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ON

"SYNERGISTIC EFFECT OF ENZYMES ON REFINING"

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Date: 17/05/2015 Manorma

CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in this progress report entitled **"SYNERGISTIC EFFECT OF ENZYMES ON REFINING"** in partial fulfilment of the requirement for the award of the degree of Master of Technology in Pulp and Paper, IIT Roorkee, Saharanpur campus is a record of my own work carried out, under the supervision of **Dr. Vivek Kumar**, Associate Professor, Department of Paper Technology, IIT Roorkee Saharanpur Campus, Saharanpur, U.P.

The matter embodied in this project report has not been submitted by me for the award of any other degree.

Date: 17/05/2015

Place: Saharanpur

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This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

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ABSTRACT

Refining or mechanical treatment is applied to the pulp to obtain better fibre and paper quality in reference of bonding in paper, strength properties. But refining demand very large amount of energy. So to reduce the energy consumption or to get better paper properties on consuming the same amount of energy new technologies are embraced in paper making. In this study the synergistic effect of blend of three enzymes (two fungal based and one bacterial based) "Amazone A" (Xylanase in nature) from GM microbes was evaluated. The result of this study was compared with that of an enzyme (Xylanase) from single source, at same enzyme dose and operational conditions. The study was carried out at unbleached mixed hardwood pulp. By application of both enzymes deniability of pulp, strength properties of sheets were increased as compared to untreated pulp. But application of xylanase from single source revealed that blend of enzymes is efficient in enhancing the effect of refining but the synergistic effect of enzymes in blend is not higher than that of effect of xylanase from single source. Drainability of pulp is higher in case blend of enzymes but when strength properties were observed the enzyme from single source showed better results. So the blend of enzyme is effective in enhancing the effect of refining but its effects are not higher that the enzyme from single source. Also the energy consumption was reduced when deniability of pulp was fixed at a particular range. So the intensity of refining can be reduced and hence the problem of fibre cutting and fines generation can be minimised.

CONTENTS

LIST OF GRAPHS:

LIST OF FIGURES:

LIST OF TABLES:

CHAPTER 1

1. INTRODUCTION

Pulp and paper industry is facing new challenges due to competitive manufacturing and rising consumer standards. Because of cost reduction pressures, many R&D laboratories are being closed, downsized, or aimed toward short-term opportunities and objectives; and external factors including environmental concerns, globalization, global warming, rising energy cost and competition limits the profitability of this industry. PPI (Pulp and paper industry) is a large industry and has been occasionally get influenced by overcapacity. To avoid the state of overcapacity and maintain a specific level of product supply, new methods are heartily welcomed to use the forest resources more sensibly and with lesser environmental impacts.

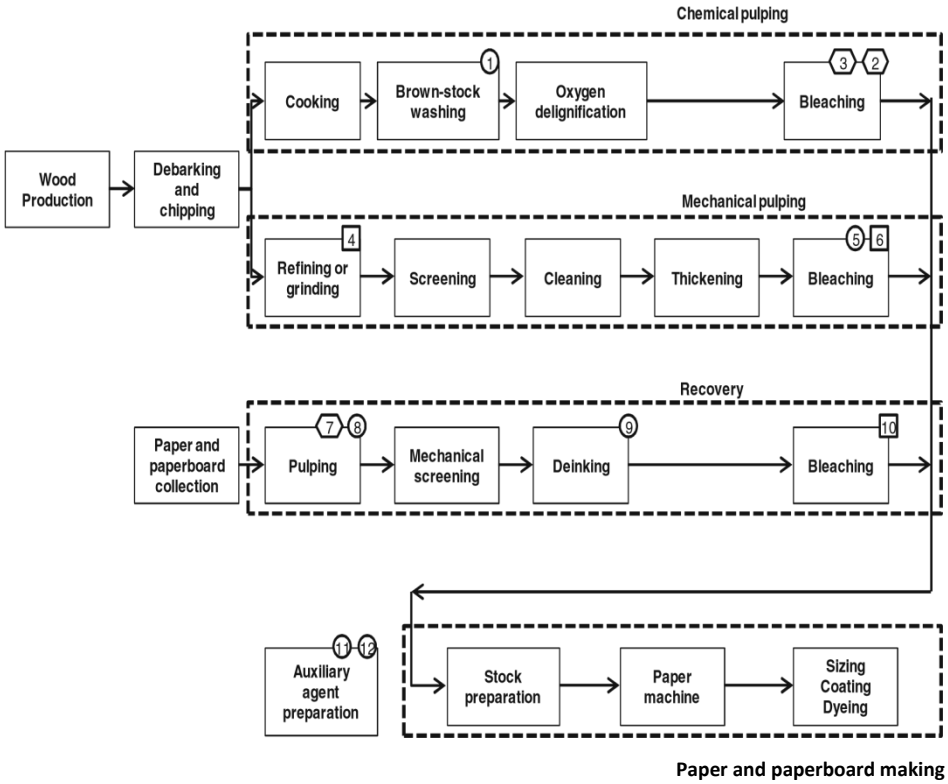
Biotechnology is capable to enhance the supply and quality of feedstock for this industry, create novel products with high values and lower cost of manufacturing. Enzyme technologies has the potential to minimise environmental problems, offers more effective processes in comparison to conventional methods and change the fiber properties in beneficial ways. Because the facilities PPI are specific to a particular task hence the emerging technologies must either fit into the existing design of plant or reduce expense up to compensable label. However, the PPI has accepted enzymes technology for the process of paper-making. The enzymes technology has the capacity to improve the economics of paper making process by improved fibre and paper quality and at the same time, to minimise the environmental consequences. Specific enzymes reduce the energy required and amount of chemicals required for the fibre modification and also prevent the development or formation of biofilms.

