PROJECT REPORT 2001-21

FINAL PROJECT REPORT

sustainable forest management network

réseau sur la gestion durable des forêts

Enzymatic and Microbial Treatment of Concentrated and Recycled Pulp Mill Effluents

J.N. Saddler, X. Zhang, R.P. Beatson, D.W. Stebbing, Y. Cai and J.J. Soong

A Network of Centres of Excellence
Un réseau de centres d'excellence

For copies of this or other SFM publications contact:

Sustainable Forest Management Network
G208 Biological Sciences Building
University of Alberta
Edmonton, Alberta, T6G 2E9
Ph: (780) 492 6659
Fax: (780) 492 8160
http://www.ualberta.ca/sfm

ISBN 1-55261-131-0

Enzymatic and Microbial Treatment of Concentrated and Recycled Pulp Mill Effluents

by

J.N. Saddler, X. Zhang, R.P. Beatson, D.W. Stebbing, Y. Cai and J.J. Soong

Chair of Forest Products Biotechnology, Wood Science Department
Faculty of Forestry, University of British Columbia

June 2001

EXECUTIVE SUMMARY

The pulp and paper industry is moving toward recycling of process waters to minimize effluent discharge into the environment. However, the increased levels of dissolved and colloidal substances (DCS) in the process waters will result from water systems closure. This will lead to a series detrimental effects on the product quality and papermachine runnabilty.

Our research carried out at Chair of Forest Products Biotechnology UBC has shown that the detrimental substances present in TMP pulp/newsprint mill process water are primarily derived from phenolics and lipophilic extractives. The level of detrimental substances will increase as the mill water system move toward the closure. The potential of using fungal enzyme treatment system to remove the detrimental DCS substances has been examined. This treatment system is capable to deal with a large amount of process water under mill operation conditions. Most of the detrimental substances have either been degraded or converted to less harmful components. The fungal enzyme treatment shows promise as one way of decreasing the detrimental substances present within process water system and can be used as an internal water treatment "kidney" for a mechanical pulp/newsprint mill with a closed water system.

ACKNOWLEDGEMENT

1. Howe Sound Pulp and Paper Company
2. Pulp and Paper Research Institute of Canada
3. BC Science Council
4. Sustainable Forestry Management Network, National Centres of Excellence

INTRODUCTION

Pulp and paper making is one of the most important and growing industries in the world, particularly in Canada where more than 66,000 employees are engaged in this sector with annual earnings reaching C$4,000 million (Pulp and Paper International, No. 1, 1997). Canada plays an important role in the world's pulp and paper with the country's total pulp and paper production in 1995 and 1996 at approximately 44,000,000 ton and 42,000,000 tons respectively. About 70% of paper & board and 25% of pulp produced in Canada is exported which accounts for 29 percent of global capacity in 1996.

A major constraint limiting the location of pulp and paper mills is the availability of an adequate water supply and of adequate receiving waters for effluent discharge. The pulp and paper making industries are major consumers of water and each ton of pulp and paper produced requires the consumption of 20-100 tons the volume of water with the effluent flow ranges between 30-200 m^3/t products. Until recently, little attempt was made to recover this water with most mill effluents released into rivers or lakes, resulting in major environment impacts on the surrounding area. Several components and/or compounds in the pulp mill waste waters are believed to be toxic and harmful to the environment. Effluents from bleached kraft pulp mill contain a wide variety of compounds, ranging from water-soluble and rapidly degraded chlorinated lignin to the persistent, highly bioaccumulative dioxins and furans which can induce cancer, deformation and other serious diseases (Kringstad, et al 1984; Crawford, et al 1991). Mechanical pulping waste waters contain large amounts of organic contaminants such as resin and fatty acids, which are released from the wood furnish. These compounds are known to be very toxic to aquatic organisms. To meet the environmental regulations, mills have had to install efficient water treatment systems to clean up the effluents before discharge into the water body. However, these systems are often expensive and add considerably to the pulp processing costs.

Water is a natural resource, which is essential to human beings. Historically it has been treated as a disposable commodity which is cheap, easy to come by and discharged after use. However, water is no longer cheap in today's world. Bringing water into the mill costs money and treating water to make it suitable for discharge also costs money. In the long term, pulp and paper mills will meet increasingly stringent environmental standards and it can be anticipated that the containment of process waters on the plant site will become the norm. Thus, closed cycle systems will become the standard in the future providing benefits in the form of, lower effluent treatment costs, energy saving and lower chemical costs. However, as a consequence of mill water system closure, not only will useful chemicals be reclaimed, it will also build up the level of contaminants, which in turn will create a series of problems in the papermaking process. In terms of the chemical composition of the recycled process waters, all the substances within the mill water system can be grouped into four categories: carbohydrates; lipophilic extractives; phenolic compounds and inorganic substances. Although carbohydrates will have minimum adverse effects on the paper and machine properties, the increased amount of carbohydrates after recycling will increase the increase and COD (chemical oxygen demand) and BOD (biological oxygen demand) in the waste water. The increased COD and BOD in water in turn caused adverse effects to the papermaking process. Although the amount of extractives is not considerable, they can result in some of the most serious problems to the overall process by the production of materials known as “pitch”. Additional issues include the fact that some of the resin and fatty acids are toxic and that the phenolic compounds can cause colour redeposition on the pulp, which will degrade the quality of products. Other components such as inorganic ions in the waste water come mainly from the water itself and the wood chip and chemicals used in pulping process. These inorganic ions are dissolved and can accumulate to a high concentration, greatly decreasing the hydrogen peroxide bleaching efficiency.

Thus it is clear that, before a typical mill water system can be closed, a process must be designed to handle the build up of dissolved and colloidal substances. Although there are various kinds of water treatment technologies available (table 1) (Glittenberg D. *et al.* 1993, Vihervaara T. *et al.* 1994, Boardman D., 1996, Lagace P. *et al*. 1996; McCubbin, 1983), most of these technologies suffer from the general handicap that they simply concentrate the constituents into a smaller volume of water which still requires further treatment before use or disposal. Membrane treatment is one of the technologies that has been tested in the pulp and paper industry at a pilot scale and is now used in a limited manner in a few mills (Webb, 1997). Besides concentrating rather than destroying the contaminants, such processes are expensive to install and maintain. Large membrane areas are required to treat large water volumes and membrane plugging is a common problem.

Table 1. Various technologies for waste water treatment

Physical treatment	Membrane filtration; Centrifuges; Air Flotation or Air stripping; Ion exchange; Reverse Osmosis; Adsorption; Gravity sedimentation; Screens and strainers; Liquid cyclones; Foam/bubble fractionation; Distillation or freeze concentration; Mechanical vapour recompression (MVR)
Chemical treatment	Ozonation; Ozone/Peroxide Oxidation; Activated Carbon Adsorption; lime treatment; Magnesium oxidize; Potassium phosphate treatment; Starch based cationic polymers; Bentonite clays, Polyaluminium Chloride (PAC) and other Anionic Trash Catchers
Biological treatment	Activated sludge, Aerated lagoons or stabilization ponds, Fixed-film reactors, Anaerobic processes, Fungal and enzyme treatment

The only established process currently being implemented for reducing the effluent in the mechanical pulp mills to very low levels is evaporation. This process is capable of giving zero-effluent mechanical pulp mills such as Millar Western mill at

Meadow Lake in Saskatchewam and Louisiana Pacific's BCTMP mill in Chetwynd. In these mills condensate from the evaporators is treated both biologically and by filtration before returning to the process. The water from the evaporators, after further concentration, is fed to a recovery boiler which generates steam for the concentrates. The solids from the recovery boiler are cooled and formed into ingots for landfill. Unfortunately, this technology is very capital intensive and is unlikely to be installed in a currently operating paper mill.

