsheets and the control (Figure 3.2).

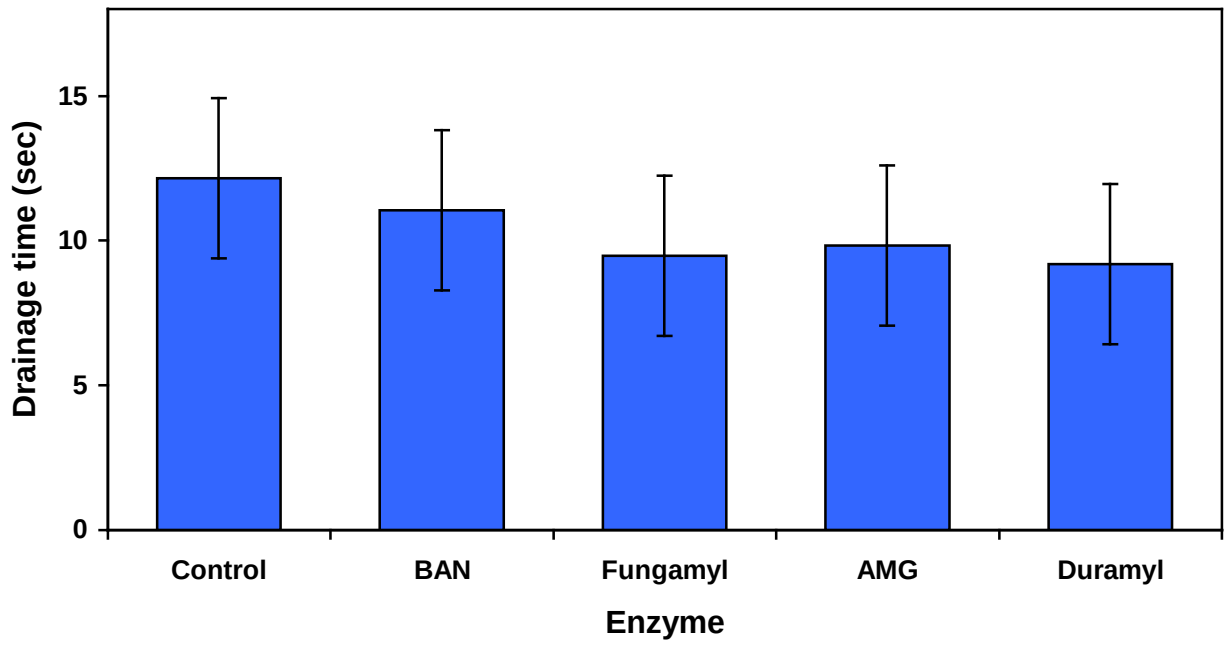

Figure 3.2. The different average drainage times of pulp handsheets made after enzyme treatment. Error bars = Q values at $p \leq 0,05$

2. Influence of amylases on drainage

2.1. Canadian Standard Freeness

An initial experiment with no enzymatic treatment showed that the Freeness test had a high repeatability. Following enzyme treatment of the pulp samples, an increase in the Freeness values was expected, as the amount of secondary starch was decreased by the enzyme, but no significant difference could be seen between the different dosages of BAN 480L (Figure 3.3). After consideration of these results an alternative method of quantifying drainage improvement due to the starch degradation was sought.

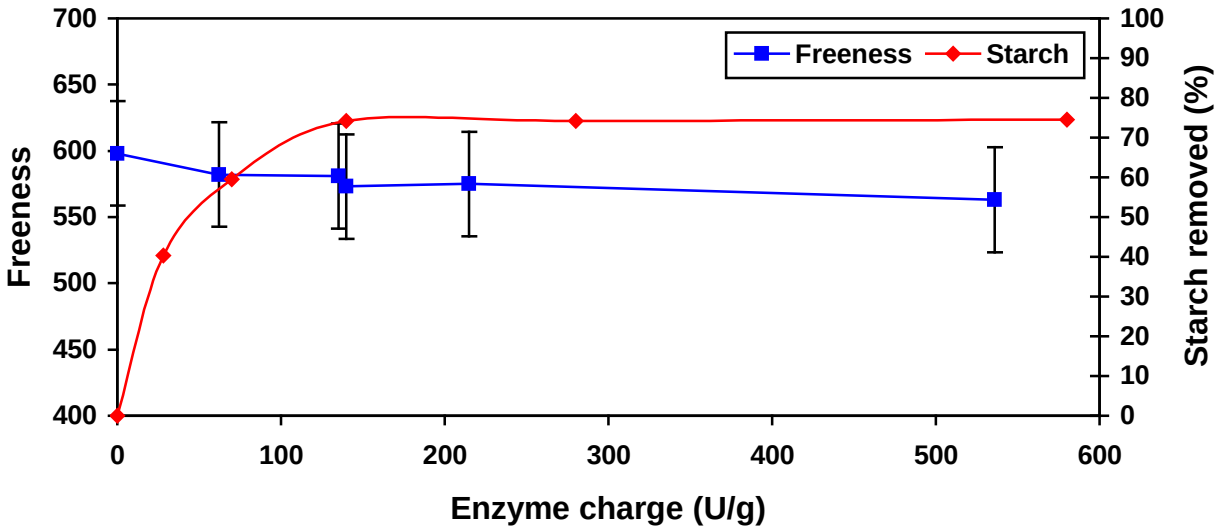

Figure 3.3. Freeness of K3 pulp after treatment with BAN 480L. Error bars = Q at $p \leq 0,05$

2.2. Drainage Time

The initial experiment with untreated pulp demonstrated that the method was highly repeatable (Appendix B1). The enzyme concentration did not influence the drainage time for any of the drained volumes (Appendix B2). The results obtained for drainage of 400 ml water is shown as an example (Table 3.4). Method 3 had significantly higher drainage rates than Method 1 and Method 2 (Table 3.4) due to pulp preparation and not due to enzymatic treatment. Method 1 and Method 2 did not differ significantly. During pulp preparation in Method 3 the pulp was disintegrated with an overhead stirrer and the pulp was not fibrillised as well as in method 2 and 3 and this lead to an increased drainage rate (Table 3.4). This means that Method 3 has no advantage for the enzymatic treatment over the other two methods.

Table 3.4. Drainage time required for drainage of 400 ml water from pulp treated with different enzyme dosages using three different methods

Dosage (U/g)	**Method 1 (sec)**	**Method 2 (sec)**	**Method 3 (sec)**
0	7.42	7.40	5.52
62	7.11	6.88	5.76
135	6.85	7.02	4.83
140	7.57	6.88	5.39
215	6.83	7.45	5.36
536	7.70	7.30	5.51
Average	**7.25 b ***	**7.15 b ***	**5.39 a ***

* Values in the same column followed by the same letter do not differ significantly ($p \leq 0,05$).

2.3. Vacuum Drainage

No significant drainage improvement ($p \leq 0,05$) was observed between different enzyme dosages of any of the enzymes or membranes (Appendix C). AMG 300L was included to degrade branched hydrolysis products, but it did not have any effect on improving vacuum drainage times in combination with the other "endo"-amylases. It appears, therefore, that the vacuum drainage test is not accurate enough to quantify any change in the vacuum drainage time due to the removal of secondary starch, even though the starch contained in the treated pulp was significantly less. The AMG 300L treatment degraded dextrins of different lengths and this treatment also showed no effect on Vacuum Drainage Time. Unfortunately, no satisfactory HPLC results were obtained due to extremely low concentrations of the formed sugars.

2.4. Water Retention Value

There were no significant differences between the Water Retention Values of the treatments (Figure 3.3). Apparently, the secondary starch contained in recycled pulp does not contribute to the water retention, or the enzyme treatment did not have a significant effect to decrease the starch. However, starch assays (Chapter 2) show that enzymes successfully degrade secondary starch, thus the first reason seems more likely.

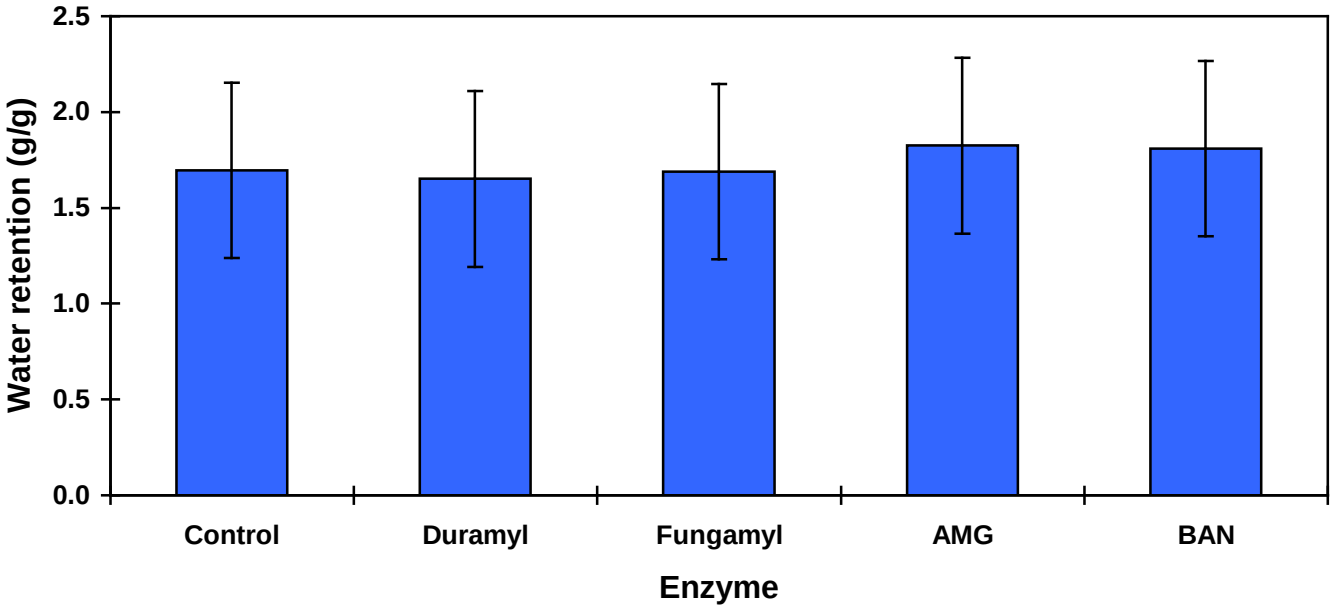

Figure 3.3. Water retention values of K4 pulp after treatment with different enzymes. Error bars = Q values at $p \leq 0,05$

CONCLUSIONS

The enzyme treatments did not have a detrimental effect on strength and other physical properties that are important to the papermaker. After numerous efforts to determine the drainage changes caused as a result of enzyme treatment on laboratory scale, no improvements could be observed. Further studies to determine possible drainage improvements are needed, because previous work showed that the starch in the pulp was significantly degraded (Chapter 2). According to literature this starch has negative effects on paper machine runnability and pulp drainage on the paper machine (Lascaris *et al.*, 1997). Pulp drainage evaluation on a small-scale paper machine could be useful to observe such improvements. Industrial conditions should, however, be reproduced as closely as possible.