The average GHG (greenhouse gas) emission of PPI is about 2.5 T CO_2 / T of paper production, which is too high in comparison of BAT (Best Available Techniques). By lowering the SEC (Specific Energy Consumption), fuel consumption will also decrease, that will result in the lowering of cost and CO_2 emission.

Hence the use of biotechnological processes, particularly the enzymatic ones, in the PPI must be accepted as a welcome relief. It includes the consumption of enzymes to produce or modify the raw materials and products for commercial use.

The main benefits of use of enzymes are:

- They have specific sites for action.
- Require milder conditions
- Conserve energy
- Low pollution than chemical processes
- Better product quality
- Mostly require low capital investment.



- ○ Enzymes uses in full-scale operation
- □ Enzyme uses at pilot scale
- ⬡ Enzymes application under study at laboratory level

Figure 1: Potential processes in pulp and paper industry where Enzyme technology has accepted (from Skals et al. 2008)

The primary focus of enzyme technology in the PPI is on following areas (Fig. 1):

(1) Bio pulping to save conservation

(2) Water treatment;

(3) To fix many problems associated with bleaching, beating & refining, deinking, drainage aid, deposits of pitch, removal of shives, stickies control, lessening of vessels in tropical hardwoods, biofilm formation and elimination of extractives from pulp etc.

The present report covers the last years and it is focused on the enzymatic application for refining in energy saving.

1.1. Refining

Refining defines mechanical separation and modification of fibres by the help of a device that uses compression and shear drives onto the wetted fibres or chips to get fibres which can now interact among themselves to make a network. For refining process only energy cost around 18% of overall manufacturing cost hence its very much energy consuming process.

Refining process can be presented by two stages:

(1) Fibre separation and

(2) Fibre development, which can run simultaneously with the first stage.

In the first one, wood chips are screwed up into individual fibres by the help of counter-rotating disks. The second stage includes causing fibrillation and enhancing flexibility of fibres for betterment of the fibre properties.

The objectives of refining are:

(1) Increasing the capability of fibers to form links among them for producing a paper sheet with higher strength properties, and improved impression properties

(2) Shortening too long fibers for better formation (for homogeneity of the paper sheet) and better sheet properties like porosity, absorbance and opacity.

Refining lowers drainage rate of water through the pulp and improves the water retention in pulp. The quantification of its results, therefore, is based on the values of measurement of the drainability of pulp and water retention ability. The CSF (Canadian Standard Drainability) is an estimation of the volume of redundant water from overflow while the pulp is drained at standard conditions. CSF is an indirect estimation of the

drainage rate of pulp and is primarily affected by the amount of fines and external fibrillation. The WRV (water retention value) is the estimation of the amount of water that corpse in the pulp when it's drained at standard conditions, and it is primarily affected by internal fibrillation. The higher WRV value and the lower CSF value indicates higher refining action.

Objectives of This Study**:**

- To compare the properties of papersheets formed after refining upto different pulp drainability, without using enzyme.
- Optimisation of different operational conditions for proper functionality of enzyme.
- To compare the strength properties of paper by using Amazon A (blend of two products, bacterial and fungal base) and refining upto fixed range of ◦SR. The enzymes are from a GM Trichoderma Reesi, a GM Penicillium funiculosum and a GM Bacillus Licheniformis.)
- To compare the paper properties by using enzyme blend and an another enzyme from single source.
- To optimise the enhancement in paper properties by using optimised dose enzyme.

CHAPTER 2

2. REVIEW OF LITERATURE

Enzymatic approach on wood chips can loosen the cell wall structure, hence, results in fibre structure separation at preferable sites in succeeding refining stages. More fibre separation in the secondary wall will result in more cellulose fibrils exposer on the upper surface of fibre, by which inter-fibre bonding will improve. As wood chips are processed at basic pH and high temperature so the enzymatic treatments require proteins exhibiting an activity in broad pH range and high thermos-stability (Haki and Rakshit2003).In case of mechanical pulping, enzyme uses on coarse fibres or wood chips, may result in significant energy conservation in refining, but there is need to develop applicable technologies at industrial scale. Enzymes can give a targeted but gentler refining. In case of mechanical refining generation of fines is the main problem which leads to decline in drainability of pulp and strength properties of paper, therefore it comes on expense for drainage additives and strength.

2.1. Enzymatic Effects on Different Types of Pulp

Main enzymes used for refining are cellulase and xylanase which act on Cellulose and Hemicellulose respectively. Other enzymes used in paper and pulp industry to aid refining are Laccase, Manganese peroxidase, Amylase, Pectinase.

The major studies on enzymes effects on refining of different types of pulp are discussed below.

2.1.1. Chemical Pulps

In early stages, when researchers started using enzymes in refining, they started working with single enzyme from wild type microbes or without any modification in the molecular structure of enzyme and they got better pulp characteristics, improved strength and impression properties of paper. They changed parameters like pH, temp., enzyme dose, fibre types, pulp type, pulp consistency, etc. and tested for their effects on

pulp and paper properties e.g. Yerkes (1968) used cellulase from white rot fungus; Noe et al. (1986) and Bharwaj et al. (1996) used xylanase; likewise Mansfield et al (1997), Kibblewhite & wong (1999), Seo B et al. (2000) and many others, used single enzymes, in a particular study, from wild type microbes.

Then in next stage of development, researchers did experiment by using blend of enzymes and tested their effects by changing parameters and they reported better results than those obtained by using single enzyme. They observed that one enzyme was enhancing the working efficiency of another enzyme in the blend (synergetic effect) e.g. Bajpai et al. (2006) used mixture of cellulase and hemicellulase; Gill et al. (2009) used mixture of cellulase and carbohydras.

But the present scenario is, researchers are using biotechnological tools and concentrating on enzymes produced by genetically modified organisms or by changing the molecular structure of enzyme to explore the maximum efficiency of enzyme. The recent studies performed on chemical pulp are summarized hereby and the major studies are surmised in table 1.