The application of biotechnology to the pulp and paper industries has attracted considerable attention during the last twenty years. Various groups have tried to use fungi and enzymes to decolorize effluents and to reduce the AOX of kraft pulp mill effluents (Singh, *et al.* 1996). A large number of fungi, particularly the species belonging to the genera Aspergillus and Penicillium are known to produce lipase which can selectively degrade lipids and other related extractives in the wood. They have been used to decrease the resin present in the wood chips and to reduce pitch in mechanical pulping process (Shimada et al, 1996; Sarkar et al, 1995; Sharyo et al, 1993). Enzymes produced by *Aspergillus niger*, *Pseudomonas fluorecens* and *Candida cylindracea* have been patented and used to diminish the level of triglycerides present in mechanical pulp and white waters (Anon, 1990). Other enzymes such as pectinased have also shown the potential to degrade detrimental organic materials present in the white water (Thornton, 1994). Biological treatment is attractive in that it can deal with vast amounts of waste water and results in the degradation of organic materials to gases and biomass. One concept that has been applied is the return of the outflow water from the biological effluent treatment system to the mill process. Although this decreases the fresh water intake to the mill, the hydraulic load on the effluent system remains constant and thus costs savings in effluent treatment are not realized. Another problem is that this type of treatment is vulnerable to the variable seasonal conditions. Another concept is to install biological treatment in-line to destroy process water organics. A combined anaerobic and aerobic treatment has been recently installed in-line, in a German recycled fiber board mill (Habetsm et al, 1997). The purified water can then be returned to the process streams. A similar concept that has been tried is to insert a fermentor with white-rot fungus into the process streams of a newsprint pulp and paper mill (Eriksson, 1985). Both of these concepts suffer from high capital costs and consume energy. Large volumes of water must be cooled to temperatures around 35°C, pH adjusted and treated for substantial time for the treatments to be effective.

It is apparent that the current technology available to the pulp and paper industry to achieve water system closure is inadequate for either economic or technical reasons. This project will assess the potential of using fungal/enzyme system as an on-line water treatment method to help a TMP/newsprint mill achieve water system closure. Fungal/enzyme treatments have the major advantage of requiring only a small stream of the process water to be cooled for generation of the fungal culture filtrate with most of the degradation of the detrimental organics being accomplished by enzymes (fungal culture filtrate) that function well at process water temperatures up to 75°C. It is probable that this type of treatment will be relatively inexpensive and environmental friendly.

SUMMARY OF DATA ANALYSIS

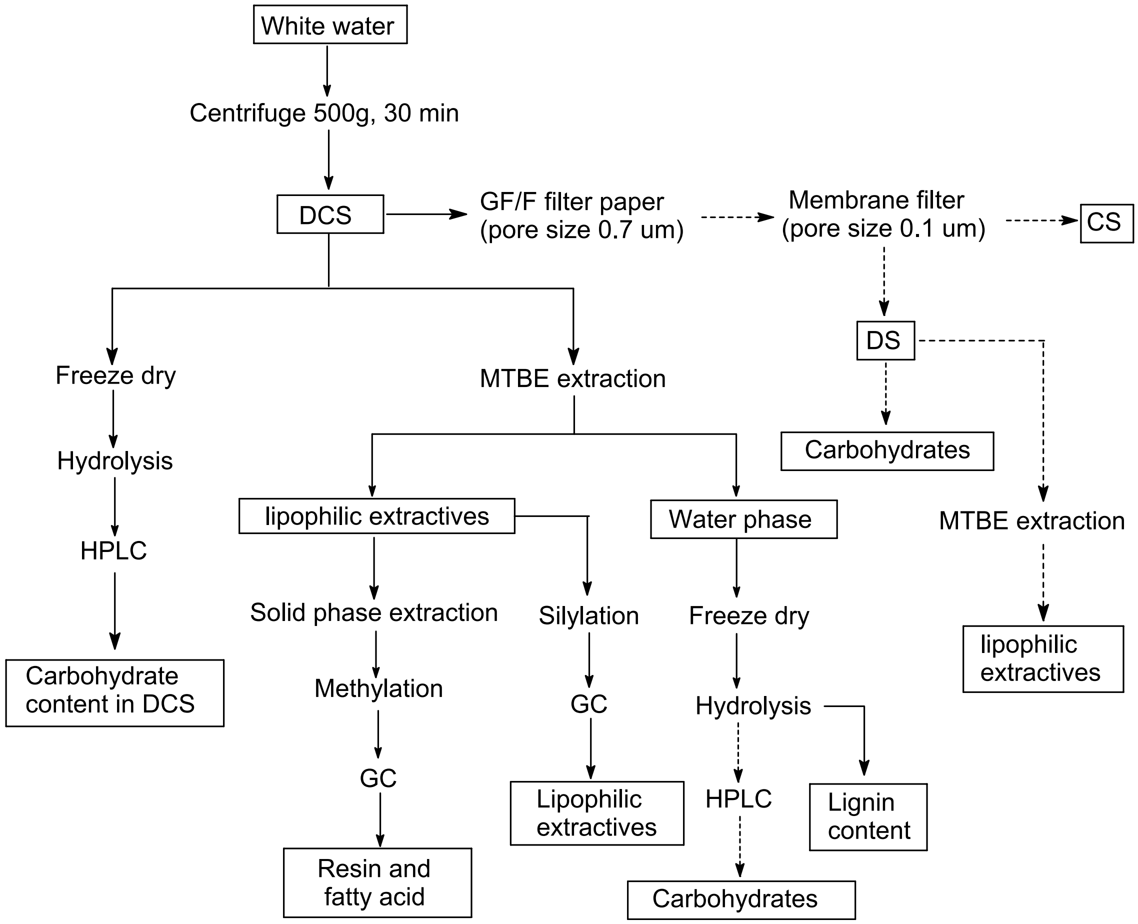
A. Characterization and Investigation of White Water Dissolved and Colloidal Substances and Their Effects on the Paper Quality and Papermaking Process

1.Development of a detailed protocol for analyzing the dissolved and colloidal substances (DCS) present in TMP pulp/newsprint mill process water

A clear knowledge of the composition of a "typical" TMP process white water will give us a better understanding of the influence that the DCS components have on the paper properties. This will also help to design appropriate treatment technologies. In the earliest stage of this project, a detailed analytical protocol (Figure 1) was set up, based on previous studies, from which white water DCS component groups: carbohydrates, lignin, inorganics and extractives (resin and fatty acids, lingams and sterols, steryl esters and triglycerides) were quantified. Other properties of the white water, such as chemical oxygen demand, total organic carbon, colour unit, colloidal particle size distribution, turbidity, water surface tension and cationic demand were also determined through this protocol.