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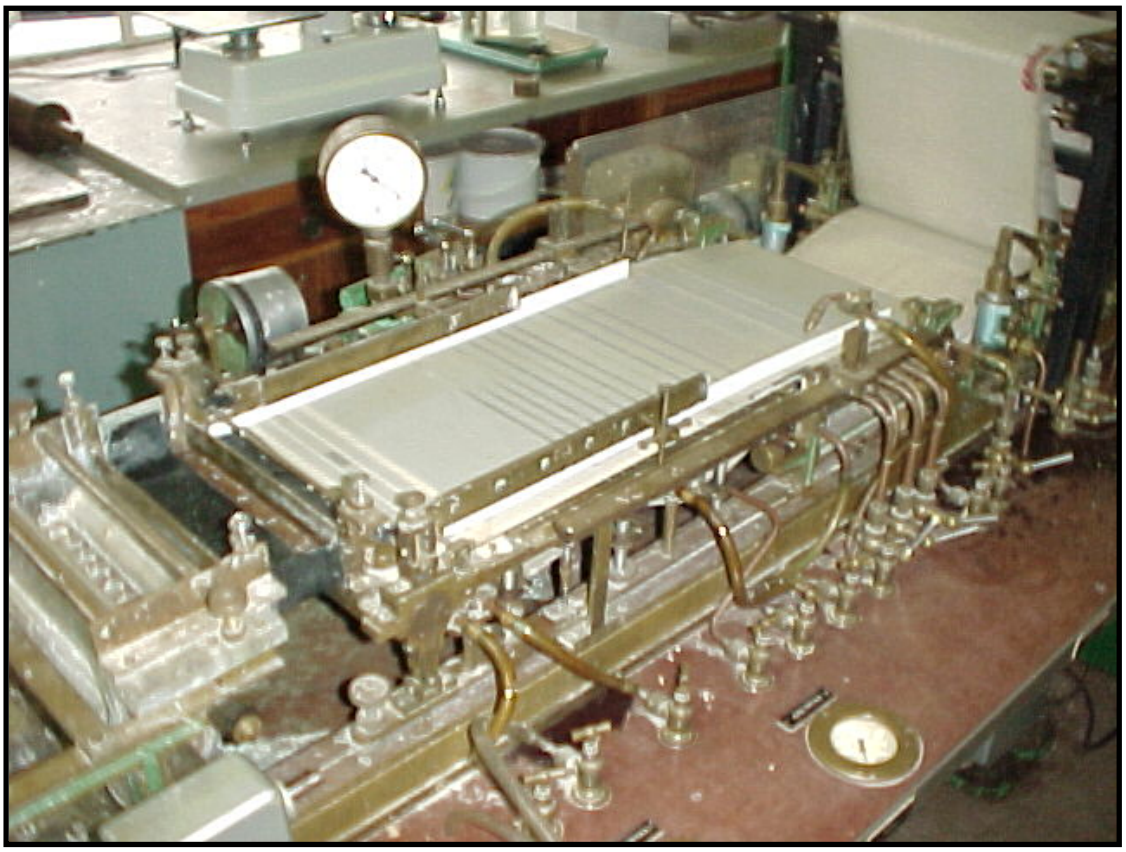
CHAPTER 4

EVALUATION OF AMYLASE-TREATED SECONDARY FIBRES ON A PILOT-SCALE PAPER MACHINE



The pilot-scale paper machine showing the forming wire and presses

ABSTRACT

Degradation of secondary starch in recycled fibre with amylase can increase the drainage rate during papermaking. During earlier studies, the secondary starch was successfully hydrolysed with the commercial amylases, but it was not possible to demonstrate drainage improvement on a laboratory scale with Vacuum Drainage, Canadian Standard Freeness, Drainage Time or Handsheet Drainage Time. The amylase treatment also did not change the strength properties of handsheets. It was, therefore, decided to evaluate the influence of amylases on starch degradation and pulp drainage on a pilot-scale paper machine. Pulp at 5 % and 20 % consistency were treated with BAN 480L, Duramyl 300L and Fungamyl 800L. Paper and backwater samples were collected at regular intervals and were evaluated for changes in moisture, starch content and Chemical Oxygen Demand (COD). The moisture was reduced in paper samples after treatment of pulp with Fungamyl 800L at 20 % consistency and the starch content was reduced with Duramyl 300L after treatment at 3 % consistency. In the backwater, the starch content was reduced after treatment with Duramyl 300L at high and low consistencies. The COD did not differ from untreated samples. It was evident that pulp treatment with amylase before papermaking can be beneficial for the papermaker, but further mill-scale evaluation is still needed.

INTRODUCTION

Recycled fibre contains starch that was added during the original papermaking process to increase strength and act as a sizing agent (Erceg, 1984). The starch contained in the recycled pulp can cause lower drainage rates and reduced machine speed (Lascaris *et al.*, 1997), but does not contribute to board characteristics. The secondary starch can be degraded by amylases to improve drainage as demonstrated at the Visy paper mill in Australia when an increase in paper production of up to 6,8 % after amylase treatment was reported (Lascaris *et al.*, 1997).

During preliminary studies it was not possible to demonstrate laboratory scale drainage improvement after enzyme treatment (Chapter 3) even though the starch contained in the pulp was successfully degraded by enzymatic action (Chapter 2). To evaluate the drainage rate before and after amylase treatment in the laboratory, Vacuum Drainage Evaluation, Canadian Standard Freeness, Drainage Time, Water Retention Value and Handsheet Drainage Time were determined but no repeatable improvements were found. The amylase treatment did not have a negative impact on the physical characteristics of handsheets such as Burst Index, Tear Index and Air Permeance (Chapter 3). The aim of this study was, therefore, to evaluate the influence of amylases on starch degradation and pulp drainage on a pilot-scale paper machine.

MATERIALS AND METHODS

1. First pilot trial

1.1. Pulp

K3 and K4 pulp was used during this trial. K4 pulp that is used in the production of fluting consists of recycled corrugated containers and kraft wrapping while K3 pulp is used for linerboard and consists of new corrugated container off-cuts. K3 and K4 pulp in the form of noodle pulp was repulped and made up to 0,5 % consistency in a 220 L stock tank where it was heated to 40 °C before enzyme treatment.

1.2. Enzyme treatment

The pulp at 0,5 % consistency was treated with BAN 480L (140 U/g pulp), Fungamyl 800L (175 U/g pulp), a combination of BAN 480L (140 U/g pulp) and AMG 300L (140 U/g pulp) at 40 °C for 30 min. Untreated pulp was used as control. AMG 300L is an amyloglucosidase that removes single glucose molecules from the non-reducing end of amylose or amylopectin. This enzyme also has limited debranching capabilities. BAN 480L and AMG 300L were applied in combination to test possible synergetic effects. Each treatment was tested in a single batch.

1.3. Papermaking

A Fourdrinier machine (Brüder-Kämmerer, Osnabrück, Germany) with a plastic forming wire with an open area of 13 % was used to make paper from treated

pulp. The paper-machine speed was set at 1,42 m/min to produce paper with a width of 210 mm and an average basis weight of 122 g/m^2. The treated pulp was pumped from the stock tank to the headbox and diluted to 0,3 % consistency by addition of backwater.

1.4. Sampling

Paper samples were taken every two minutes after the paper passed through the press section of the paper machine. The samples were placed in sealed plastic bags to prevent evaporation. These samples were used to gravimetrically determine the moisture content of the paper on a wet basis. Backwater samples (approximately 25 ml) were also collected every two minutes to determine the starch content. The backwater samples were placed in a boiling water bath for 30 min to denature the enzyme.

2. Second pilot trial

2.1. Pulp

Due to the high cost of pilot trials, only K4 pulp was used during the second trial. The consistency of K4 noodle pulp was adjusted to 20 % by adding water and the predetermined enzyme dosage. The pulp and enzyme were mixed with a mechanical mixer (Malvis Engineering Limited, London, UK) for 90 sec. The pulp was then placed in sealed plastic bags and incubated at 40 °C for 30 min in a thermostatically controlled water bath before repulping in the stock tank to 0,5 % consistency. The diluted pulp was then pumped to the headbox and further diluted to approximately 0,3 % consistency with the addition of backwater.

2.2. Enzyme treatment

The pulp was treated with BAN 480L, Duramyl 300L, and Fungamyl 800L at dosages of 400, 140, and 400 U/g pulp, respectively. Untreated pulp was used in the control runs.

2.3. Papermaking

Paper was made as described for the first pilot trial.

2.4. Sampling and statistical analysis

Paper and backwater samples were taken and treated in the same manner as described for the first pilot trial. In addition to determining the starch content of the backwater samples the Chemical Oxygen Demand (COD) and starch content of the paper samples were also determined. Each treatment and papermaking run was replicated three times and 10 to 15 samples collected. The average values of the 10 to 15 samples were used as representative values for each replication and each data set was subjected to one-way analysis of variance. The means of the various treatments were tested for significant differences with Tukeys test (Winer, 1971) at 95 % confidence.

3. Third pilot trial

3.1. Pulp

Only K4 pulp was used during the third trial and the noodle was made up to a consistency of 3 % (30 L) in the stock tank. The pulp was heated to 40 °C for enzyme treatment. After treatment for 30 min the pulp was diluted with cold water to 0,5 %

(180 L) and after thorough mixing the pulp was pumped to the headbox for final dilution to 0,3 % with backwater.

3.2. Enzyme treatment

The pulp in the stock tank was treated at low consistency with BAN 480L, Duramyl 300L and Fungamyl 800L with dosages of 140, 140 and 175 U/g pulp, respectively. Control runs were done without the addition of enzyme. Each treatment was replicated three times.

3.3. Papermaking

Papermaking was done as described for the previous pilot trials.

3.4. Sampling and statistical analysis

Paper and backwater samples were treated as described for the previous trials. The COD of the backwater and starch content of the paper samples were also determined. The means of 10 to 15 samples were used as representative for the replication of every treatment and the data was subjected to one-way analysis of variance. The means of the various treatments were tested for significant differences with Tukeys test (Winer, 1971) at 95 % confidence.

4. Starch content of paper

The starch contents of the paper samples were determined by following Tappi Test Method T419 (Starch in paper) (Anon, 1988). The samples were disintegrated in hot water (100 ml) with a blunted electric blender (Kenwood Chef 750 W KM300, Kenwood LTD Havant, UK). After disintegration and hot-water extraction, the

samples were vacuum filtered through Whatman 541 filtration paper (Whatman International Ltd, Maidstone, Kent UK) and the HCl extraction was done (25 ml 6N HCl for 3 min repeated twice and then 25 ml of concentrated HCl for 20 sec). The starch concentration was determined as described previously (Chapter 2).

5. Starch content of backwater

The starch contents of the backwater samples were determined by following the prescriptions of Tappi Method T419 (Starch in paper). No extraction was needed and the samples (150 ml) were centrifuged for 10 min at 9820 *g* on a Beckman J2-MC centrifuge using a JA14 rotor (Beckman-Coulter, Fullerton, California, USA). The starch content was assayed as described previously (Chapter 2).

6. Chemical Oxygen Demand (COD) of backwater

COD was determined according to the Hach Method 8000 (Oxygen Demand, Chemical Method 8000) (Anon, 1997). Samples (100 ml) were homogenised with a Heidolph Diax 600 (Heidolph Instruments GmbH & Co., Schwabach, Germany) at 10 000 rpm for 30 sec. After homogenisation, 0,2 ml of the sample was transferred to a Hach high range plus (0-15000 mg/L) COD reactor vial (Hach, Loveland, Colorado, USA) and the vial was placed in the Hach COD reactor (Hach, Loveland, Colorado, USA) at 150 °C for two hours. A control sample containing 0,2 ml dH_2O was used. After digestion, the colorimetric measurement was taken with a Hach Calorimetric Reader (Hach, Loveland, Colorado, USA) (620 nm).

RESULTS AND DISCUSSION

1. First pilot trial

Only the combination of BAN 480L and AMG 300L slightly reduced the moisture of the paper (0,13 percentage points) made from K3 pulp (Table 4.1) (Appendix D1). The enzymatic treatment of K4 pulp produced much better results. After treatment with Fungamyl 800L the moisture was reduced by 4,5 percentage points, the combination of BAN 480L and AMG 300L reduced the moisture by 4,0 percentage points and the treatment of pulp with BAN 480L reduced the moisture by 2,7 percentage points (Table 4.1).

Table 4.1. Change in moisture and starch content compared to control samples after enzyme treatment.

	Pulp	**BAN 480L**	**Fungamyl 800L**	**BAN 480 L + AMG 300L**
Moisture of paper (% points)	K3	0,0	0,0	-0,1
	K4	-2,7	-4,5	-4,0
Starch in backwater (% points)	K3	+28,4	+21,8	+27,6
	K4	+27,4	+7,9	+8,4

During this trial all the enzyme treatments on K3 and K4 pulp increased the starch concentration in the backwater samples (Table 4.1). This could possible be ascribed to partially degraded starch that was released from the web and drained with the backwater. Unfortunately these experiments were not replicated and further pilot trials were required to allow for statistical analysis of high and low consistency pulp treatments. It was also decided to exclude K3 pulp from further pilot trials due to the high cost of conducting pilot-scale-trials.

2. Second pilot trial

Paper treated with Fungamyl 800L contained 0,8 percentage points less water after pressing when compared to control samples, but the other enzymes did not decrease the moisture content significantly during this trial (Table 4.2) (Appendix D2). Fungamyl 800L was also the most efficient to remove the starch (-12,8 percentage points) from pulp and BAN 480L showed no improvement in starch content.

Table 4.2. Change in moisture and starch content compared to control samples after enzyme treatment of pulp at high consistency.