Cadena et al. (2010) studied that the endoglucanase Cel9B from *Paenibacillusbarcinonensis* is a very effectivebiocatalyst, which decreases the energy costsassociated with pulp refining. In this work, domains which are responsible for this enzyme's action on cellulose surfaces or the specific structural domain for rising the new cellulases molecule for maximum biorefining efficiency is identified. The recombinant enzymesFn3–CBD3b, GH9–CBD3c, and CBD3b were used, these are trimmed forms of Cel9B, which permit us to review the particular impact of the fibronectin-like domains, catalytic, and cellulose binding domain of the whole enzyme with TCF kraft pulp(Eucalyptus globulus) on refining. On basis of physico-mechanical properties, the trimmed form having the GH9–CBD3c (catalytic domain) had major effect on morphology of fiber. When its effects were compared with theCel9B (whole enzyme) then it showed that the trimmed enzyme adds to enhancing strength of papersheet by enhanced burst and tensile strength and moreover the trimmed form of enzyme is more efficient in improving tear resistance than the whole enzyme. So that, theGH9–CBD3c domain of whole enzyme (Cel9B) has biorefining action. Beside that CBDs (cellulose binding domains) are less effective toward refining of pulp but results of this work reveals that CBD3b make changes in fibre surfaces as a result affect the paper properties.

Ko et al. (2010) studied the refining effects on BKP (Bleached Kraft Pulp) with two Cellulases enzymes, one produced by GMO *Paenibacillus.campinasensis* and the other one a commercial product named FiberCare®. For this study three different types of pulp were used: unbleached, fully bleached and oxygen bleached. Conditions for enzyme treatment for cellulase were 0–20 IU/g, 40 °C, and 7 pH, and for FiberCare® were 185.2 IU/mg, 40 °C, and 6 pH. The pulp consistency and retention time for both enzymes were 10 %, and 60 min resp. The cellulase from *P.campinasensis* reduced drainability of fully BKP of eucalyptus when 3,350 and 2,350 revolutions were applied in PFI mill and that accords to 10 % and 37% refining energy conservation resp. and these results were better when compared with that of FiberCare® treated pulp.

Table 1. Major studies performed on enzymatic refining of Chemical pulp.

Year	**Researcher**	**Enzyme Used**	**Material Used**	**Major Effects**
1968	Yerkes	Cellulases (from white-rot fungus)	Chemical pulp or cotton linters	Improved strength and reduced beating time
1986	Noe et al.	Xylanase	Bleached chemical pulp	Reduced energy demand
1996	Bhardwaj et al.	Hemicellulase	Unbleached kraft pulps (softwood, bamboo and mixed pulp)	Reduction in beating time by 17-25%
1997	Mansfield et al.	Cellulase and xylanase	Douglas fir kraft pulp	Changes in fiber both at micro and macro structural level
1999	Mansfield et al.	Cellulase	Douglas fir kraft pulp	Enhance in-plane strength of handsheets
1999	Kibblewhite and Wong	Endoglucanases and xylanase	Radiate pine kraft pulp	Increased tear index-apparent density and tear index-tensile index properties of handsheets
2000	Seo B. et al.	Cellulase	Chemical pulp	Shortened refining time by causing extensive fibre

				cutting and improved drainage without significantly lowering fiber length.
2001	Sigoillot	Manganese peroxide	Alkaline peroxide pulp derived from popular	Decrease in refining energy and increase in drainability by 25%
2002	Torres *et al*	Cellulases (Commercial enzymes ComC CelB from Paemibacillus sp. Strain BP-23)	Dried and never dried eucalyptus pulp	Improved paper properties and counterbalancing the hornification effect caused by drying
2006	Bajpai et al.	Mixture of cellulase and hemicellulase	Different type of pulps bleached kraft, long fiber fraction bamboo, old corrugated container, etc.	Reduced refining energy requirement by 18-45%
2006	Shamim et al.	Cellulase/ hemicellulose	Unbleached and bleached mixed (hardwood and bamboo) chemical pulp	Increased drainability value, reduced beating time and also reduced energy by 15-20%
2008	Sandeep et al.	Hemicellulase	Bleached mixed hardwood pulp containing Acacia and MTH	6-30% energy savings, improved drainage and strength properties.
2009	Gill et al.	Cellulase and carbohydrase mixture	Bleached *Eucalyptus globulus* Kraft pulp	Improved pulp drainability (°SR) up to 80% when same amount of energy used for refining.
2010	Ko et al	Commercial Cellulase (fiber care)	Bleached Kraft pulp	Decreased drainability, when applied 3,350 and 2,350 PFI revolutions, from CSF 621 to 400 ml. Thus 10 % and 37% refining energy savings, respectively

2010	Cadena *et al*	Whole cellulase Cel9B from *P.barcinonensis* And truncated form of Cel9B (GH9-CBD3b, Fn3-CBD3b, and CBD3b)	TCF kraft pulp	Reduced associated energy cost and truncated enzyme Cel9b is responsible for bio refining and more effective in improving strength properties than whole enzyme.
2010	Sigoillot *et al*	Commercial Cellulase	Softwood bleached kraft pulp	Improved Fiber swelling and reduced refining intensity by 33%
2011	Liu *et al*	Commercial cellulases with (N2 51101) and without (Refinase M) CBDs	Died, bleached spruce kraft pulp	Improved °SR by 20°SR at same beating conditions / for fixed °SR the energy saved is 4434 kWh/t and 5200kWh/t. N2 51101 is more sensitive to beating time
2011	Ko *et al*	Commercial cellulase (FiberCare®)	Bleached kraft pulp from *Eucalyptus globulus*	Fibrillation by PFI refining clearly increased the fiber widths so more available surface area
2012	Du et al	Pulpzyme HC®	Bleached softwood pulp	Improved refining efficiency, fiber length, knot index and curl index of fiber.
2012	Meyer et al	Xylanase, cellulase, pectinase and laccase	Norway spruce pulp	70% fewer shives so savings on reject treatment and energy saving
2013	Andre et al	Formulation of cellulase and hemicellulase	Black spruce (Picea mariana) pulp	Overall energy savings as high as 15% and lowering the overall specific energy consumption (SEC)
2014	Bhardwaj et al	commercial cellulase	Bleached mixed hardwood pulp	18% reduction in refining energy to reach CSF 300±10 and the enzyme pretreatment affected web consolidation and also improved tensile index and burst index by 15% and 13% resp.