Several white water samples including mill white water collected from a typical TMP/newsprint mill and "model" recycled white waters (one to five times recycled white waters) made in PAPRICAN pilot plant have been analyzed using this protocol. The results showed that (Zhang, *et al* 1999, Zhang, 2000): 1) white water from a mechanical pulp/newsprint mills using softwood furnishes contained a large amount of water soluble carbohydrates which are mainly derived from galactoglucomannan and arabinogalactan. 2) A considerable amount of phenolics were also present in the white waters and they could be separated into two fractions, lignin and lignans due to their solubility in the MTBE. The lignin fraction had a molecule weight distribution up to 3,000 while the molecule weight of lignans was below or around 1,000. 3) The extractives in the white water are defined as MTBE extractives which contain four major fractions, resin and fatty acids, lignans and sterols, sterly esters and triglycerides. The concentration of each of these extractions was different due to the differences in the white waters. This was probably due to the different furnishes and different processes used and also influenced by seasonal effects. 4) A high inorganic content was found in the mill white water samples. 5) Despite differences in the chemical nature of these DCS components, all of these substances were physically present either as colloidal particles or dissolved substances. The sizes of colloidal particles ranged from 250 nm to 1000 nm. 6) The investigation of the distribution of each of the DCS component in either the dissolved or colloidal fractions showed that lignin and ester-bonded extractives, such as sterol esters and triglycerides were the main constituents of the colloidal particles, while the neutral polysaccharides and lignans were predominantly dissolved in the white water.

Figure 1. Analytical protocol for determining various DCS components present in white water samples



2. Investigation of the effects of DCS components on paper properties

Although the general effects of the white water dissolved and colloidal substances on paper properties have been discussed in previous research, due to the diversity of different white waters and different DCS components, the specific detrimental effects that each of the DCS component groups have on the papermaking process have not yet been clearly defined. This type of information is critical to the development and evaluation of a white water treatment process. In my study, different types of "model" recycled white waters were prepared in the PAPRICAN pilot plant and several mill white waters were collected from the Howe Sound Pulp and Paper mill, with the intention of obtaining different ranges of DCS components in these white water. Also, a modified handsheet forming procedure was developed to make pulpsheets for testing physical properties. Conventional TMP handsheet forming required circulation of handsheet machine water to maintain the fines content. However, if we make handsheets using white water, this type of procedure would result in the removal of the colloidal materials from the white water through filtration in the first fine-poor handsheets. To avoid the removal of the colloidal material from the white water while maintaining the fines content, the pulp slurry used for the physical testing was enriched with fines from the original pulp. The fines content in the original pulp and the fines lost during the sheet forming on the sheet machine were calculated, so that the added fines will compensate for the lost of fines during sheet forming. In this manner, I was able to show the effects of the DCS component on the paper properties without the interference from fines.

2.1. Chemical nature of DCS components affecting paper properties (Zhang, et al 1999)

A significant difference in the distribution of the various DCS component groups (carbohydrate, lignin, resin and fatty acid, lignan, steryl ester and triglyceride) present in both the "model 5 times recycled white water" and "mill white water" from cloudy white water tank were observed. The paper properties of the handsheets prepared by using these two white waters were tested and compared to the paper sheets formed when using distilled water from which the effects of the different DCS components on the paper properties were investigated. The results showed that:

1) The concentration of dissolved and colloidal substances increased significantly with white water recycling (Figure 2).
2) The lignin component of the DCS was primarily responsible for the decrease in papersheet brightness (Table 2) whereas the presence of RFAs reduced wet web strength properties, as a consequence of their ability to decrease water surface tension (Figure 3).
3) The presence of lipophilic substances caused a decrease in sheet density and interfibre bonding which resulted in reduced tensile strength (Table 3).
4) The presence of enhanced amounts of carbohydrates in the highly recycled white water acted to counteract some of reduced bonding caused by the lipophilic extractives.

Table 2. The optical properties of the handsheets formed when using distilled water (DW), 5-time recycled white water (HRWW) and mill white water (MillWW).

	Optical properties obtained after handsheet forming using		
	DW	HRWW	Mill WW
Brightness, %ISO	48	39	51
Scattering coefficient (620 nm, m^2/kg)	69	64	74
Absorption coefficient (457 nm, m^2/kg)	19	31	18

Table 3. The dry strength properties of the handsheets formed when using distilled water (DW), 5-time recycled white water (HRWW) and mill white water (MillWW).

	Properties obtained after handsheet forming using		
	DW	HRWW	MillWW
Thickness (μm)	179±3	212±5	204±4
Density (g/cm^3)	0.328±0.007	0.298±0.010	0.292±0.006
Tensile index, N•m/g	33.1±0.7	26.0±0.7	23.3±1.0
Tear index, mN•m^2/g	26.9±1.4	25.7±1.2	25.4±0.3
Scott bond, kJ/m^2	38±2	29±1	27±2

Figure 2. The increase in concentration of total dissolved and colloidal substances (TDCS), total extractives (TE) and total non-extractive substances (TNE) during white water recycle.

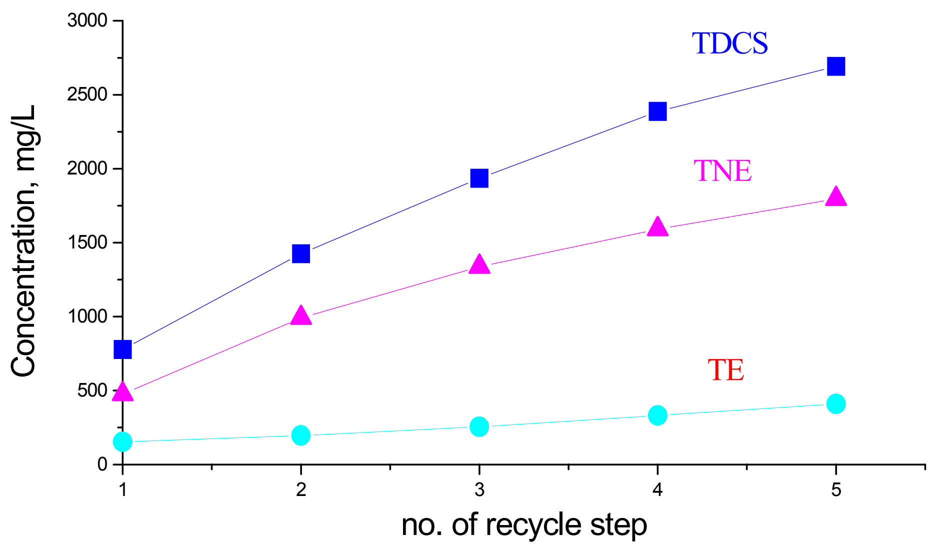
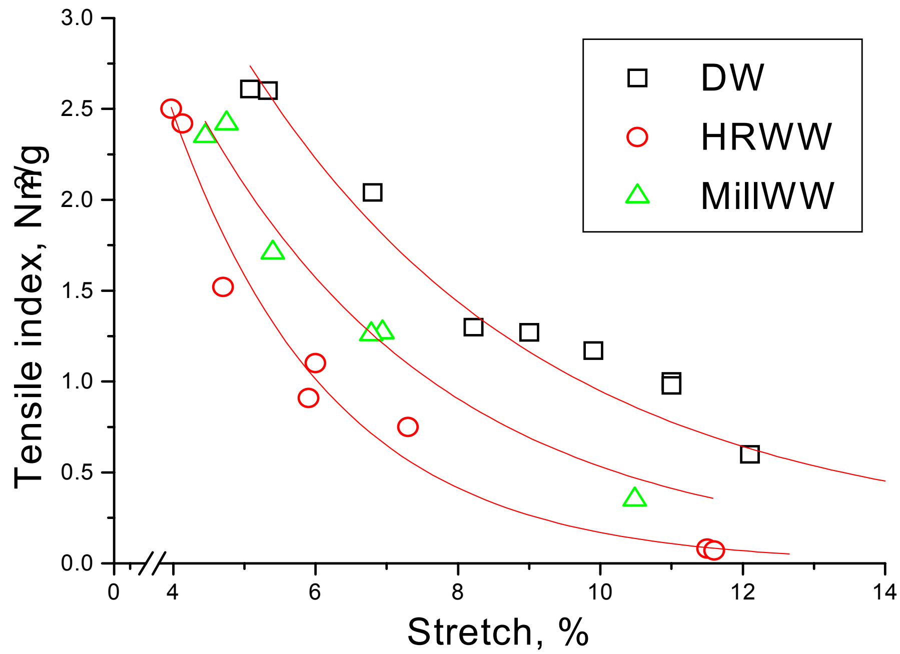