	BAN 480L	**Fungamyl 800L**	**Duramyl 300L**
Moisture of paper (% points)	-0,6	-0,8*	-0,2
Starch in paper (% points)	0,0	-12,8	-2,4
Starch in backwater (% points)	+17,4	+0,5	-50,9*

* Values significantly ($p \leq 0,05$) different from control treatments.

Duramyl 300L caused a significant reduction in starch content of backwater (50,9 %), but BAN 480L and Fungamyl 800L increased starch levels (Table 4.2). The increase in starch content was possibly due to the release of partially degraded starch from the web and increasing the concentration of the starch in the backwater.

Enzymatic treatments did not cause any significant changes to the COD values of the drained water (Figure 4.1). The low volumes of enzyme used to degrade the starch had no direct effect on the COD, but when more fines are released from the web due to starch breakdown COD values could increase. The effect of primary starch is also uncertain and a mill-scale evaluation needs to be done.

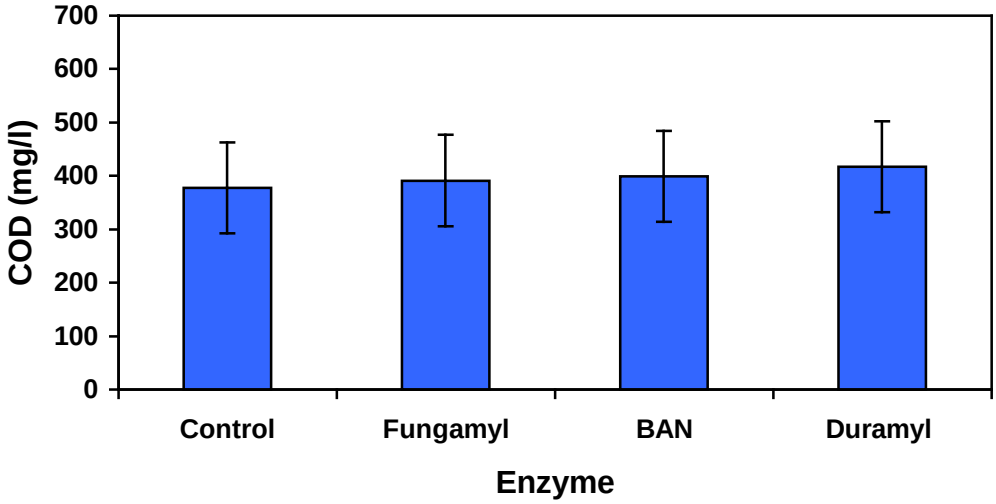


Figure 4.1. Mean Chemical Oxygen Demand of the backwater samples from enzyme treated pulp. Error bars = Q values at $p \leq 0,05$

The second pilot trial showed that Fungamyl 800L can be introduced in high-consistency unit operations to degrade secondary starch. The removal of the starch could lead to drainage-rate increases in a paper mill but the conditions in a paper mill differ too much from the pilot scale machine to make an accurate estimation.

3. Third pilot trial

The highest reduction in moisture content of paper was achieved with Duramyl 300L (-2,0 %) (Table 4.3) (Appendix D3). Duramyl 300L also produced the best results with a 45,0 percentage points decrease in the starch content. All three enzymes decreased the starch content of the paper samples significantly (Table 4.3). Duramyl 300L reduced the starch content of the paper samples significantly more than the other two enzymes when enzyme treatment was done at low consistency.

Table 4.3. Change in moisture and starch content compared to control samples after enzyme treatment of pulp at low consistency. .

	BAN 480L	Fungamyl 800L	Duramyl 300L
Moisture of paper (% points)	1,1	1,1	2,0
Starch in paper (% points)	28,6 *	22,6 *	45,0 **
Starch in backwater (% points)	17,1 *	9,7 *	59,8 **

* Values significantly ($p \leq 0,05$) different from control treatments

** Values significantly ($p \leq 0,05$) different from control and other values in the same row

The starch content of the backwater samples were all significantly reduced by the enzymes, with Duramyl 300L performing significantly better than the other treatments by removing 59,8 percentage points more starch than the control (Table 4.3). The COD values were not influenced by the enzyme treatment at low consistency (Figure 4.2) possibly because of the small volume of enzyme used during the treatment.

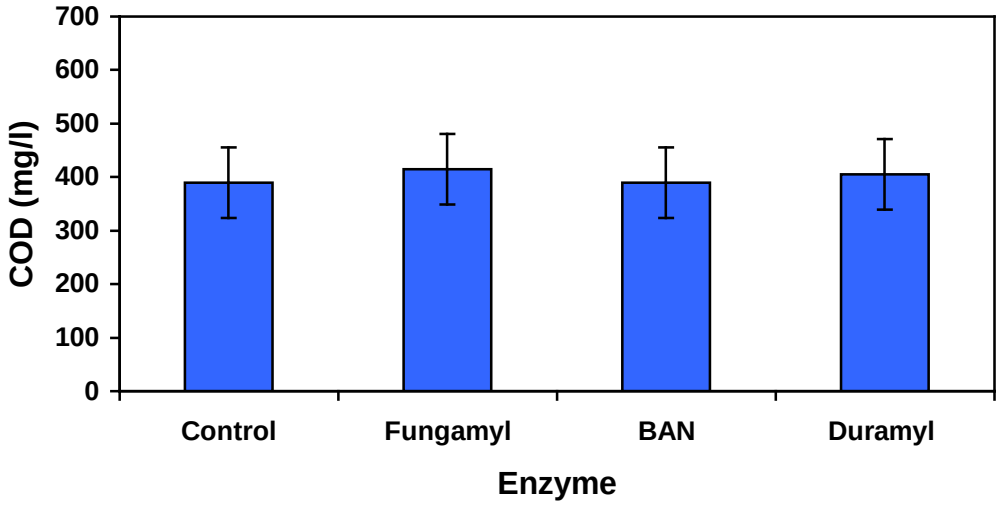


Figure 4.2. Mean Chemical Oxygen Demand of the backwater samples from enzyme treated pulp. Error bars = Q values at $p \leq 0,05$

CONCLUSIONS

The results from the first pilot trial suggested that K4 pulp is better suited for drainage evaluation on a pilot-scale paper machine. Fungamyl 800L was the only enzymatic treatment to achieve a significant reduction in the moisture content of the paper (0,8 percentage points) in pilot trials when it was applied to pulp at 20 % consistency. Fungamyl 800L can therefore, be introduced in high-consistency unit operations to degrade secondary starch.

Duramyl 300L was the most successful in drainage improvement during low-consistency treatment. The removal of 2,0 % water after pressing as achieved by Duramyl 300L could lead to an increase in production of between 7 and 14 %, or savings in energy consumption in the drying section of a paper machine. By reducing moisture by 3,6 % in the Visy mill Lascaris *et al.* (1997) increased production by 6,8 % during the mill trial. All the enzymes reduced starch levels in the paper samples and the backwater during the low consistency treatment. The drainage should, therefore, be improved on a mill-scale as demonstrated previously (Lascaris *et al.*, 1997).

When the second and third pilot trials are compared, all the enzymes performed better on pulp at low consistency (3 %) treatment, possibly because of a higher rate of enzymatic diffusion between the fibres, bringing it into contact with more starch. These results indicate that enzymatic treatment should rather be introduced in low-consistency unit operations but further investigation on a mill-scale is required to confirm these observations. I recommend Duramyl 300L for application

at a mill trial, because of its better stability over different pH levels, its wide temperature tolerance (Chapter 2) and its superior ability to degrade the residual starch in the pulp and backwater. The present study indicates that the relatively low cost and the small volumes of enzyme used to treat pulp could make this process economically feasible.

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CHAPTER 5

MILL-SCALE EVALUATION OF DURAMYL 300L TO IMPROVE DRAINAGE OF RECYCLED FIBRE

The Macpulper at Sappi Cape Kraft

ABSTRACT

The secondary starch contained in corrugated containers does not contribute to strength when the fibre is recycled, but it can be degraded with amylases to improve drainage and production rates. This study describes the mill-scale evaluation of an enzymatic process for the improvement of drainage that was conducted at Cape Kraft, South Africa. An amylase (Duramyl 300L) was applied to K4 pulp that was used to produce fluting (195 g/m^2). The Macpulper was selected as application point for the enzyme, because it is a semi-continuous process with easy access for application. Parameters such as pH, temperature, starch content, Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) and moisture content were measured at a number of sampling points. The machine speed, steam consumption in the drying section and moisture content of product were recorded in the control room and strength properties determined. The tested enzyme degraded starch effectively (66 %) under mill conditions, leading to improved drainage as reflected in the decreased moisture contents of trim samples and product. However, machine speed or steam consumption did not improve. The enzymatic treatment did not influence strength properties. COD and TDS of backwater increased, but the impact on effluent and microbial fouling cannot be predicted. I recommend that the trial be repeated for a longer time and that the process must be evaluated for other fluting grades. The enzyme dosage must still be optimised and a longer time allowed before and after application to better observe the effect of the treatment. Future studies should include the impact of the process on effluent treatment and microbial fouling.

INTRODUCTION

The residual starch contained in corrugated containers does not contribute to strength when the fibre is recycled (Lascaris *et al.*, 1997). The residual starch is dispersed during repulping to form an amorphous layer on fibres, which contributes to drainage resistance. This residual starch can be degraded with amylases that are commercially available at a relatively low cost (Chapter 2 and Chapter 3). Pilot trials have shown that amylases can be applied to K4 pulp to increase drainage and production rates (Chapter 4). Duramyl 300L was the most effective enzyme on K4 pulp, but BAN 480L was less expensive and also benefited the paper-making process. This chapter describes the mill-scale evaluation of an enzymatic process for the improvement of drainage that was conducted at Cape Kraft. Different results were expected during this trial because the mill differs from the pilot scale machine in various process parameters such as size, speed, enzyme treatment temperature and single ply fluting on the pilot scale paper machine compared to multiple ply fluting produced in the mill. A further important difference is that the mill sprays starch on the paper as filler and binding agent but no starch was added during the pilot scale experiments.

Sappi Cape Kraft produces paper from recycled fibre. The recycled fibre is repulped in a Macpulper. The paper machine produces multiple ply fluting and after the stock is distributed through the slices it is sprayed with uncooked starch to aid inter-ply bonding. The starch is gelatinised in the drying section. Strength tests are done at regular intervals on the finished product.

A mill trial with amylase by Visy Paper in Australia resulted in a 5,3 % increase in dry-end speed and 6,9 % increase in production (Lascaris *et al.,* 1997). Strength properties were variable, but board passed all quality control tests. No primary starch was, however, added during this trial and it can be expected that addition of wet-end starch could ameliorate negative changes (personal communication: N Franks, Novozymes USA). Possible benefits of enzyme treatment can be reduced consistency in the headbox, increased machine speed, saving of energy during drying (Lascaris *et al.,* 1997) and reduced turbidity of clarifier effluent (Schwonke and Davis,1973).

MATERIALS AND METHODS

1. Enzymatic treatment

Duramyl 300L was selected for the trial despite its relatively high cost. The enzyme, when compared to the other enzymes tested, produced good results overall and was the most effective in laboratory and pilot-scale experiments to degrade starch. The physical conditions at the point of application include temperature, pH and shear, and under all these simulated conditions in the laboratory Duramyl 300L produced satisfactory results. The enzyme (25 kg) for the trial was donated by Novozymes (Denmark) and on arrival at our laboratories the enzyme activity was determined according to the Novo method described earlier (Chapter 2). The pulp was treated at a dosage of 104 x 10^3 KU/tonne. The volume of the enzyme treatment was 400 ml/tonne in comparison to the 2,25 L/tonne used by Lascaris *et al.* (1997).

2. Trial plan

The Macpulper was selected as application point for the enzyme because it is a semi-continuous process with easy access for application. The capacity of the Macpulper is 1,2 bdt fibre and 480 ml of enzyme was added to each batch while the pulper was filling. On average, the residence time was 8 min, but exposure of the pulp to the enzyme in other unit operations allowed for further degradation of starch. Application in the Macpulper also allowed for treatment of both the filler and top-line pulps that are further refined later on in the production process. It was possible, however, that some of the enzyme was lost with rejects and not carried forward but the exposure time during repulping and in the dump chest would have been enough to degrade some of the secondary starch.

Twenty-eight batches of K4 pulp were treated for production of 195 g/m^2 fluting. The first enzyme was dosed at 14:37 on 21 January 2003 and the last at 23:00, but sampling and measurement started from 13:15 and continued until 03:00 on the next day (Table 5.1).