2.1.2. Secondary Fibres

As it is known to us that with each recycle, the raw materials quality deteriorates because of increase in drainage resistance of recycled pulp and undesirable changes that occur in the fibre properties. The first cause difficulty in sheet formation process, reduces the runnability of paper machine, and more drying energy consumption, it's because of higher drainage resistance of the recycled fibers than virgin fibers, due to its repeated pulping and exposure to drying process. The second influences interfiber bonding and hence the paper strength. (Rasmi and Nishi 2010).

In the recycling process the enzymes use has been accomplished because by treatment with enzymes like cellulases and hemicellulases properties of recycled fibbers' can be improved.

Poorna and Prema (2007) studied that the impact of crude enzyme on treatment of waste paper utilization made a notable change on fibre surfaces; the untreated pulp surface appeared smoother than the surface of enzyme pre-treated fibres. Fibbers of treated pulp hold up under a peeling process that gave rise to filaments and flakes of materials separated from fiber surfaces because of xylan hydrolysis. By enzyme treatment fiber swelling increased, which favoured refining, which consequently resulted in improved physical properties. Enzyme addition before refining can enhance strength properties at a fixed level of refining.

Bajpai (2010) observed another case where a blend of enzymes cellulase and hemicellulase covered in the commercial product by the name FiberzymeLBR® had clear impact on a double-sorted OCC (Old corrugated containers) pulp (consistency 4 %). The operational were 50 °C temp, 5-7 pH interval, enzyme dose was 0.05 %, and180 min retention time; by this treatment the energy consumption was reduced by 30 % to get 429 ml CSF.

Valchev and Bikov (2011) showed the effect of FiberCare®D (enzyme having endoglucanase activity) treatment on the refining degree and dewatering time of Duropak–Trakia–Papir OCC pulp (6–10 %consistency) and revealed that the enzyme dose 0.05–0.2 % were applied at temp. 60 °C, in 4-7 pH range, 60 min retention time, enhanced the refining degree up to 25 %, and the dewatering of pulp by 20–45% resp.

In paper production, steam consumption in dryer could be decreased by over 4 %. The impact of enzyme treatment exhibits that with increasing enzyme dosage breaking length also increased slowly, while burst index and tear index reduced. Possibly at low enzyme charges, the FiberCare® D treatment adds to better paper structure independently.

Table 2. Major studies performed on enzymatic refining of Secondary fibres.

2006	Bajpai et al.	Mixture of cellulase and hemicellulase	Old corrugated container	Reduced refining energy requirement by 18-45%
2009	Shaikh and luo	Cellulases	Old newsprints, old corrugated containers and mixed waste	Increase in drainability was reported by 13-40%
2010	Huang et al	Cellulase 5 (Endoglucanase)	Old corrugated containers	Refining degree, ring pressure index and tensile index were 44.4%, 18.6% and 25.2% larger than without pretreatment
2010	Bajpai et al	Cellulase/ hemicellulase	Old corrugated containers	Reduction in refining time by 15% to reach 429mL CSF
2011	Valchev and Bikov	FiberCare D	Old corrugated containers	Improved pulp dewatering by 20-45% and refining degree up to 25% and reduced dryer steam consumption by over 4%
2011	Huang et al	Amylase	Old corrugated containers	Increased mechanical strength of secondary fiber. Tensile index and crush index were increased by 15.6% & 18.3%

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Pulp:

For the experimental purpose three types of unbleached mixed hard wood pulp were used from nearby paper mill. The initial drainability of pulp A, B and C were 15 °SR and 10, 8 °SR respectively.

3.2. Enzyme:

For this study the two enzymes were used. One was a blend of three enzymes (Amazone A) and all three enzymes in the blend are xylanase by nature but from different sources. The sources of these enzymes were: GM Trichoderma Reesi, GM Penicillium funiculosum and GM Bacillus Licheniformis. The second enzyme used for comparison purpose, was xylanase from single source. The activity of blend of enzymes was 100800 IU/ml and that for xylanase from single source was 23000 IU/g, and operational conditions for both the enzymes were pH 5-6, temp. 50-60.

3.3. Reagents and Solutions:

- Distilled Water,
- Acetic acid solution,
- Tri-Sodium citrate solution,
- Acetic acid -Tri-Sodium citrate buffer solution, pH value 5.5
- Xylan Solution, strength 0.5%
- DNS Reagent

3.4. Instrument and Equipment:

- Disintegrator (Universal Engineering Corp.)
- Analytical balance: sense quantity 0.001 g
- PFI Mill refiner (J. Hensgstler K.G.)
- British Sheet former (1092; Mavis Engineering Co. Ltd)
- Hot air oven (HAC405; Popular Traders Ambala Cantt.)
- PH meter: capable of precision 0.01 (OR900 Multiparameter, ORLAB)