Figure 3. Tensile strength versus stretch plots of the wet web formed by distilled water (DW), 5-times recycled white water (HRWW) and mill white water (MillWW)



2.2.Does the physical form of the DCS components affect paper properties? (Zhang, 2000)

During our investigation on the effects of different DCS components on the paper properties, it was recognized that the physical properties of the paper sheets was not only determined by the chemical nature of DCS components, but it was also influenced by the physical form of these substances present in the white water. After 0.22 µm membrane filter was used to separate the newsprint mill white water colloidal particles from the dissolved substances, the chemical and physical constitutions of these two fractions were then determined. Both the original white water and white water containing only the dissolved substances, obtained after membrane filtration, were used to prepare handsheets. The physical properties of these two types of handsheets were tested and compared to the handsheets made using distilled water. In this manner, the separate effects of the dissolved and colloidal fractions in white water on the paper properties were determined. The results showed that:

1) The white water contained a significant amount of colloidal substances ranging in size from 250nm to 1000nm.
2) The lignin and ester-bonded extractives (steryl esters and triglycerides) were the main constituents of the colloidal particles, while the neutral saccharides and lignans were predominantly dissolved in the white water.

3) Dissolved substances mainly caused the reduction in the paper strength properties, whereas the colloidal substances were primarily responsible for the impairment of paper surface and optical properties (Figure 4 and 5).

Figure 4. The Dry Strength Properties of Handsheets Made Using Distilled Water (DW), Filtered White Water (FWW) and White Water (WW)

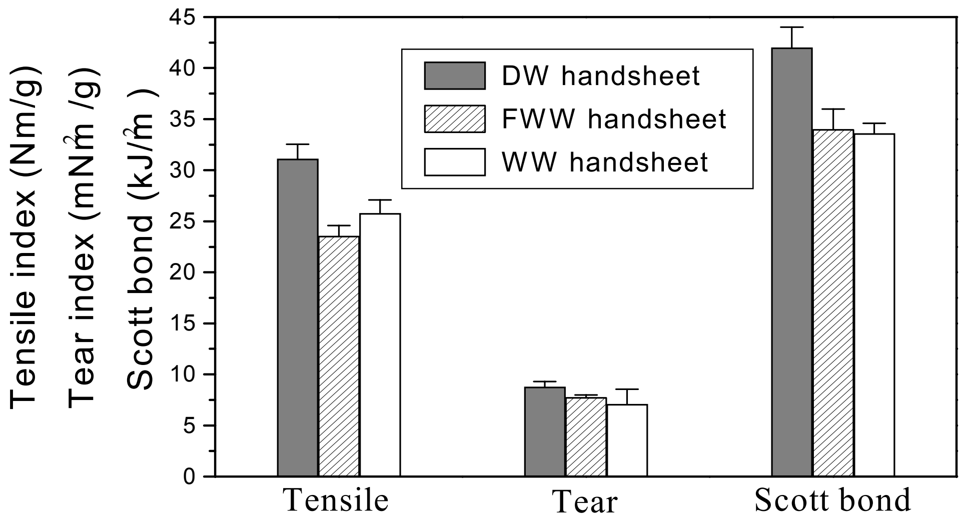
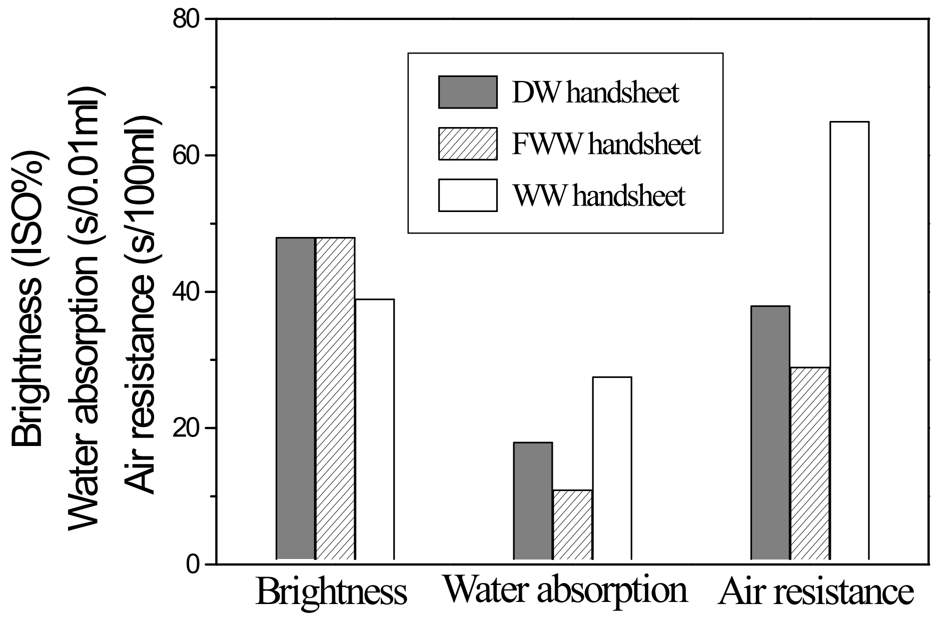