Table 5.1. Frequency and positions sampled during the mill trial to evaluate enzymatic drainage improvement

Parameter	**Sampling points**				
	Macpulper	**Filler Line Vertical screen**	**Top Line Vertical screen**	**Reverse Press**	**Trim**
Starch	Batch	30 min	30 min	30 min	20 min
COD				30 min	
TDS				30 min	
Moisture					20 min
Temperature	Batch				
PH	Batch				

3. Sampling, measurement and analysis

Pulp was sampled at the outlet of the Macpulper to determine pH and temperature of each batch. The pH was measured with a handheld pH meter (Hanna Instruments, USA) and temperature with a mercury thermometer. Samples (approximately 250 ml) were placed in a boiling water bath for 30 min to denature the amylase. The starch contents of the paper samples were determined by following Tappi Method T419 (Starch in paper) (Anon, 1988).

Thin stock was sampled (approximately 250 ml) from the vertical screens in the filler and top lines, because the screens are the last accessible points before primary starch is added during papermaking. The amylase was denatured in a boiling water bath and the starch content determined according to Tappi Test Method T419 (Starch in paper) on return to the laboratory.

Backwater was sampled at the reverse press for starch analysis, determination of Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS). COD was determined according to the Hach Method 8000 (Oxygen Demand, Chemical Method 8000) (Anon, 1997) with the “High Range Plus” reagent. TDS was determined with a hand-held probe (Hanna Instruments, USA). The reverse press was selected because it is routinely used for microbial sampling and it allows sampling before backwater is combined with other effluent streams. The COD and TDS was measured to indicate possible impacts of the enzymatic process on microbial fouling, since carbohydrates that were previously retained in the pulp in the form of residual starch will be released to the backwater as dissolved sugars.

The machine speed, steam consumption in the drying section and moisture content of K4 on the Jumbo roll were recorded every 10 min by the mill staff in the control room. Strength properties (Scott bond and Flat crush resistance) were determined by mill technicians for every Jumbo roll according to Work Instruction documents of the mill (Horne, 2002a; Horne, 2002b).

RESULTS AND DISCUSSION

The temperature of the pulp in the Macpulper varied between 51 °C and 54 °C and the pH fluctuated between 6,35 and 6,57. Previous experiments (Chapter 2) have indicated that the cost of an effective dosage of BAN 480L would be approximately 50 % higher than Duramyl 300L under these conditions. The results from the pilot trial experiments (Chapter 4) differed from the mill trial results as was expected, probably because of a managed wet-end chemistry at the mill-scale paper machine.

After the production was changed to 195 Flute (at 13:00) the process was stabilised before the machine speed was increased from 285 m/min to 290 m/min (at 15:55) and later (at 16:10) to 295 m/min. These speeds are in the range of the normal operational conditions. Steam consumption remained constant at 5,2 bar in the drying section.

The starch content in the last Macpulper batch before the trial started was 3,95 mg/L (Figure 5.1). The starch content was immediately decreased by application of Duramyl 300L and while enzyme was applied, it remained at an average of 1,33 mg/L. The starch content did not increase immediately when the application of amylase was ceased, since repulping is a semi-continuous process and approximately

10 % of enzyme was retained in the Macpulper. After 12 untreated batches the residual enzyme appeared to be removed from the system when the starch content reached 3,35 mg/L. The starch content of the Trim was also reduced after application of amylase, but when compared to the starch concentrations of the Macpulper the starch content was very high due to the presence of primary starch (Figure 5.1).

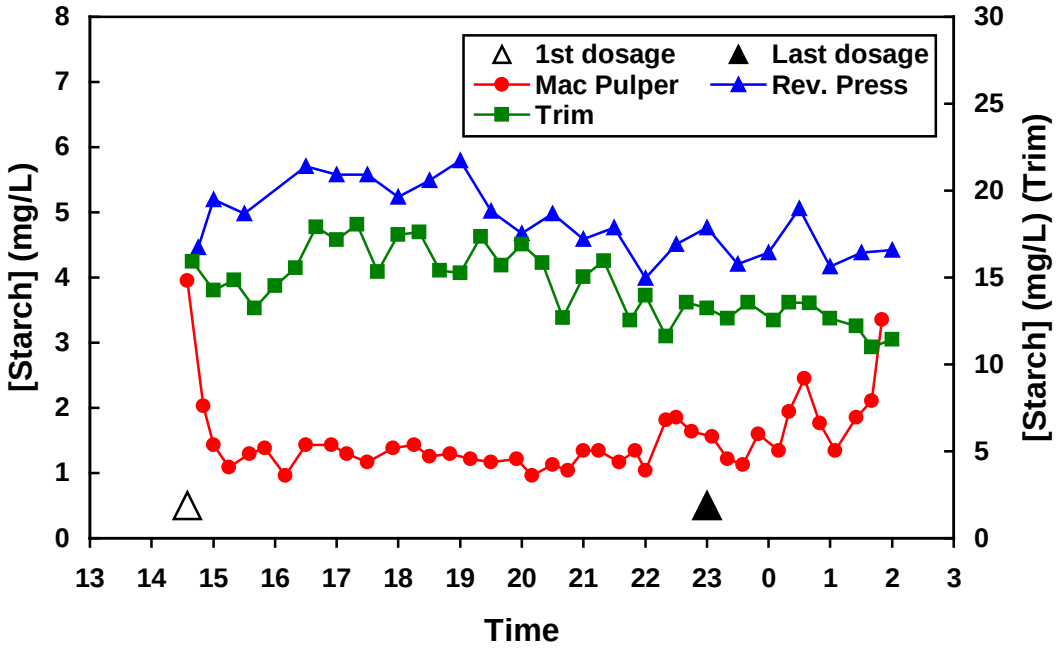

Figure 5.1. The influence of enzyme treatment on the starch content of the different pulp samples during the trial.

Two hours after the enzyme was first applied, the starch content in the backwater at the reverse press started to decrease (Figure 5.1) at a rate of 0,125 mg/L/h (R^2=0,65). However, in the thin stock at the vertical screens, the reduction in starch content was visible after only 20 min (Figure 5.2). The starch content in some samples were much higher when enzyme application was stopped, but variability between these samples was too large to confirm any trends.

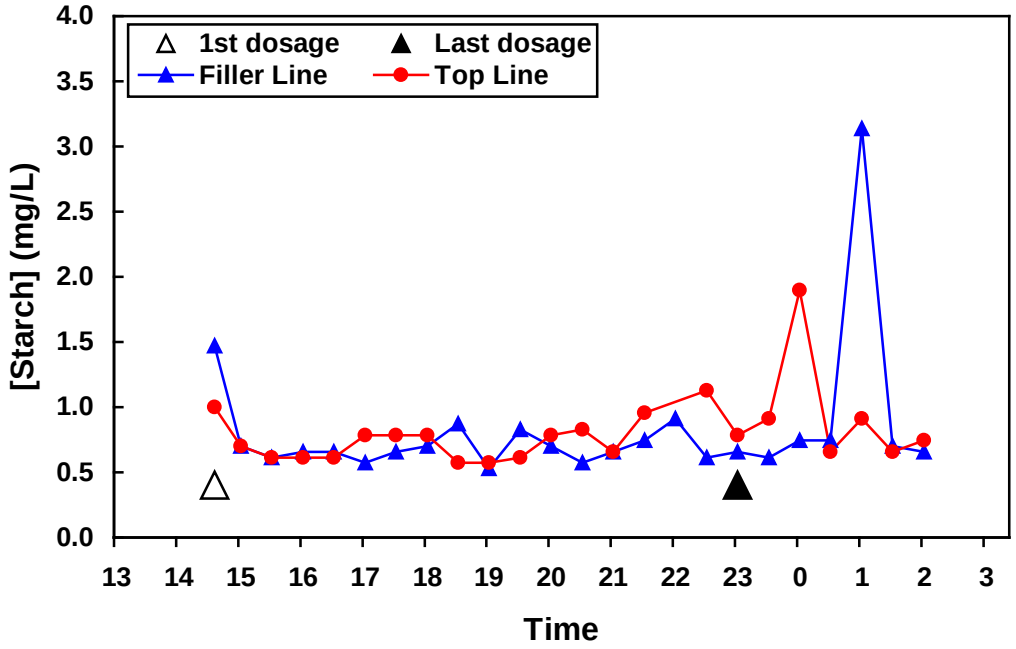

Figure 5.2. The influence of enzyme treatment on the starch content of thin-stock samples during the trial.

The moisture content of the Trim samples was reduced (Figure 5.3) during the trial, but was only weakly correlated (R^2=0,19) to the starch content of the same samples (Figure 5.4). The moisture content of board after the drying section remained fairly constant throughout the trial, but increased rapidly when the enzymatic treatment came to an end.

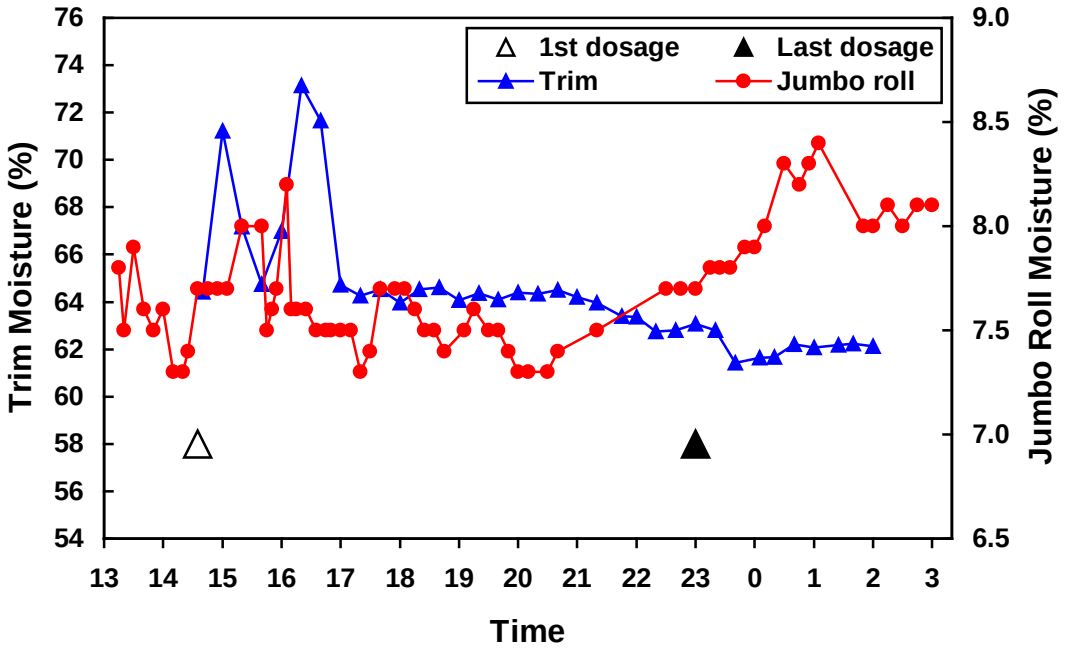

Figure 5.3. The influence of enzyme treatment on moisture content of the Trim and the Jumbo roll during the trial.

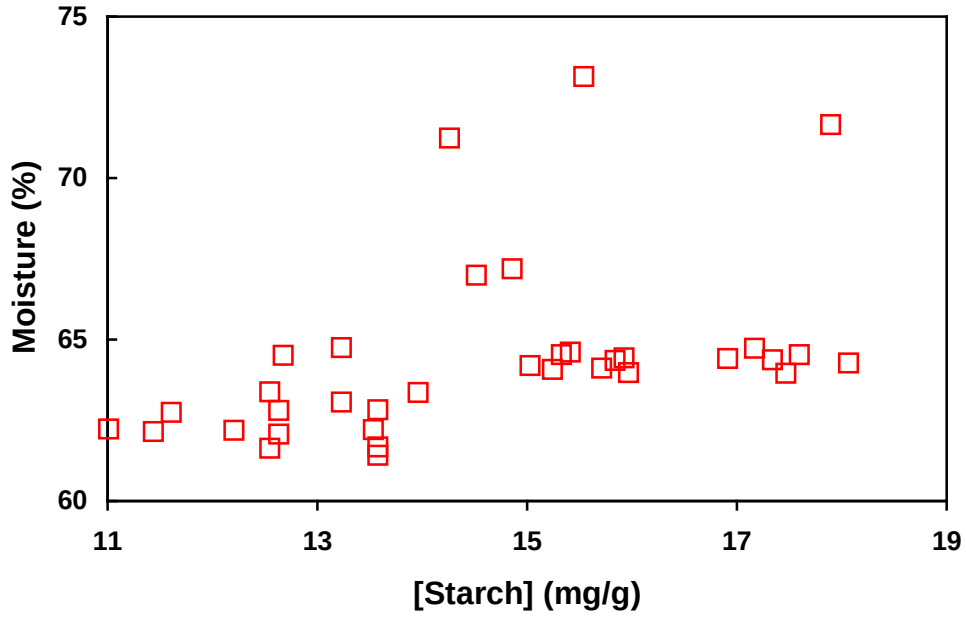

Figure 5.4. Relationship between starch content and moisture of Trim samples.