- Refrigerator
- Magnetic stirring apparatus
- Electromagnetic vibrator
- Buchner Funnel (for vacuum filtration)
- Thermostat water bath: temperature controlled between 30 to 60℃, capable of precision 0.1℃
- Stopwatch: errors no more than 5s
- Spectrophotometer (UV- Visible spectrophotometer)
- Micropipettes: precision 1 µl
- Glassware
- Measuring Cylinders
- Instruments for fiber and sheet properties measurement
    - Hydraulic press (Lorentzen & Wettres)
    - Schopper-Riegler type Drainability Tester (Lorentzen & Wettres)
    - Morfi fiber analyzer (Tech Pap)
    - Electro-Hydraulic tensile strength tester (Lorentzen & Wettres)
    - Tear tester (AB Lorentzen & Wettres Maskinaffar)
    - Mullen Burst tester (Lorentzen & Wettres)

3.5. Enzyme Activity Determination:

The enzyme activity was determined on the basis of analysis of reducing sugars formed by enzyme action on substrate. Enzyme scised the glycosidic bonds between two carbohydrates or between a carbohydrate and non-carbohydrate moiety of substrate. The enzyme activity of both enzymes, blend of enzymes and enzyme from single source, was determined by DNS (3, 5-dinitrosalicylic acid) method proposed by G.L. Miller. To determine the enzyme activity, first Acetic acid -Tri-Sodium citrate buffer solution of pH value 5.5 was prepared. This buffer solution was used in preparation of substrate (xylan) solution and dilutions of enzyme. After this xylan solution of 0.5% strength is prepared by dissolving 0.1g xylan in 20ml Acetic acid -Tri-Sodium citrate buffer. This solution was put into water bath at 50°C and kept there till the water in waterbath start boiling. Then the solution was taken out from waterbath and kept on electro-magnetic stirrer till the temperature of solution reach at room temperature. Then the dilutions of enzyme (150000, 180000 and 200000 times) were prepared in buffer

solution. After that eight reaction mixture tubes for enzyme blend were prepared as shown in the table 3. Then all reaction tubes were incubated into waterbath at 50°C for 20 min after 20 mins of incubation 2ml of DNS solution is added into each reaction mixture tube to stop the reaction. Then all tubes were incubated into boiling water for 5mins immediately after adding DNS into tubes. After 5min all tubes were put under running tap water to cool down the solution upto room temp. Then the absorbance of all solutions were measured at 540nm. A_{540} of enzyme and substrate control were subtracted from the absorbance of enzyme-substrate reaction mixture. Then by comparing this absorbance with that of standard curve of xylose the activity of enzyme under analysis is calculated.

Table 3: The dilutions and absorbance for enzymes blend

Particulars	Enzyme dilutions and their absorbance at 540 nm (A_{540})						Substrate control	Blank
	150,000 times		180,000 times		200,000 times			
Enzyme Control and its A_{540}	200µl E +800µl B	-0.007	200µl E +800µl B	-0.007	200µl E +800µl B	-0.007	800µl S +200µl B & A_{540}- 0.165	1ml
Reaction Mixture and its A_{540}	200µl E +800µl S	1.274	200µl E +800µl S	1.149	200µl E +800µl S	1.024		

By considering the absorbance value of 200,000 times enzyme dilution and comparing it with standard plot of xylose, enzyme activity value obtained was 100800 IU/ml.

In the same way activity for xylanase from single source was determined but the dilutions level was different. The dilutions and absorbance of particular dilution for xylanase from single source is shown in the table 4. By considering the value of absorbance at 50,000 times dilution and comparing it same standard plot for xylose activity was calculated. The activity value for this enzyme was obtained as 23000 IU/g.

Table 4: The dilutions and absorbance for xylanase from single source.

Particulars	Enzyme dilutions and their absorbance at 540 nm (A_{540})						Substrate control	Blank
	25,000 times		50,000 times		100,000 times			
Enzyme Control and its A_{540}	200µl E +800µl B	0.002	200µl E +800µl B	0.001	200µl E +800µl B	0	800µl S +200µl B & A_{540}- 0.154	1ml
Reaction Mixture and its A_{540}	200µl E +800µl S	1.881	200µl E +800µl S	0.923	200µl E +800µl S	0.534		

3.6. Pulp-Enzyme Pre-treatment:

First of all the operational conditions (pH and dose), for enzymes blend were optimised. For optimisation process the condition which is to be optimised is changed by keeping all other conditions fixed. Firstly pH was optimised in the range 4-8 by applying the enzyme dose 45 IU/g OD pulp and pulp consistency, temp and time was kept at 4%, 50 °C and 180 min resp. throughout the experiment (Nishi K. Bhardwaj, 1996). The pH of pulp was maintained at particular value with the help of H_2SO_4 (2N) and NaOH (1N). The pH was optimised on the basis of highest pulp drainability on least energy consumption or PFI revolutions and its come at 5.5 pH value. After that enzyme dose was optimised on the same basis. The dose was applied in the range 45- 195 IU/g OD pulp, keeping the difference of 30 IU/g OD pulp between two values and 150-180 IU/g OD pulp, the difference between two values was 15 IU/g OD pulp (pH 5.5, temp 50 °C and time 180 min). The optimised dose obtained was 150 IU/g OD pulp.

After optimisation process, pulp was treated with blend of enzymes at optimised conditions before refining. The optimised conditions for enzyme pre-treatment were temp 50°C, pH 5.5, treatment time 180min, pulp consistency 4% and enzyme dose 150 IU/ g of O.D pulp. After 180min, the residual enzyme was washed out from pulp to stop the further enzyme action and refining of pulp was carried out for further analysis.

3.7. Fibre Quality Analysis:

After Enzyme pre-treatment the washed pulp was refined in PFI mill at different no. of revolutions to obtain required value of pulp °SR (around 40). °SR was measured by Schopper-Riegler type Drainability Tester according to standards ISO 5267/1. This test quickly provides an idea of the refining, relating to the speed of the drainage of the diluted paper suspension. For this test 1000 ml diluted pulp sample of consistency 0.2% is drained. The speed of drainage is related to the surface conditions and the swelling of fibres and provides a useful indicator, of the amount of mechanical treatment (refining) of pulp. The scale of measurement in °SR is (a) drainage of 1000 ml corresponds to 0 °SR, (b) drainage of 0 ml corresponds to 100 °SR and (c) drainage of each 10 ml of water corresponds to 1 °SR.