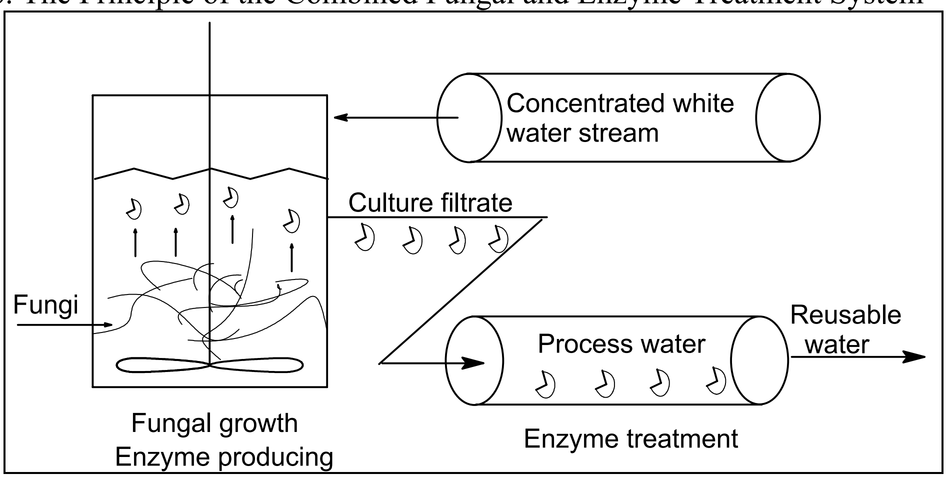
Figure 5. The Optical and Absorption Properties of Handsheets Made Using Distilled Water (DW), Filtered White Water (FWW) and White Water (WW)



B. Assessment of the Potential of Fungal and Enzyme Treatment For DCS Removal From TMP/newsprint Mill Process Water

The dissolved and colloidal substances present in the TMP/newsprint mill process waters have been shown to be mainly derived from the woody components. Various microorganisms have been known to be the natural degraders of these substances, particularly the white rot fungi which are known to be able to degrade extensive amounts of wood materials. Biological effluent treatment systems are widely used in the pulp and paper industries. Compared to alternative physical and chemical treatment processes, biological treatment systems are more capable of dealing with large quantities of water, more able to remove target contaminants and require less facility and operational costs. As the mill water system is gradually closed, it is also likely that biological systems will prevail over physiochemical processes as they will have less impact on the pulp and papermaking process. Thus, there is a considerable potential to use biological treatment systems in treating mill process waters, once the pulp and paper mill water system is closed. In terms of predominant biological system, there are two major types of microorganism, the bacteria and fungi. However, as most fungal cells physically resemble those of plant fibers, treatment of process water using select fungal species may have the additional benefits of increasing pulp yield as well as removing the detrimental substances. However, the long retention time and limited temperature range of fungi make a direct microbial treatment difficult to be used in a mill situation. However, compared to fungal treatment, enzyme treatments have the advantage of quicker reaction times and tolerant to higher temperatures. One of the objectives of this thesis was to assess whether combined fungal/enzyme treatment system could be used as an internal treatment "kidney" in a integrate paper mill with a closed water system. The principle of this system is shown in (Figure 6). It is proposed that a small portion of the white water could be cooled down and fed into a bioreactor used to grow the fungi. The various water components will be consumed during fungal growth and many of the enzymes required to break down the DCS substrates will be released into the water. By collecting the culture filtrates at the appropriate time, a highly active fungal culture filtrate (FCF) could be obtained. Because the DCS substances present in white water are the only nutrition source for the fungi, the enzymes produced in the culture filtrate should be specific for the DCS components. The FCF produced will be continuously decanted into the process water so that enzymes can react with the DCS components while the whole paper making process continues.

Figure 6. The Principle of the Combined Fungal and Enzyme Treatment System



1. Screening fungi for activity against detrimental DCS (Cai, et al 1998)

One of the first objectives of this part of the thesis was to identify suitable fungal strains for the treatment system. Twenty-three fungal strains were screened through a two step-process. The first screening was based on the growth of these fungi on agar plates containing white water without supplementation of additional nutrients. This initial step helped us eliminate some of the strains which could not survive on the unsupplemented white water due to factors such as the deficiency of key nutrients or inhibition effects from some white water DCS components. The secondary screening was used to determine the ability of the remaining strains to degrade extractives, which are one of major detrimental substances present within the white water. From the two screening steps, the white-rot fungus *Trametes versicolor* showed both the highest growth on white water and highest activity against the DCS components present in the TMP white water (Figure 7 and 8).

Figure 7. The Ability of Fungal Growth on a Media Containing White Water Only

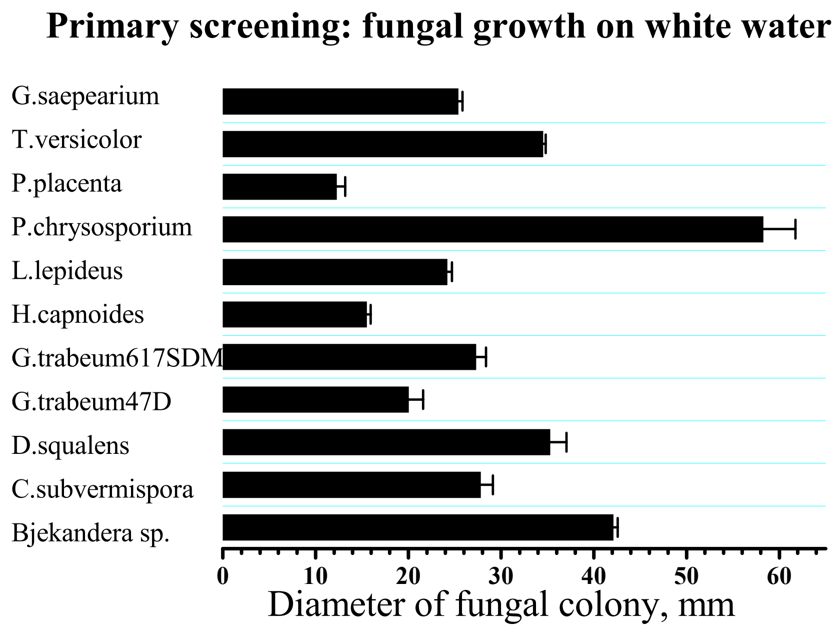
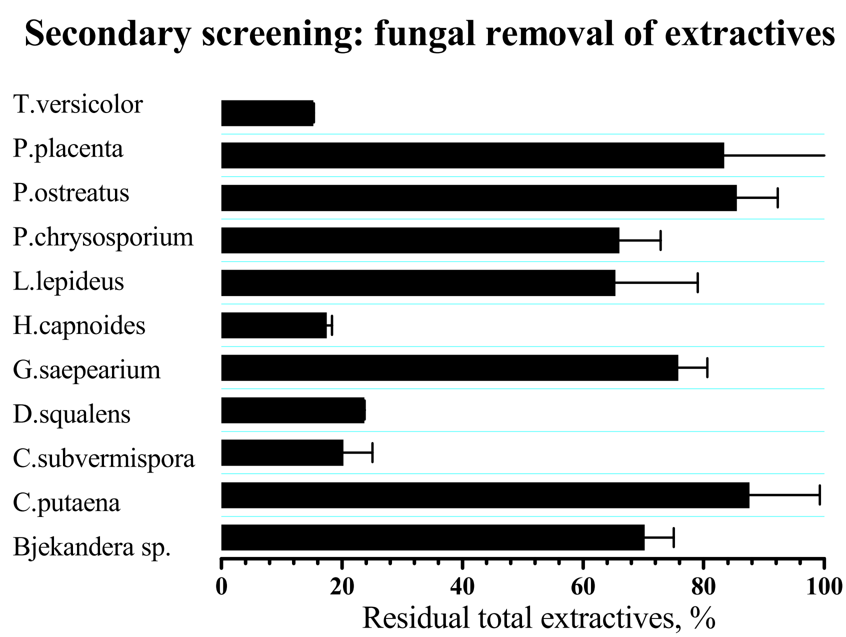