There was a trend for strength properties (Scott Bond and Flat Crush) of board to decrease during the trial (Figure 5.5). However, the downward trend continued even after application of enzyme was stopped and it is possible, therefore, that these

changes were related to machine speed and not enzyme application. All of the board produced during the trial conformed to product specifications.

The COD and TDS showed an increase during the trial (Figure 5.6), indicating that starch degradation has an impact on effluent quality. Longer trials will be required to determine the influence of these parameters on microbial fouling as well as effluent treatment processes.

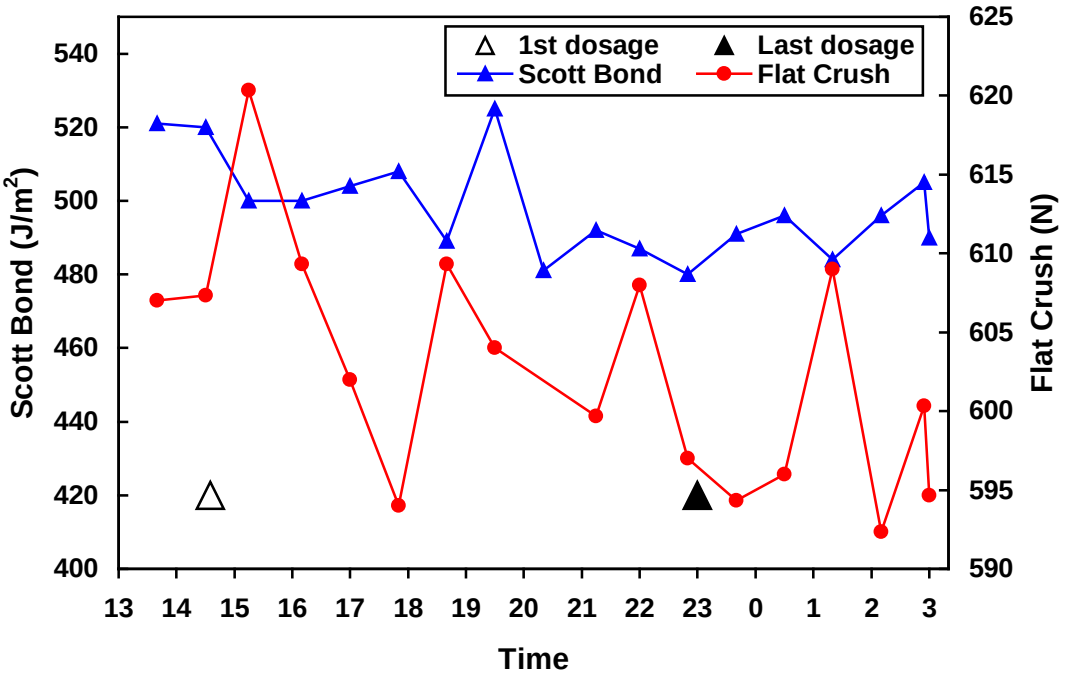

Figure 5.5. The influence of enzyme treatment on strength properties of the board produced during the trial.

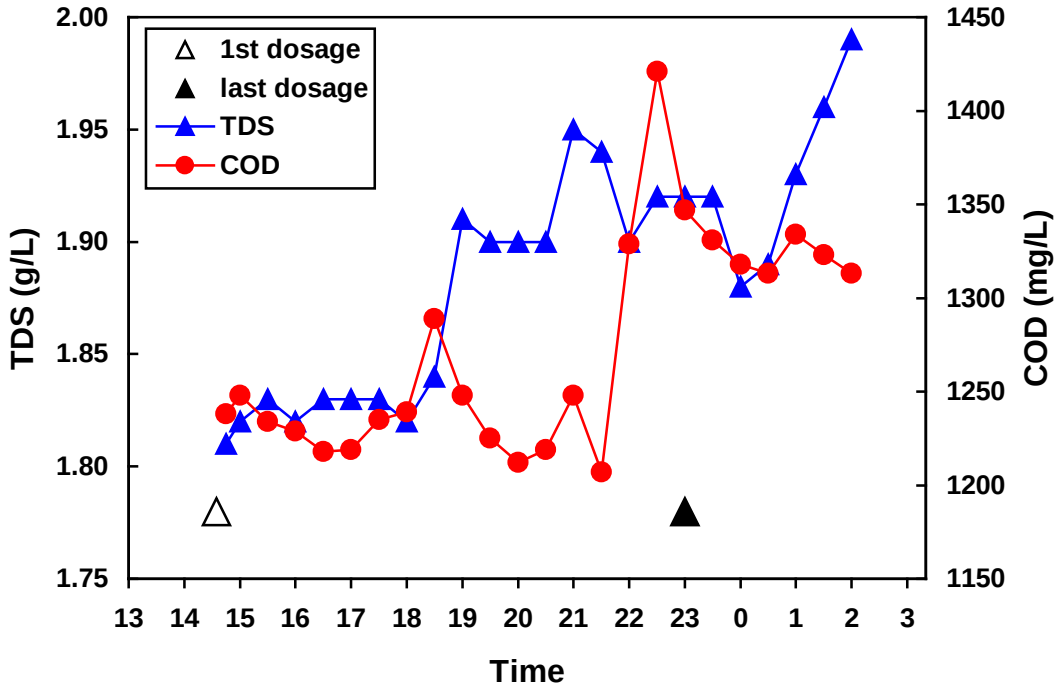

Figure 5.6. The influence of enzyme treatment on Total Dissolved Solids and Chemical Oxygen Demand during the trial.

CONCLUSIONS

Duramyl 300L was efficient in degrading starch under mill conditions and led to improved drainage as reflected in the decreased moisture contents of trim samples and product. Unfortunately the improved drainage did not improve machine speed or steam consumption. The strength properties of the finished product did not seem to be influenced by the enzyme treatment. COD and TDS of backwater increased, but the impact on effluent and microbial fouling cannot be predicted.

It is recommended that the trial be repeated for a longer time. The process must also be evaluated for other fluting grades. The enzyme dosage must be optimised to ensure an economic process. The small volume of enzyme used for the treatment (400 ml/tonne) in comparison to Lascaris *et al.*, (1997) (2,25 L/tonne) is of

great economic importance when evaluating the results. With dosage optimisation the volume of enzyme used will be less, making this process more profitable. Longer time must be allowed before and after application to better observe the effect of the treatment. Future trials should also assess the impact of the process on effluent treatment and microbial fouling.

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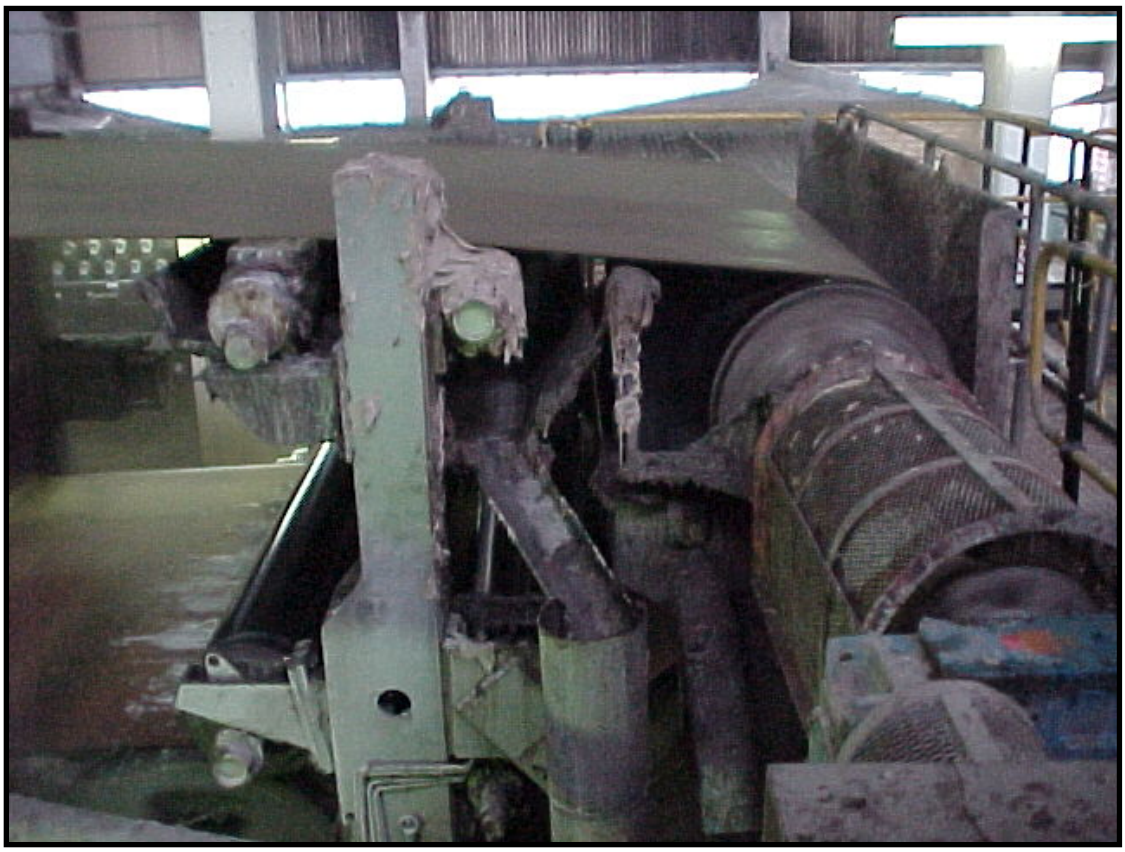
CHAPTER 6

GENERAL CONCLUSIONS



Reverse press and wire at Sappi Cape Kraft

Research into biotechnological applications of enzymes for the pulp and paper industry is receiving a lot of attention. There is a worldwide increase in recycling and the use of recycled fibres for the production of paper. Unfortunately recycled fibre has a very high fines content and also contains residual starch applied in the initial papermaking process. These two factors contribute to the low drainage rate of recycled fibre, but the situation can be improved with enzymes. Cellulases break down fines to increase drainage, but cellulase can also attack the cellulose fibres, thus decreasing the strength of the paper. Amylase enables the degradation of the secondary starch in the recycled fibre, thereby increasing the drainage rate without affecting the strength properties.

BAN 480L, Duramyl 300L and Fungamyl 800L were able to sufficiently degrade secondary starch in pulp at 40 °C without the addition of $CaCl_2$ but Termamyl 120L was excluded from further studies due to its low activity at 40 °C. BAN 480L, Duramyl 300L and Fungamyl 800L all displayed activity over a range of temperatures and pH values. These enzymes also displayed good tolerance to shear forces. Experiments were conducted to evaluate the suitability of the enzymes for application in the paper industry and Duramyl 300L was identified as the most suitable to mill conditions.

The physical properties of the handsheets made with treated pulp indicated that the enzymes would not have a negative influence on the strength properties of paper produced. The laboratory scale drainage improvement after starch degradation could not be quantified using a number of standard techniques. Apparently these tests

were not repeatable enough to measure small drainage improvements. The secondary starch was successfully degraded and, therefore, pilot scale drainage evaluation was needed.

The drainage improvements on the pilot scale paper machine were small but the starch in the backwater and in the paper was reduced significantly. A moisture reduction of only 2 percentage points after the pressing section could translate to an increase in production of between 7 and 14 %, or savings in energy consumption in the drying section of the paper machine. The Chemical Oxygen Demand (COD) of the treated backwater was not significantly increased. Duramyl 300L was most successful to degrade the starch and produce paper with reduced moisture content after the pressing section. The relatively low cost and the small volumes of enzyme used to treat pulp made this process economically feasible, even without optimisation. The influence of Duramyl 300L on pulp was, therefore, evaluated on a mill scale.