After that fibre analysis is done with the help of Morfi fibre analyser by taking 25mg/L of pulp suspension. By fibre analysis the parameters like lengthwise fibre distribution, fibre length, fiber width, fines average length and average area were observed.

For analysis of changes in fibre morphology due to enzyme treatment and refining the SEM (Scanning Electron Microscopy) of fibres was carried out. Under SEM four types of fibres were observed (a) the untreated fibres after disintegration, (b) fibers after enzyme treatment, (c) Untreated fibers after refining, and (d) Enzyme pre-treated fibres after refining. SEM of all four samples under different magnification showed changes in fibre morphology like smooth surface of untreated pulp, external fibrillation (cracks, pores and peeling) on treated fibre surface and internal fibrillation after refining and also the combined effect of enzyme and refining as shown in the figure 2.

3.8. Sheet Formation and Strength properties measurement:

After analysis of fibre the paper sheets, from the pulp sample which had °SR in rang 35-45, were formed according to TAPPI standards T 205 sp-95. By taking 0.33% pulp consistency the paper sheets of about 60 gsm were prepared with the help of British sheet former. Then sheets formed were pressed under 5 psig pressure for 5min in hydraulic press and after 5 min pressure is removed and sheets were taken out. Then the sheets were separated from blotting papers and taken at mirror polished drying discs.

After that, those drying discs having wet sheets on their surface were stacked between drying rings for around 24 hrs for air drying. After sheets drying the strength properties of sheets formed were measured with the help of laboratory instruments.

The tear, tensile and burst index of sheets were calculated on the grammage basis. The Tear strengths was measured by tear tester (AB Lorentzen & Wettres Maskinaffar) according to TAPPI standards 'T 414 om-98', then this strength was divided by grammage to obtain tear index ($mN*m^2g^{-1}$).

The tensile strength was measured by electro-hydraulic tensile tester (Lorentzen & Wettres) according to 'T 494 om-96' and this value was divided by grammage to calculate tensile index (Nmg^{-1}) of paper.

Burst strength of paper was measured by Mullen burst tester (Lorentzen & Wettres) according to TAPPI standards 'T 403 om-97' and value obtained was divided by grammage to calculate the burst index ($KPa\ m^2\ g^{-1}$). The values of strength indexes are summarised in tables 13 & 15.

CHAPTER 4

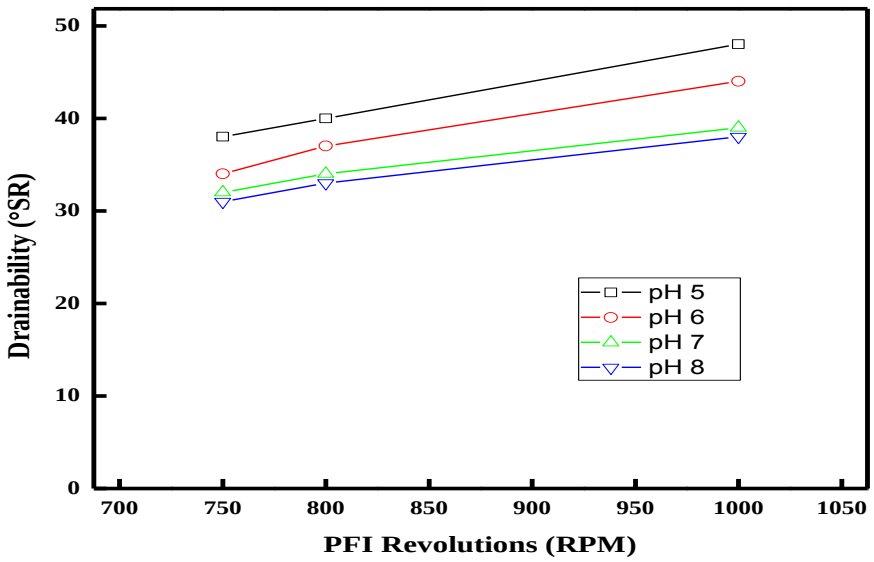
4. RESULTS AND DISCUSSION

In order to test the synergistic effect of enzymes on refining, the unbleached mixed hardwood pulp was refined after enzymatic pre-treatment with blend of enzymes. Firstly, the pulp was refined sequentially from 0 to 2500 rpm to obtain the ◦SR value of pulp in the range of 35-45 ◦SR. Then a comparative study was carried out by using another enzyme from single source and considering the parameters like ◦SR, fibre analysis and sheets strength properties analysis. Results of this study are summarised in the table blow and compared by plotting this values.

4.1. pH Optimization

Table 5: pH optimisation with pulp A (First run)

pH	Revolutions	°SR
5	750	38
	800	40
	1000	48
6	750	34
	800	37
	1000	44
7	750	32
	800	34
	1000	39
8	750	31
	800	33
	1000	38
Initial °SR of pulp 15, enzyme dose 45 IU/g OD pulp, temp 50 °C, pulp consistency 4% and treatment time 180 min,		