Figure 8. The Ability of Fungal Removal of Extractives Present in Mill White Water

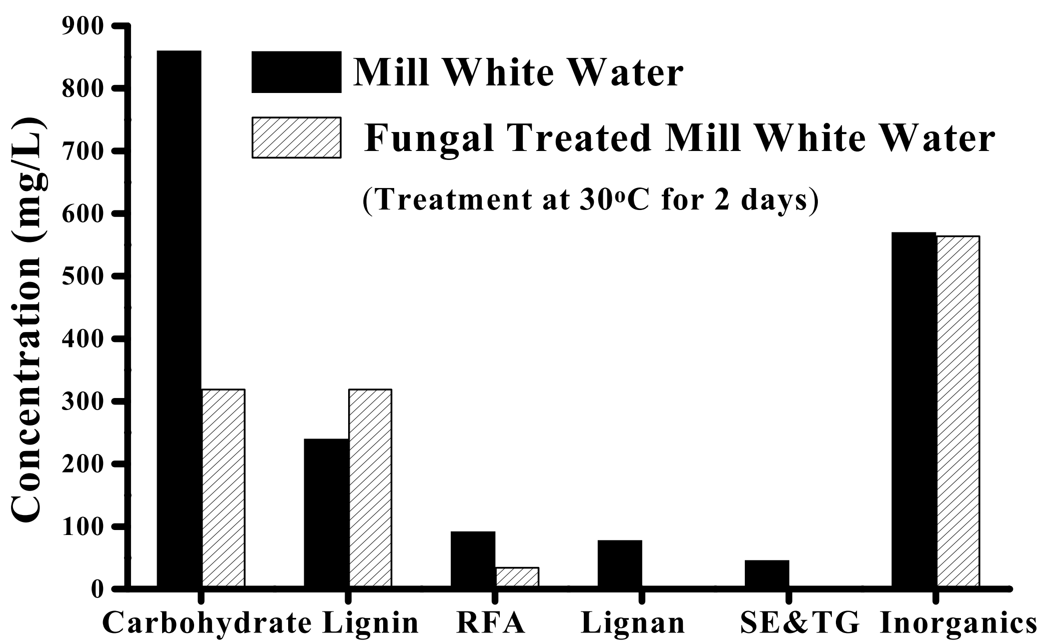


2. Fungal enzyme treatment of DCS components present in the mill white water and model recycled white water

2.1. Effects of fungal removal of DCS components

When the efficiency of fungal removal of the white water DCS components was tested, significant decrease in the total dissolved and colloidal substances, carbohydrates and extractives was detected after 2 days of fungal treatment under 30°C (Figure 9). Of the carbohydrate fractions, the mannans and glucans were extensively degraded before a reduction in galactans was noticed. *Trametes versicolor* was able to degrade almost all of the fatty acids, lignans and ester bonded extractives. There was also good removal of resin acids by *Trametes versicolor* which was probably due to the initial sorption of the resin acids on to the fungal mycelium and then further modification by enzymes such as laccases. An increase in acid insoluble lignin content was observed after 3 days of treatment, probably due to the polymerization of low molecular weight phenolics (e.g. lignans). No evidence of lignin degradation was observed after 14 days of fungal treatment. However, this fraction is expected to be less stable in the white water. The stability of white water colloidal particles after fungal treatment will also be further determine in the future work.

Figure 9. The Efficiency of Fungal Removal of Dissolved and Colloidal Substances Present in Mill White Water



2.2. Enzyme production during Trametes versicolor growth on the white water

As mentioned earlier, fungal enzymes rather than fungi themselves are the major agents in effective for white water treatment. Therefore, the efficient production of fungal enzymes during *Trametes versicolor* growth on white water is of great importance. Several different white water streams, including mill cloudy white water, chip washer white water, model recycled white water and membrane filtered white water were used to grow *Trametes versicolor* for 7 days. The activities of enzymes produced in the fungal culture filtrates (FCF) were measured daily. The use of different white water streams containing different concentrations of DCS components enabled us to obtain a better understanding of the effects that the various DCS concentrations and ratio of each component had on the enzyme production profiles. This in turn could help us define a FCF with higher enzyme activities. During this 7-day growth period, the highest oxidative enzyme activities were detected after 2 or 3 days of fungal growth; the esterase activities (based on the hydrolysis of PNPP) and hydrolytic enzyme activities (based on the hydrolysis of konjac Glucomannan) increased dramatically in the first 2 or 3 days and then level off. Although the levels of each enzyme produced in the FCF by *Trametes versicolor* growth on different streams were slightly different, the overall enzyme production profiles were similar. One exception is that there is a one day delay for oxidative enzyme to reach maximum activity in the FCF produced by *Trametes versicolor* growth on model recycled white water, which contained a lower lignan content compared to the rest of the white water streams.

The mill white water collected from cloudy white water tank was found to be enriched in extractives and it resulted in a considerable enzyme activity after growth of fungi. This stream is also abundantly available in the mill and usually has a long settlement time. These facts made this type of stream favourable for fungal growth after cooling down to ambient temperature. Thus the fungal culture filtrate produced from mill cloudy white water was selected for future enzyme treatments.

2.3. The efficiency of fungal culture filtrate removal of DCS components (Zhang, et al, 2000)

Fungal culture filtrate (FCF) produced by *Trametes versicolor* grown on mill cloudy white water was used to treat the model recycled white water and mill white water. The conditions for the FCF treatments were 45ºC for 3 hours and the ratio between FCF and white water was 1 to 2. FCF treatment of white water resulted in good degradation of most of the DCS components. Carbohydrates were extensively hydrolyzed into monomers while the lignin content increased as a result of lignan polymerization (Figure 9). The FCF was also active in degrading most of the extractives present in the white water, with a reduction of more than 90% for the lignans, steryl esters and triglycerides and about 20% for the resin and fatty acids (Figure 11). The change in the white water properties such as water surface tension, cationic demand and the properties of colloidal particles will be further investigated.

Figure 10. The Polymerization of Phenolics Present in the Model Recycled White Water After 3 hours Fungal Culture Filtrate Treatment at 65°C