Duramyl 300L was successful in degrading the secondary starch and lead to improved drainage as reflected in the decreased moisture contents of trim samples and fluting. However, machine speed and steam consumption did not improve. The enzymatic treatment did not significantly influence strength properties, but COD and Total Dissolved Solids (TDS) of backwater increased. The impact of these increases on effluent and microbial fouling cannot be predicted.

I recommend further trials conducted for longer periods and that the process be evaluated for other fluting grades due to the successful degradation of the starch in the Macpulper and the decrease in moisture in the trim samples showing improved

drainage. The enzyme dosage must still be optimised and a longer time must be allowed before and after application to better observe the effect of the treatment. The small volume of enzyme used for the treatment (400 ml/tonne) in comparison to Lascaris *et al.*, (1997) (2,25 L/tonne) is of great economic importance when evaluating the results. With dosage optimisation the volume of enzyme used will be less, making this process more profitable. Future studies should determine the impact of the process on effluent quality and microbial fouling.

SUMMARY

Keywords: Amylase, Biotechnology, Drainage, Enzyme, Recycled fibre, Starch

The use of recycled fibre to produce paper is increasing due to improved processes and consumer pressure. However, recycled fibre has slow drainage rates because of fines and residual starch. Slow drainage reduces machine speed, production rate, energy demands of the drying section and water consumption. Amylases have been used previously on mill scale to degrade residual starch in paper and improve drainage.

This study evaluated commercial amylases to degrade starch and improve drainage. The enzymes BAN 480L, Duramyl 300L, Fungamyl 800L, Termamyl 120L and AMG 300L were used to treat recycled pulp and the influence of pH, temperature and shear forces was tested on the enzymes. BAN 480L and Duramyl 300L displayed activity over a wide temperature range, while Duramyl 300L had activity over a wide pH range. Termamyl 120L, BAN 480L, Duramyl 300L and Fungamyl 800L showed good tolerance to shear forces.

The influence of BAN 480L, Duramyl 300L and Fungamyl 800L was tested on strength properties of pulp. Enzymatic treatment had no negative effect on Bursting Index, Tearing Index, Air Permeance and Handsheets Drainage Time. In some cases, strength properties improved. Laboratory-scale drainage evaluation (Canadian Standard Freeness, Drainage Time, Vacuum Drainage Time and the Water Retention Value) showed no significant improvements despite reduction of starch content.

Pilot trials were conducted with different pulp grades and at different consistencies. Paper and backwater samples were evaluated for changes in moisture, starch content and Chemical Oxygen Demand (COD). The COD levels of backwater did not increase after enzymatic treatment. The reductions in starch and moisture content of paper indicated that amylases could improve papermaking, but that mill-scale trials were required to quantify possible benefits.

Duramyl 300L was applied in a mill trial to treat K4 pulp for production of 195 Flute. Temperature, pH, starch, COD, Total Dissolved Solids (TDS), and moisture were measured at different sampling points. The machine speed, steam consumption, moisture and strength properties of the product were also recorded. Secondary starch degradation of 66 % was achieved and moisture of the trim and jumbo roll was reduced. Machine speed and steam consumption did not improve and no significant influence on strength properties was found. The backwater COD and TDS increased, but were still within control limits. Longer trials should be conducted in future to focus on optimisation of enzyme dosages and production of other fluting grades. The impact of the enzymatic treatment on effluent quality and microbial fouling should also be investigated.

OPSOMMING

Sleutelwoorde: Amilase, Biotegnologie Dreinering, Ensiem, Herwinde vesel, Stysel

Die gebruik van herwinde vesel om papier te produseer neem toe as gevolg van verbeterde prosesse en druk van verbruikers, maar herwinde vesel lei tot stadige dreineringstempo's as gevolg van kort vesels en residuele stysel. Stadige dreinering verminder die spoed van die papiermasjien, die produksietempo, energievereistes van die drogingsproses asook waterverbruik. Amilases is voorheen in 'n meule gebruik om residuele stysel in papier af te breek en sodoende dreinering te verbeter.

In hierdie studie is kommersieële amilases evalueer om stysel af te breek en dreinering te verbeter. Die ensieme BAN 480L, Duramyl 300L, Fungamyl 800L, Termamyl 120L en AMG 300L is gebruik om die herwinde pulp te behandel en die invloed van pH, temperatuur, en sleurkragte is op die ensieme getoets. BAN 480L en Duramyl 300L het ensiemaktiwiteit oor 'n wye reeks temperature getoon, terwyl Duramyl 300L ook aktiwiteit oor 'n wye pH reeks getoon het. Termamyl 120L, BAN 480L, Duramyl 300L en Fungamyl 800L het goeie weerstand teen sleurkragte getoon.

Die invloed van BAN 480L, Duramyl 300L en Fungamyl 800L op die sterkte-eienskappe van pulp is getoets. Ensimatiese behandeling het geen negatiewe effek op Barsindeks, Skeurindeks, Lugdeurlating en Dreineringstyd van toetsvelle gehad nie. In sekere gevalle is die sterkte-eienskappe verbeter, ten spyte van die afbraak van stysel. Laboratoriumskaal dreineringstoetse ('Canadian Standard Freeness',

Dreineringstyd, Vakuumdreingeringstyd en Water Retensiewaarde) het nie betekenisvol verbeter nie,

Proewe op loodsskaal is onderneem met verskillende grade van pulp en teen verskillende konsistensies. Papier en monsters van dreineringswater is geneem en die voginhoud, styselinhoud en Chemiese Suurstof Behoefte (COD) is bepaal. Die COD-vlakke van die dreineringswater het nie toegeneem na ensimatiese behandeling nie. Die verlaging van stysel- en voginhoud het aangedui dat amilases moontlik tot ‘n verbeterde papiermaak proses kan lei, maar dat ‘n evaluasie op volskaal wel nodig is om die moontlike voordele te kwantifiseer.

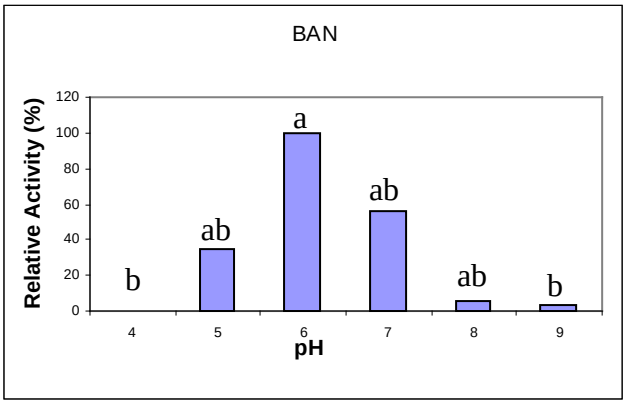
Duramyl 300L is aangewend in ‘n meuleproef om K4-pulp te behandel vir die produksie van 195 riffelbord. Temperatuur, pH, stysel, COD, Totale Opgeloste Vastestowwe (TDS) en voginhoud is gemeet by verskillende punte. Die spoed van die papiermasjien, verbruik van stoom, voginhoud en sterkte-eienskappe van die produk is ook aangeteken. Ses-en-sestig persent van sekondêre stysel is afgebreek en die voginhoud van die afvalpulp en jumbo-rol is verminder. Die spoed van die papiermasjien en die verbruik van stoom het nie verbeter nie en geen betekenisvolle verandering van sterkte-eienskappe is waargeneem nie. Die COD en TDS van dreineringswater het toegeneem, maar was steeds binne perke. Langer meulproewe moet in die toekoms onderneem word om te fokus op optimalisering van die ensiem-toediening en produksie van ander grade van riffelbord. Die impak van ensimatiese behandeling op die kwaliteit van uitvloeisel en opbou van mikrobes moet ook vasgestel word.

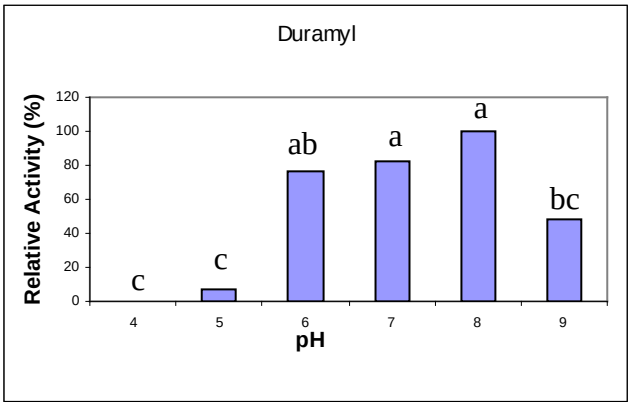
APPENDICES

Water sampling point at Sappi Cape Kraft

APPENDIX A

A1. Influence of pH on amylase enzymes (2 min incubation)





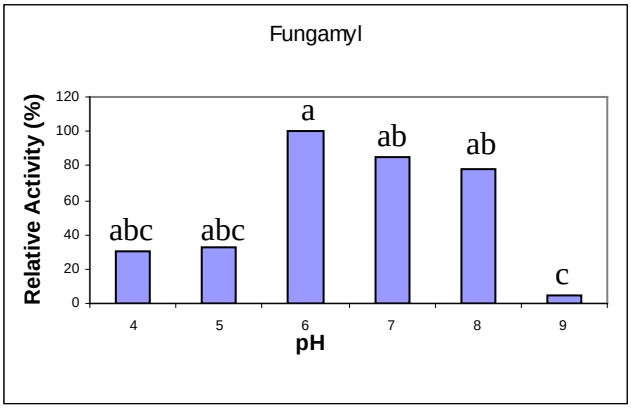
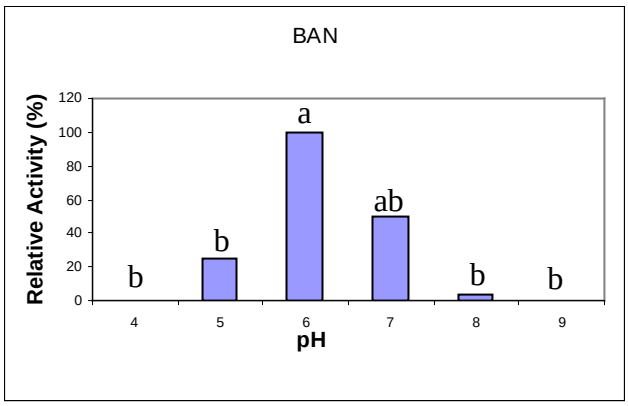
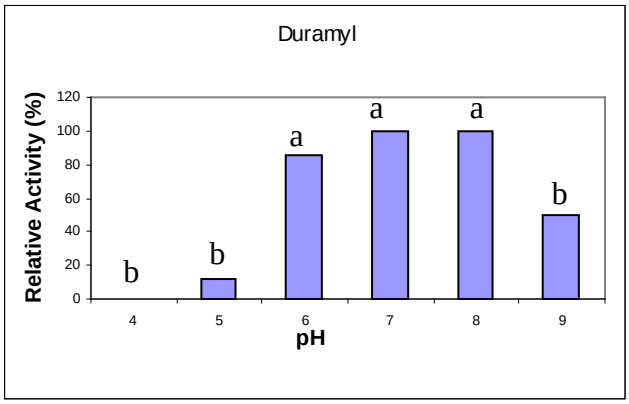


Figure A1. The influence of pH on the different enzymes after pulp incubation for 2 minutes. Bars with the same letters do not differ significantly $p \leq 0,05$.

A2. Influence of pH on amylase enzymes (4 min incubation)





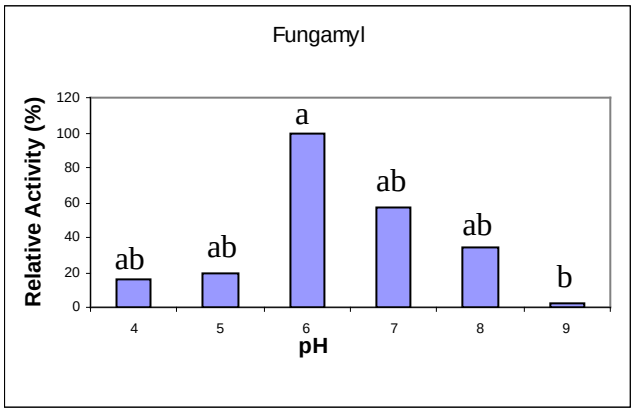


Figure A.2. The influence of pH on the different enzymes after pulp incubation for 4 minutes. Bars with the same letters do not differ significantly $p \leq 0,05$.