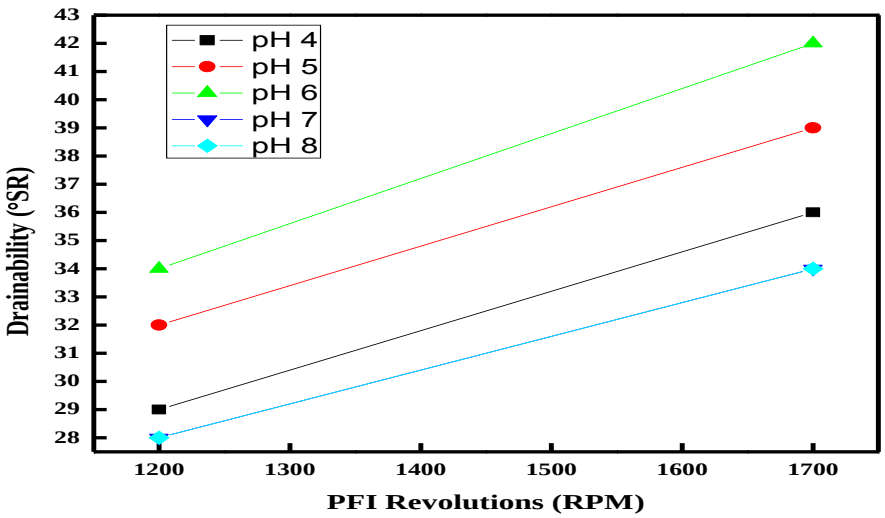
Graph 1: Plot of Revolutions vs °SR at different pH

By studying the table and graph shown above It can be said that the unbleached hardwood pulp with initial °SR 15 was pre-treated with enzyme dose 45 IU/g OD pulp. The pH of pulp was varied in the range 5-8 for different samples and treated for 3 hrs at 50 °C and after pulp washing refining was carried out at different PFI revolutions as indicated in the table 5. The best results were considered on the basis of higher pulp drainability at lesser PFI revolutions or energy consumption. Hence the table 5 indicates that, the best results were obtained at pH 5. For the conformation of pH optimisation, another run was carried out on different pulp with initial °SR 10 in the pH range 4-8 and using same operational conditions of enzyme dose and pre-treatment time and values obtained are summarised in table 6 and plotted as shown in the graph 2.

Table 6: pH Optimisation with pulp B (Second run)

pH	Revolutions (RPM)	°SR
4	1200	29
	1700	36
5	1200	32
	1700	39

6	1200	34
	1700	42
7	1200	28
	1700	34
8	1200	28
	1700	34
Initial °SR of pulp 10, enzyme dose 45 IU/g OD pulp, pulp consistency 4%, temp 50 °C and treatment time 180 min.		



Graph 2: Plot of Revolutions vs °SR for second run of pH optimisation

The output after second run of pH optimisation revealed that the enzyme showed highest efficiency at pH 6.

So by considering the results of both runs of pH optimisation it was concluded that enzyme was working at its best with pH values 5 & 6 on pulp A & B respectively. By taking the mean of both optimised pH, 5.5 was found optimised pH for the best functioning of enzyme.

4.2. Enzyme Dose Optimization:

After pH optimization the enzyme dose was optimized by keeping others parameters like pH, temp, time, consistency constant. The experiment of enzyme dose optimization was carried out at pulp B (initial °SR 10) with time 180 min, temp 50 °C, pH 5.5 and pulp consistency 4%. The results of enzyme optimisation are summarised in table 7. The values in table 7 shows that the enzymes blend enhanced the refining efficiency in comparison to untreated pulp, when only °SR was taken into consideration. But the drainability was not increasing in proportion with increasing enzyme dose. Initially the drainability was increased in very slow fashion and at enzyme charge 165 IU/g OD pulp, there was a sharp increase and after that the drainability starts decreasing. Hence, the maximum drainability was achieved at enzyme charge 165 IU/g of OD pulp. So this value was taken into consideration for further analysis.

Table 7: Enzyme dose optimization with pulp B (first run)

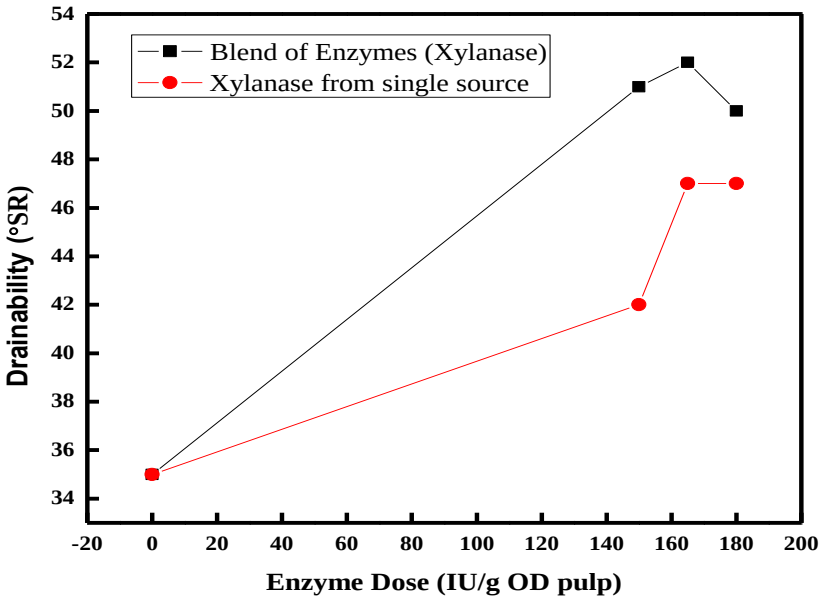
Enzyme dose (IU/g of OD Pulp)	Drainability (°SR)
0	40
45	42
75	43
105	42
135	44
165	55
195	54
Initial °SR 10; pH 5.5, PFI revolutions 1700, temp 50; time 180 mins.	

To confirm the results of dose optimisation the experiment was repeated with different PFI revolutions and applying the narrower range (difference between two doses was 15 IU/g OD pulp) of enzyme dose.

A parallel experiment was carried out by using an enzyme from single source on the same pulp for comparative studies. The other parameters were kept same as that for the blend of enzymes.