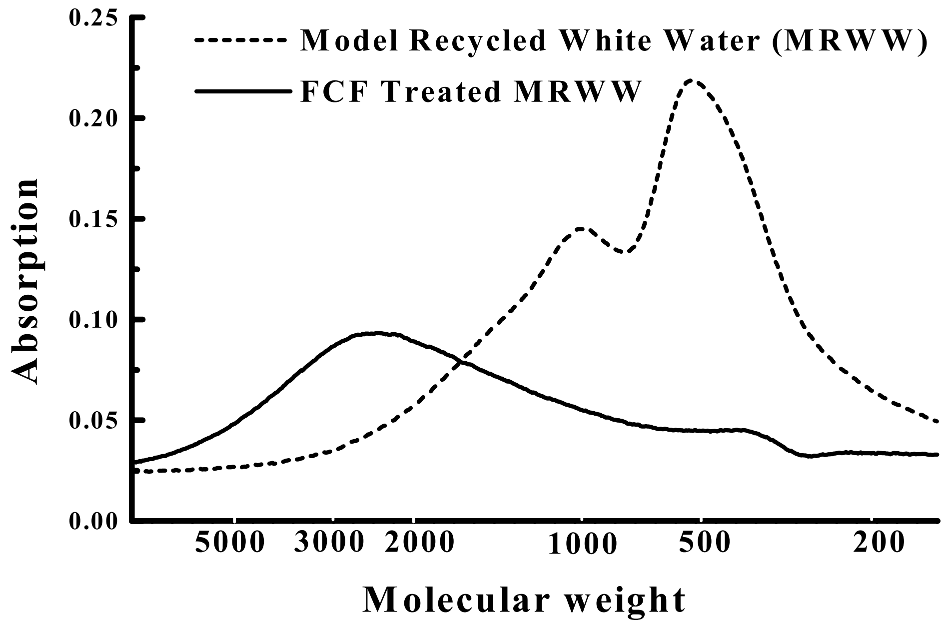
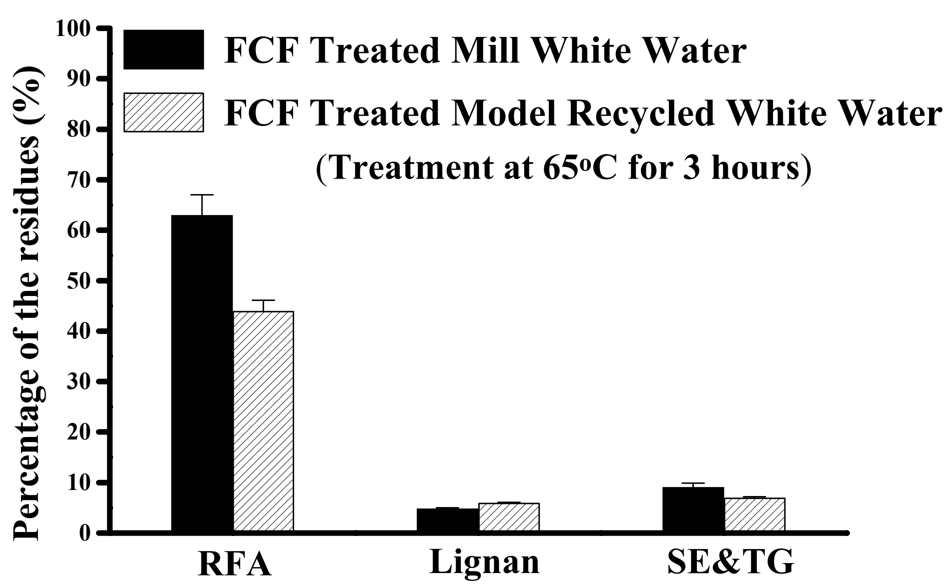


Figure 11. The Removal of Extractives Present in the Model Recycled White Water and Mill White Water After 3 hours Fungal Culture Filtrate Treatment at 65°C



2.4. Treatments of white waters with commercial sources of enzymes found in the FCF (Zhang, 2000)

To obtain a better understanding the impact of the FCF as well as different groups of enzymes on process waters, the model recycled white water and colloidal free white water were treated with commercial sources of the three main types of enzymes (hemicellulase, oxidative enzymes and lipases) found in the *Trametes versicolor* culture filtrate. The active hydrolysis of carbohydrates by cellulase/hemicellulase and degradation of lipids by lipase/esterase has been well documented in the literature and correlated well with our results. However, results not initially anticipated were obtained from the laccases treatment of white water. The polymerization of low molecular weight phenolics to higher molecular weight lignin type of materials was proved when using laccases provided by VTT food and biotechnology lab and Novo Nordisk ltd.. The laccase treatment also resulted in significant degradation of the ester bonded extractives such as SE and TG. The laccase-catalyzed reactions of lipophilic extractives were further investigated using three extractive model compounds, methyl linoleate, cholestryl linoleate and trilinolein, and one laccase from VTT Food and Biotechnology Lab and two laccases from Novo Nordisk Ltd. All three laccases degraded trilinolein well, however, degradation of methyl linoleate was only detected after VTT laccase treatment.

FUTURE WORK

The potential of using combined fungal and enzyme treatment to alleviate DCS problems from water system closure has become evident through this work. However, the end products from DCS degradation and white water properties after fungal and enzyme treatment need to be well quantified and the true nature of the "recalcitrant" remaining after fungal and enzyme treatment needs to be defined. Since the mechanisms involved in cellulase/hemicellulase hydrolysis of carbohydrates and esterase/lipase hydrolysis of lipids are clearly documented, our future work will be focused on investigating the reaction mechanism involved in laccase catalyzed reactions with lipophilic extractives.

Another important aspect that needs to be defined is the half life of each of the enzyme groups present in the fungal culture filtrate. This will help in the design of an appropriate bioreactor for fungal growth and enzyme production. This experimental will be carried out by monitoring enzyme activities of FCF under different temperatures over extended periods of time.

Finally, any improvements obtained on paper properties through fungal and enzyme treatment will be determined by examining the changes in white water properties, such as water surface tension, cationic demand, and the composition and stability of white water colloidal particles, after fungal enzyme treatment.

CONCLUSIONS

As results of this project research, the detrimental effects that dissolved and colloidal substances present in mechanical pulp/newsprint mill process waters have on the paper properties have been well defined. It has been shown that, not only the chemical natures of different DCS components, but also the physical forms they are present in the white water influenced the resulting paper properties. *Trametes versicolor* has been shown to actively grow on white water DCS components. Treatment of white water with *Trametes versicolor* resulted in a considerable reduction on total dissolved and colloidal substances and, more importantly, various enzymes were produced during this growth. The treatment of white water with fungal enzyme solution (fungal culture filtrate) led to extensive degradation of DCS components. Most of the DCS were broken down into smaller molecules, which had no or little impact on the paper properties, while others were modified and became readily removed by further treatment processes. This combined fungal and enzyme treatment "kidney" has been shown promise as one way of controlling the build up of detrimental substances in a TMP/newsprint mill closed water system. The actual mechanisms involved in fungal and enzymatic degradation of each DCS group were investigated. Although most of these initial results agreed well with other documented research, the reaction of laccases with ester bonded extractives present in white water is worthy of considerably more research.

REFERENCES

Anon, Pap. Trade J. Vol. 170, No. 8, 1986 p48-49.

Boardman D., 1996 Papermakers Conference, Philadelphia PA, USA, p533-538

Cai, Y.J., Zhang, X., de Jong, E., Beatson, R.P., and Saddler, J. N., 7th International Conference on Biotechnology in the Pulp and Paper Industry, Vancouver, BC Canada, June 16-19, Vol. C:155-158, 1998

Crawford, R.J. et al, Tappi J. 74(4), 1991

Eriksson, K.E.L., "Swedish developments in biotechnology related to the pulp and paper industry", Tappi J. 39(6):46-55, 1985

Glittenberg D; Hemmes J-L and Bergh N-O, 1993 Papermakers Conference, Atlanta GA, USA, Book 2, p639-668

Habets, H. A. et al, 82nd Annual Meeting, Technical Section, CPPA, p313.