APPENDIX B

B1. Repeatability of drainage time of pulp

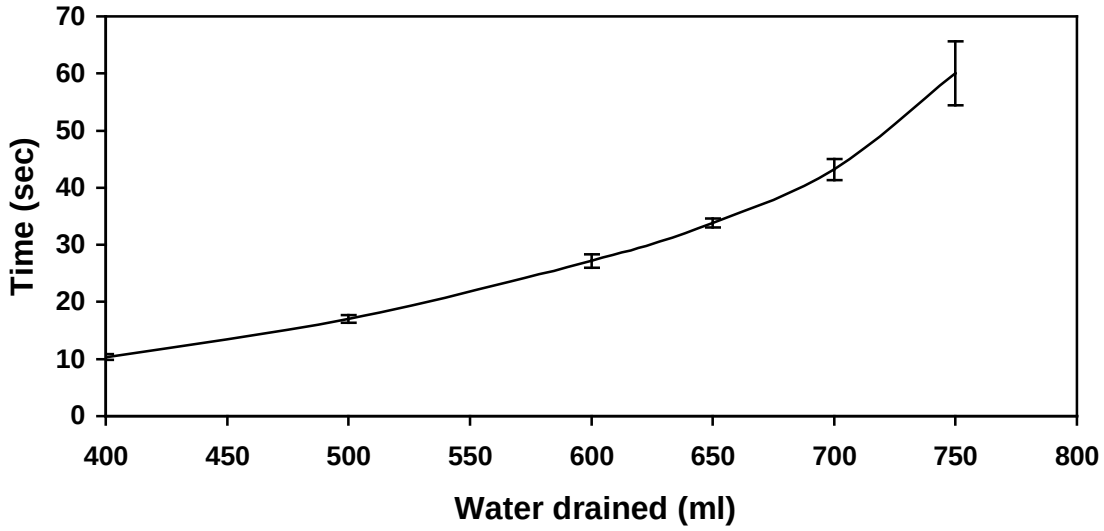


Figure B.1. The average drainage time of untreated pulp. Error bars show standard deviation ($p \leq 0,05$).

B2. Drainage time of treated pulp

Table B.1. Drainage time (sec) for pulp treatment with different enzyme charges using three different methods

Water Volume (ml)	Charge (U/g)	Method 1 (sec)		Method 2 (sec)		Method 3 (sec)	
400	0	7.4		7.4		5.5	
	62	7.1		6.8		5.7	
	135	6.8		7.0		4.8	
	140	7.5		6.8		5.3	
	215	6.8		7.4		5.3	
	536	7.7		7.3		5.5	
Average		**7.2**	**b**	**7.1**	**b**	**5.3**	**a**
500	0	12.1		11.6		8.8	
	62	11.5		11.4		8.9	
	135	11.4		11.4		7.6	
	140	12.3		11.0		8.5	
	215	11.1		12.1		8.5	
	536	12.1		11.7		8.6	
Average		**11.8**	**b**	**11.5**	**b**	**8.5**	**a**
600	0	18.8		18.1		13.1	
	62	15.8		17.6		13.1	
	135	17.4		18.1		11.5	
	140	18.8		17.0		12.9	
	215	16.9		18.8		12.5	
	536	18.4		15.7		13.5	
Average		**17.7**	**b**	**17.5**	**b**	**12.7**	**a**
650	0	23.7		23.2		17.8	
	62	20.6		22.1		16.6	
	135	21.7		22.0		15.2	
	140	23.0		21.0		15.8	
	215	20.7		23.6		16.6	
	536	23.7		22.7		16.1	
Average		**22.2**	**b**	**22.4**	**b**	**16.5**	**a**
700	0	28.5		28.1		20.2	
	62	26.6		27.3		19.9	
	135	26.5		26.9		17.5	
	140	28.2		25.8		19.5	
	215	25.7		29.3		18.9	
	536	28.7		28.5		19.9	
Average		**27.4**	**b**	**27.6**	**b**	**19.3**	**a**
750	0	35.7		35.1		24.3	
	62	30.5		33.9		24.8	
	135	33.9		33.6		20.8	
	140	34.6		32.0		24.3	
	215	31.8		36.5		23.5	
	536	35.5		35.5		24.1	
Average		**33.7**	**b**	**34.9**	**b**	**23.7**	**a**

APPENDIX C

C1. Influence of BAN 480L and Fungamyl 800L on Vacuum Drainage Through a Whatman 541

Experiment 1

Table C.1. Influence of different enzyme dosages on K3 and K4 pulp Vacuum Drainage Time (sec) while drained through a Whatman 541 membrane.

Treatment	Dosage (U/g)	Vacuum Drainage Time (sec)							
		K3 pulp				K4 pulp			
		R 1	R 2	R 3	Average	R 1	R 2	R 3	Average
BAN 480L	0	42.7	58.6	35.9	**45.8**	36.8	70.3	83.1	**63.4**
	65	29.0	37.3	37.9	**34.7**	100.9	62.1	56.0	**73.0**
	140	35.7	35.4	46.2	**39.1**	82.3	52.9	85.8	**73.7**
	220	40.8	37.5	38.4	**38.9**	51.6	52.3	59.3	**54.4**
	350	31.6	36.4	35.6	**34.5**	51.2	45.9	49.2	**48.8**
	550	29.4	53.8	35.8	**39.7**	100.4	52.1	53.0	**68.5**
Fungamyl 800L	0	35.8	46.2	43.8	**41.9**	53.0	69.2	59.6	**60.6**
	20					45.6	54.7	70.3	**56.9**
	80	30.9	35.1	42.1	**36.0**	60.5	114.6	74.3	**83.1**
	110					80.7	64.7	55.7	**67.1**
	140					72.6	67.6	63.9	**68.0**
	230	48.0	42.0	42.6	**44.2**				
	400	40.3	39.5	37.2	**39.0**				
	650					51.1	60.6	66.0	**59.2**
	1000	50.2	38.7	43.8	**44.2**	55.3	60.1	62.3	**59.2**

C2. Influence of BAN 480L and AMG 300L on vacuum drainage through a Whatman 113

Experiment 2

Table C.2. Influence of different enzyme dosages on the Vacuum Drainage Time (sec) of K3 pulp when drained through a Whatman 113 membrane.

Treatment		Dosage (U/g)		Vacuum Drainage Time (sec) R1	R2	R3	Average
BAN 480L			0	31.7	139.0	89.4	**86.7**
			70	57.0	60.6	43.4	**53.7**
			140	44.8	122.3	51.4	**72.8**
			220	50.9	119.7	88.7	**86.4**
			350	45.4	101.5	68.2	**71.7**
			550	50.4	107.0	73.9	**77.1**
BAN 480L	1020	AMG 300L	0	48.1	66.2	60.0	**58.1**
	1020		0.35	89.1	120.6	116.2	**108.6**
	1020		0.7	61.1	82.7	79.7	**74.5**
	1020		1.4	60.8	82.4	79.4	**74.2**
	1020		3.5	47.2	63.9	61.6	**57.6**
	1020		7	83.5	113.0	108.9	**101.8**
	1020		14	40.5	54.8	52.8	**49.4**
	1020		35	66.5	90.1	86.8	**81.1**
	1020		70	36.4	49.3	47.5	**44.4**
	1020		140	41.0	55.6	53.6	**50.1**
	1020		350	52.4	70.9	68.4	**63.9**
	1020		550	38.7	52.4	50.5	**47.2**

C3. Influence of BAN 480L and AMG 300L on vacuum drainage through a wire mesh

Experiment 3

Table C.3. Influence of different enzyme dosages on the Vacuum Drainage Time (sec) of K3 pulp when drained through a wire mesh.

Dosage (U/g)				Vacuum Drainage Time (sec)			
				R 1	R 2	R 3	Average
BAN 480L			0	17.4	18.1	13.3	**16.3**
			1.4	27.3	28.4	21.0	**25.6**
			3.5	16.9	17.5	12.9	**15.8**
			7	19.7	20.4	15.1	**18.4**
			14	24.2	17.9	23.3	**21.8**
			35	26.5	19.6	25.6	**23.9**
			70	17.0	12.5	16.4	**15.3**
			140	24.4	18.0	23.5	**22.0**
			210	18.3	24.8	23.9	**22.4**
			350	11.4	14.9	15.5	**13.9**
			550	17.0	22.2	23.0	**20.7**
			700	17.7	23.1	24.0	**21.6**
	1020		0	18.2	18.9	14.0	**17.0**
	1020		0.7	16.4	17.0	12.6	**15.4**
	1020		1.4	20.9	21.7	16.0	**19.5**
	1020		3.5	17.2	17.8	13.2	**16.1**
	1020		7	16.9	12.5	16.3	**15.2**
BAN 480L	1020	**AMG 300L**	14	21.4	15.8	20.6	**19.3**
	1020		35	19.3	14.3	18.6	**17.4**
	1020		70	17.6	13.0	16.9	**15.8**
	1020		140	14.1	19.1	18.4	**17.2**
	1020		350	15.9	20.8	21.6	**19.4**
	1020		700	14.4	18.7	19.4	**17.5**

C4. Influence of BAN 480L, AMG 300L and Termamyl 120L on vacuum drainage through a wire mesh

Experiment 4

Table C.4 Influence of different enzyme dosages at 60 °C on the Vacuum Drainage Time (sec) of K3 pulp when drained through a wire mesh.

Dosage (U/g)				Vacuum Drainage Time (sec)			
				R 1	R 2	R 3	Average
BAN 480L	1020	AMG 300L	0	21.4	22.2	16.4	**20.0**
	1020		28	19.0	19.7	14.6	**17.8**
	1020		70	24.0	24.9	18.4	**22.5**
	1020		140	23.4	17.3	22.6	**21.1**
	1020		280	21.9	16.2	21.2	**19.8**
	1020		700	27.5	20.3	26.5	**24.7**
Termamyl 120L	1020	AMG 300L	0	17.7	24.0	23.1	**21.6**
	1020		28	18.6	24.3	25.2	**22.7**
	1020		70	17.0	22.2	23.0	**20.7**
	1020		140	19.0	24.8	25.8	**23.2**
	1020		280	25.5	26.5	19.6	**23.9**
	1020		700	22.5	23.3	17.2	**21.0**

C5. Influence of BAN 480L, AMG 300L and Termamyl 120L on vacuum drainage through a Whatman 113

Experiment 5

Table C.5 Influence of different enzyme dosages at 60 °C on the Vacuum Drainage Time (sec) of K3 pulp when drained through a Whatman 113 membrane.

Dosage (U/g)				Vacuum Drainage Time (sec)			
				R 1	R 2	R 3	Average
BAN 480L	1020	AMG 300L	0	32.8	34.0	25.1	**30.6**
	1020		28	37.7	39.1	28.9	**35.2**
	1020		70	44.1	45.7	33.8	**41.2**
	1020		140	34.7	25.6	33.4	**31.2**
	1020		280	38.6	28.5	37.2	**34.8**
	1020		700	45.5	33.6	43.8	**41.0**
Termamyl 120L	1020	AMG 300L	0	28.4	38.4	37.1	**34.6**
	1020		28	16.4	21.4	22.2	**20.0**
	1020		70	30.0	39.2	40.6	**36.6**
	1020		140	28.0	36.6	38.0	**34.2**
	1020		280	35.5	36.8	27.2	**33.2**
	1020		700	45.2	46.9	34.6	**42.2**

APPENDIX D

D1 First pilot trial

Table D.1. Moisture content of paper samples collected during the first pilot trial.

	Control Moisture Content (%)	BAN 480 Moisture Content (%)	Fungamyl 800L Moisture Content (%)	BAN 480L + AMG 300L Moisture Content (%)
K3 pulp	73.21	72.24	71.10	70.61
	71.58	72.63	72.04	71.71
	70.87	72.69	71.83	71.25
	70.84	72.52	72.38	71.04
	70.82	72.59	72.16	71.27
	70.51	72.62	71.82	70.86
		72.56	72.15	71.46
		72.44	71.75	71.15
		72.87	71.81	70.92
		72.88	72.06	71.32
		72.80	71.97	71.40
		72.82	71.86	71.60
		72.64	72.01	71.13
Average	**71.31**	**72.64**	**71.92**	**71.21**
	Moisture Content (%)	**Moisture Content (%)**	**Moisture Content (%)**	**Moisture Content (%)**
K4 pulp	74.40	73.44	65.76	72.92
	74.46	73.34	68.84	72.05
	73.73	72.63	71.46	69.85
	73.26	71.07	70.47	69.91
	72.48	70.46	69.90	69.48
	71.60	69.81	69.70	69.91
	72.13	70.49	68.93	69.79
	72.11	70.60	70.15	69.72
	72.20	70.06	70.12	69.55
		70.65	70.05	69.18
		70.89	70.21	69.47
		70.51	69.72	69.39
		69.48	70.37	69.31
		70.25	69.85	69.78
Average	**72.93**	**70.98**	**69.68**	**70.02**

Table D.2. Starch content of backwater samples collected during the first pilot trial.

	Control Backwater Starch (mg/L)	**BAN 480 Backwater Starch (mg/L)**	**Fungamyl 800L Backwater Starch (mg/L)**	**BAN 480L + AMG 300L Backwater Starch (mg/L)**
K3 pulp	8.70	13.53	9.35	9.35
	10.19	12.65	10.05	13.07
	10.79	13.91	11.17	8.89
	10.52	13.07	10.47	11.63
	10.84	13.30	10.93	12.74
	10.52	12.98	11.49	10.75
		12.19	11.82	10.65
		13.07	11.54	12.28
		13.12	11.77	11.07
		13.02	11.44	11.17
		12.98	11.21	10.79
		13.07	11.58	11.07
Average	**10.26**	**13.07**	**11.07**	**11.12**
	Backwater Starch (mg/L)	**Backwater Starch (mg/L)**	**Backwater Starch (mg/L)**	**Backwater Starch (mg/L)**
K4 pulp	5.41	6.24	10.93	7.73
	5.59	7.12	7.78	7.40
	4.94	7.82	7.08	6.34
	5.22	6.80	6.89	5.87
	4.85	7.12	7.31	5.96
	4.76	6.34	6.75	6.43
	5.92	5.92	6.1	6.34
	5.92	7.12	6.61	7.50
	5.50	7.12	3.64	7.22
		7.12	6.61	6.15
		6.89	7.68	7.91
		7.31	6.71	6.85
		6.99	6.24	6.94
		6.15	0.81	
Average	**5.34**	**6.86**	**6.51**	**6.82**

D2 Second pilot trial

Table D.3. Moisture content of different paper samples during the second pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Moisture Content (%)			
	R 1	**R 2**	**R 3**	
Control	73.87	71.99	72.13	
	73.05	72.03	71.85	
	72.67	72.05	72.18	
	72.61	71.85	72.00	
	72.38	71.82	71.73	
	72.19	72.05	72.07	
	72.27	72.05	72.26	
	72.23	72.08	71.98	
	72.15	72.27	72.19	
Average	**72.60**	**72.02**	**72.04**	**72.22 b**
BAN 480L	71.01	71.99	72.08	
	71.13	71.94	72.14	
	71.16	71.59	70.41	
	71.23	71.91	71.77	
	71.15	71.93	71.84	
	71.24	71.88	71.81	
	71.95	71.95	71.84	
	72.01	71.69	71.67	
		71.52		
Average	**71.36**	**71.86**	**71.69**	**71.64 b**
Duramyl 300L	72.12	72.26	72.26	
	71.98	72.11	72.34	
	71.94	72.02	72.16	
	71.63	71.66	71.95	
	72.00	72.03	72.02	
	71.84	71.91	72.29	
	71.95	71.49	72.16	
	71.94	71.83	72.24	
	71.95	71.53	100.00	
Average	**71.93**	**71.87**	**72.18**	**71.99 b**
Fungamyl 800L	71.50	71.72	71.18	
	71.53	71.62	70.90	
	71.47	71.52	71.25	
	71.46	71.71	70.88	
	71.86	71.22	71.14	
	72.02	71.17	71.29	
	71.50	73.02	71.07	
	71.29	71.48	70.86	
	71.36	71.46	71.15	
Average	**71.55**	**71.66**	**71.08**	**71.43 a**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference

Table D.4. Starch content of different paper samples during the second pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Paper Starch (mg/L)			
	R 1	**R 2**	**R 3**	
Control	1.13	1.64	3.48	
	2.97	1.13	0.61	
	1.01	1.52	3.36	
	1.39	0.88	3.23	
	0.83	1.34	3.18	
	0.78	3.13	1.29	
	0.65	1.16	3.00	
	3.40	1.04	1.56	
	0.53	2.88	1.04	
Average	**1.41**	**1.64**	**2.31**	**1.78 a**
BAN 480L	1.47	3.48	3.14	
	0.96	2.97	2.62	
	3.36	3.02	1.35	
	1.22	2.89	3.23	
	3.18	2.84	1.17	
	1.12	2.79	3.13	
	0.99	3.00	2.66	
	1.39	3.05	3.40	
	2.88	2.54	0.87	
Average	**1.78**	**2.94**	**2.48**	**2.40 a**
Duramyl 300L	2.45	2.71	0.96	
	0.44	2.20	1.94	
	2.59	0.84	2.33	
	2.46	0.71	2.20	
	2.15	2.41	0.66	
	0.61	2.36	2.11	
	1.98	2.23	0.48	
	2.63	0.87	2.37	
	0.36	2.11	1.85	
Average	**1.74**	**1.83**	**1.66**	**1.74 a**
Fungamyl 800L	2.37	2.16	1.04	
	0.53	1.64	1.85	
	2.03	0.92	2.25	
	0.79	2.12	1.90	
	2.07	1.86	0.74	
	1.81	0.69	2.02	
	1.89	1.68	0.56	
	0.96	2.07	2.28	
	0.44	1.56	1.77	
Average	**1.43**	**1.63**	**1.62**	**1.56 a**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference

Table D.5. Starch content of different backwater samples during the second pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Backwater Starch (mg/L)			
	R 1	**R 2**	**R 3**	
Control	7.16	8.06	8.02	
	6.73	7.72	8.1	
	7.29	7.80	7.59	
	6.77	6.09	7.72	
	6.95	7.42	7.46	
	6.52	8.31	7.89	
	7.07	7.16	9.64	
	8.31	6.52	8.02	
	7.42	4.81	7.84	
Average	**7.14**	**7.10**	**8.03**	**7.42 b**
BAN 480L	6.09	7.72	6.69	
	7.25	8.96	9.17	
	6.48	7.89	9.43	
	7.80	9.68	7.42	
	10.97	10.07	8.79	
	7.54	9.21	9.08	
	12.12	9.26	8.87	
	11.74	8.66	8.36	
		9.04	8.31	
Average	**8.75**	**8.94**	**8.46**	**8.72 b**
Duramyl 300L	3.10	5.19	4.34	
	4.04	4.21	4.25	
	3.57	3.35	3.31	
	3.22	3.91	3.82	
	2.67	3.01	3.74	
	3.74	3.87	3.57	
	3.57	3.40	3.74	
	3.01	3.95	4.25	
	3.22	2.45	3.74	
Average	**3.35**	**3.70**	**3.86**	**3.64 a**
Fungamyl 800L	7.63	7.46	8.02	
	8.10	8.23	6.26	
	8.44	8.36	7.25	
	7.63	8.96	8.27	
	8.49	7.59	6.69	
	7.07	7.29	6.73	
	6.65	6.90	6.30	
	7.42	8.66	6.30	
	7.03	5.71	7.97	
Average	**7.61**	**7.68**	**7.09**	**7.46 b**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference

D3 Third pilot trial

Table D.6. Moisture content of different paper samples during the third pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Moisture Content (%)			
	R 1	**R 2**	**R 3**	
Control	75.48	68.81	68.73	
	75.84	72.92	70.65	
	75.76	73.04	69.48	
	75.51	72.93	69.45	
	75.36	72.96	72.65	
	75.06	72.98	70.13	
	75.15	72.86	70.80	
	75.2	72.21	72.38	
	75.06	72.83	69.09	
Averages	**75.31**	**72.46**	**70.39**	**72.72 a**
BAN 480L	73.12	70.30	67.10	
	73.03	71.96	70.36	
	72.52	71.25	70.49	
	72.48	71.43	70.44	
	72.39	71.96	69.33	
	72.37	71.60	71.58	
	72.54	71.53	68.30	
	72.46	71.19	70.97	
	72.08	82.41	70.06	
Averages	**72.41**	**72.63**	**70.11**	**71.72 a**
Duramyl 300L	72.11	70.55	68.35	
	72.92	71.63	70.24	
	72.89	71.58	70.65	
	72.90	71.46	70.55	
	73.62	71.68	70.55	
	72.85	73.38	70.47	
	72.90	71.72	70.31	
	72.95	71.45	70.05	
	79.33	71.71	70.17	
Average	**73.32**	**71.55**	**70.14**	**71.67 a**
Fungamyl 800L	71.5	71.72	71.18	
	71.53	71.62	70.90	
	71.47	71.52	71.25	
	71.46	71.71	70.88	
	71.86	71.22	71.14	
	72.02	71.17	71.29	
	71.50	73.02	71.07	
	71.29	71.48	70.86	
	71.36	71.46	71.15	
Average	**71.55**	**71.66**	**71.08**	**71.43 a**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference

Table D.7. Starch content of different paper samples during the third pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Paper Starch (mg/L)			
	R 1	**R 2**	**R 3**	
Control	1.56	1.81	1.51	
	1.04	1.30	1.48	
	1.43	1.69	1.88	
	1.30	1.56	1.75	
	1.26	1.51	1.70	
	1.21	1.46	1.60	
	1.08	1.33	1.52	
	1.47	1.73	1.91	
	0.96	1.21	1.98	
Averages	**1.26**	**1.51**	**1.70**	**1.49 a**
BAN 480L	1.34	1.17	1.17	
	1.24	0.94	1.08	
	1.63	1.33	1.48	
	1.50	1.20	1.35	
	1.77	1.13	1.43	
	1.41	1.11	1.25	
	1.28	0.98	1.12	
	1.67	1.37	1.51	
	1.26	1.17	1.30	
Averages	**1.46**	**1.16**	**1.3**	**1.31 b**
Duramyl 300L	1.13	1.34	0.87	
	0.9	0.83	0.64	
	1.29	1.22	1.03	
	1.16	1.09	0.91	
	1.3	1.04	0.86	
	1.06	0.99	0.81	
	0.94	0.86	0.68	
	1.33	1.26	1.07	
	0.91	0.74	0.87	
Average	**1.11**	**1.04**	**0.86**	**1 c**
Fungamyl 800L	1.30	1.51	1.34	
	1.14	1.24	1.21	
	1.53	1.63	1.61	
	1.40	1.50	1.48	
	1.47	1.34	1.56	
	1.31	1.41	1.38	
	1.18	1.28	1.25	
	1.57	1.67	1.64	
	1.30	1.51	1.38	
Average	**1.36**	**1.46**	**1.43**	**1.42 b**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference

Table D.8. Starch content of different backwater samples during the third pilot scale evaluation. Different letters following the average indicate a significant difference from treatments ($p \leq 0,05$)

	Backwater Starch (mg/L)			
	R 1	**R 2**	**R 3**	
Control	7.80	7.03	8.23	
	7.59	6.60	9.60	
	8.57	6.69	9.17	
	12.42	7.46	8.36	
	10.11	7.16	9.30	
	7.50	8.57	8.74	
	7.97	7.03	9.38	
	7.29	6.95	10.20	
	11.35	7.63	7.84	
Averages	**8.96**	**7.20**	**9.12**	**8.43 c**
BAN 480L	7.07	6.30	7.29	
	7.63	7.25	7.59	
	8.10	6.52	8.44	
	6.43	7.84	7.16	
	7.59	6.69	7.63	
	9.43	8.44	10.84	
	7.37	6.95	7.72	
	7.12	7.12	7.59	
	7.03	7.93	8.57	
Averages	**7.49**	**7.28**	**7.99**	**7.59 b**
Duramyl 300L	3.40	4.21	2.15	
	3.99	5.15	1.98	
	4.68	3.57	2.88	
	2.03	4.08	2.84	
	4.25	3.48	2.37	
	6.69	0.79	5.32	
	2.03	2.75	2.97	
	2.07	4.89	3.14	
	4.46	4.85	4.38	
Average	**3.73**	**3.89**	**3.27**	**3.63 a**
Fungamyl 800L	7.25	7.03	7.42	
	8.02	6.60	7.29	
	7.59	6.69	6.43	
	7.76	7.46	6.39	
	7.89	7.16	6.82	
	8.40	8.57	8.31	
	7.42	7.03	6.48	
	7.16	6.95	6.95	
	9.81	7.63	7.42	
Average	**8.22**	**7.25**	**7.22**	**7.56 b**

Values followed by the same letter indicate no significant ($p \leq 0,05$) difference