Kringstad, K. P. and Linstrom, K., Environ. Sci. Technol., 18:236, 1984

Lagace P., Stuart P. R. and Kubes G. J., Pulp & Paper Canada 97:1, 1996

McCubbin, N., The basic technology of the pulp and paper industry and its environmental protection practices, 1983, p130-136

Sarkar, J.M. et al, 1995 Papermakers conference, Chicago, IL, USA, p175-182

Shimada, M. et al, Biotechnology in the pulp and paper industry sixth international conference, Vienna, p17-21 1996

Sharyo, M. et al, Jpn. Tappi J. Vol.47, No.10, p41-45, 1993

Thornton et al, J. Wood Chem. and Tech. 14(2), 159-175 1994

Vihervaara T and Kaki J., 1994 Papermakers Conference, San Francisco CA, USA, Book 2, p529-533

Webb, L. Pulp and Paper International, June: 43-46, 1997

Zhang, X., Stebbing, D., Beatson, R.P., Saddler, J. N., Chalmers, B.G. and Ling, J., 86 PAPTAC Annual Meeting, Montreal, QC Canada, Jan. 31 – Feb. 3rd, Vol. B:99, 2000

Zhang, X., Pulp and Paper Canada 101(3): 59-62, 2000

Zhang, X., Beatson, R.P., Cai, Y.J. and Saddler, J. N., Journal of Pulp And Paper Science 25(6): 206-210, 1999

ARTICLES IN PEER-REVIEWED JOURNALS

Zhang, X., Stebbing, D., Saddler, J. N. and Beatson, R.P., "Enzyme Treatments of the Dissolved and Colloidal Substances Present in Mill White Water and the Effects on the Resulting Paper Properties." J. Wood Chemistry and Technology, accepted (2000)

Zhang, X., "The Effects of White Water Dissolved and Colloidal Fractions on Paper Properties and Effects of Various Enzyme Treatments on the Removal of Organic Components." Pulp and Paper Canada 101(3): 59-62 (2000) (*Winner Paper for 1999 C. Howard Smith Award from the Pulp and Paper Technical Association of Canada*)

Zhang, X., Beatson, R.P., Cai, Y.J. and Saddler, J. N., " Accumulation of Specific dissolved and Colloidal Substances During White Water Recycling Affects Paper Properties." Journal of Pulp And Paper Science 25(6): 206-210 (1999).

Stebbing, D., "The Potential of Fungal Culture Filtrate to Reduce Extractive Levels in Newsprint White Water System." Pulp And Paper Canada 103(3): 46-49 (1999). (*Winner Paper for 1998 National Undergraduate Student Problem Solving Award from the Pulp and Paper Technical Association of Canada*)

RESEARCH PAPERS INCLUDED IN CONFERENCE PROCEEDINGS

Zhang, X., Stebbing, D., Beatson, R.P., Saddler, J. N., Chalmers, B.G. and Ling, J., "The removal of detrimental dissolved and colloidal substances by a combined fungal and enzyme treatment" 86 PAPTAC Annual Meeting, Montreal, QC Canada, Jan. 31 – Feb. 3rd, 2000. Vol. B:99 *(Oral Presentation)*

Beatson, R.P., Zhang, X., Stebbing, D. and Saddler, J. N., "The dissolved and colloidal fractions of white water: impact on paper properties and degradation by enzymes" 10th International Symposium on Wood and Pulp Chemistry, Yokohama, Japan, June 7-10, 1999. Vol. I:200-203 *(Oral Presentation)*

Saddler, J. N., Zhang, X., Stebbing, D., Beatson, R.P., Chalmers, B.G. and Ling, J., "The Effects of white water dissolved and colloidal fractions on paper properties and effects of various enzyme treatments on their removal" PACWEST Conference, Whistler, BC Canada, May 19-22, 1999, Session 4A, Paper 2 *(Oral Presentation)*

Zhang, X., Beatson, R.P., Chalmers, B.G., Francis, D.W. and Saddler, J. N., "Paper quality problems caused by the different organic components of newsprint white water. Can they be solved by enzymes?" In Proceedings of the 1999 Sustainable forest Management Network Conference p680, Edmonton Albert, February 14-17, 1999. *(Oral Presentation)*

Zhang, X., Beatson, R.P., Stebbing, D. and Saddler, J. N., " The different effects of white water dissolved and colloidal fractions on the paper properties" In Proceedings of the 1999 Sustainable forest Management Network Conference p811, Edmonton Albert, February 14-17, 1999.

Zhang, X., Cai, Y.J., Stebbing, D., Beatson, R.P., and Saddler, J. N., "Influence of accumulated dissolved and colloidal substances on paper properties and the potential of enzyme treatment for component removal" 7th International Conference on

Biotechnology in the Pulp and Paper Industry, Vancouver, BC Canada, June 16-19, 1998, Vol. C:151-154

Cai, Y.J., Zhang, X., de Jong, E., Beatson, R.P., and Saddler, J. N., "Fungal treatment of organic contaminants present in the white water of a thermomechanical pulp mill" 7th International Conference on Biotechnology in the Pulp and Paper Industry, Vancouver, BC Canada, June 16-19, 1998, Vol. C:155-158

A.N. Serreqi, H.R. Gamboa, R. Leone, R. Arao, R. Beatson, J.N. Saddler and C. Breuil, Chair of Forest Products Biotechnology, Department of Wood Science, Forest Science Centre, University of British Columbia, 4041-2424 Main Mall, Vancouver, BC, Canada V6T 1Z4

Xiao Zhang, SFM BC workshop, Vancouver BC, Sept. 22nd 1999, "Fungal and Enzyme Treatment of TMP/newsprint Mill Process Waters"

PRESENTATIONS BEEN MADE ON WHITE WATER PROJECT FROM April 1999 to March 2000

ORAL PRESENTATIONS

Zhang, X., Stebbing, D., Beatson, R.P., Saddler, J. N., Chalmers, B.G. and Ling, J., "The Removal of Detrimental Dissolved and Colloidal Substances by a Combined Fungal and Enzyme Treatment" 86 PAPTAC Annual Meeting, Montreal, QC Canada, Jan. 31 – Feb. 3rd, 2000. Vol. B:99

Beatson, R.P., Zhang, X., Stebbing, D. and Saddler, J. N., "The Dissolved and Colloidal Fractions of White Water: Impact on Paper Properties and Degradation by Enzymes" 10th International Symposium on Wood and Pulp Chemistry, Yokohama, Japan, June 7-10, 1999. Vol. I:200-203

Saddler, J. N., Zhang, X., Stebbing, D., Beatson, R.P., Chalmers, B.G. and Ling, J., "The Effects of White Water Dissolved and Colloidal Fractions on Paper Properties and Effects of Various Enzyme Treatments on Their Removal" PACWEST Conference, Whistler, BC Canada, May 19-22, 1999, Session 4A, Paper 2

Zhang, X., "Fungal and Enzyme Treatment of TMP/newsprint Mill Process Waters" SFM BC workshop, Vancouver BC, Sept. 22nd, 1999

POSTER PRESENTATIONS

Zhang, X., Beatson, R.P., Stebbing, D. and Saddler, J. N., " The Different Effects of White Water Dissolved and Colloidal Fractions on the Paper Properties" 86 PAPTAC Annual Meeting, Montreal, QC Canada, Jan. 31 – Feb. 3rd, 2000