Table 8: Comperison of enzymes effect on refining (pulp drainabilty)

Enzyme Dose (IU/g of OD pulp)	°SR of pulp pre-treated with blend of enzymes	°SR of pulp pre-treated with single sourced xylanase
0	35	35
150	51	43
165	52	47
180	50	47
Initial °SR 10; pH 5.5, PFI revolutions 1300, temp 50; time 180 mins.		



Graph 3: Comparison of Enzymes mixture efficiency with single sourced enzyme

The values in table 8 shows that both enzymes enhanced the refining efficiency when only °SR was taken into consideration. But the drainability was not increasing in proportion with increasing enzyme dose after a particular value of enzyme charge. When pulp was treated with blend of enzymes initially the drainability was increased in very slow fashion at enzyme charge 150 to 165 IU/g OD pulp but after that it started decreasing but in case of enzyme from single source drainability increased slowly and

then get stable between enzyme doses 165 to 180 IU/g OD pulp. The values of table 8 were plotted in graph 3 for qualitative analysis.

4.3. Effect of Hold up Time on Enzyme Pre-treated Washed pulp:

In this study another observation was made, if the washed pulp after enzyme treatment was kept for overnight prior to refining then it gave better results in comparison to the condition where the pulp was refined immediately after washing step. But if this hold up time was given for untreated pulp then this time didn't made any significant difference in pulp drainability. So the reason behind the enhanced efficiency can be that, enzyme treatment induced changes to the external surface of fibre (flakes and peeling) which made the interior surface of the fibre more accessible to water hence the increased swelling of fibre. So the effect of refining was enhanced when pulp was hold up for overnight after enzyme pre-treatment followed by washing step. Results of this comparative study, in which effect of hold-up time on untreated and enzyme pre-treated pulp after washing step are summarised and plotted in table and graph 6 resp.

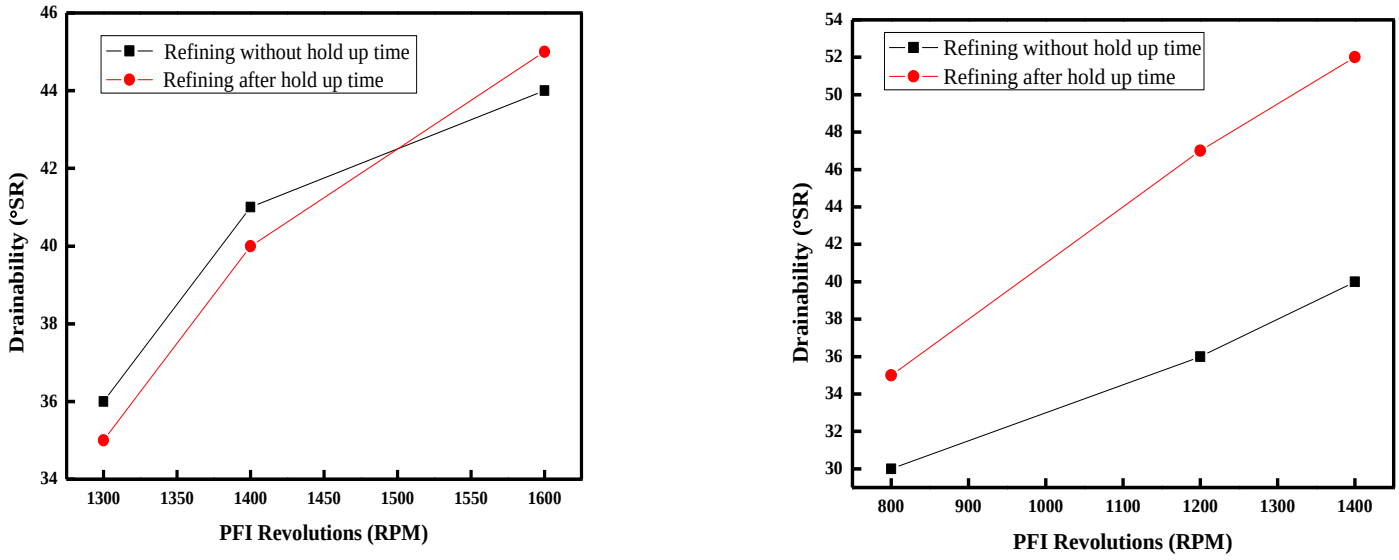
Table 9: Effect of hold up time before refining (without enzyme pre-treated pulp)

Revolutions	Refining without any time gap washing	Refining after 12hrs of washing
1300	36	35
1400	41	40
1600	44	45

It can be seen from above table that hold up time has no significant effect on pulp drainability when pulp was not treated with enzyme. The drainability of both the pulps are almost same when refined with or without a hold-up time. So the effect of hold-up time is because of enzyme functioning as it can be seen from table 9 when pulp was pre-treated with enzyme.

Table 10 Effect of hold up time before refining on enzyme pre-treated washed pulp

Revolutions	Refining without immediately after washing	Refining after 12hrs of washing
800	30	35
1200	36	47
1400	40	52
Initial °SR 10, Dose 150 IU/g of OD pulp, pH 5.5, temp 50, time 180 min		



Graph 4: Plots showing effects of hold-up time on untreateated (L) and enzyme pre-treated (R) pulp on refining respectively.

By comparing both graphs it can be concluded that enzyme have done some changes in outer surface of fibre so when pulp was kept for overnight after washing then it absorb more water than without untreated pulp and refining act more efficiently on that pulp. So higher pulp drainability was obtained on refining at same level of PFI revolutions.

4.4. Effect Of Enzyme On Fiber Morphology:

Enzyme treatment induced changes in outer surface of fibre so mainly effect the external fibrillation by acting upon hemicellulose of cell wall. To observe the changes induced by enzyme on fibre surface four samples were viewed under SEM (Scanning Electron Microscope). (a) Untreated, (b) Enzyme pre-treated, (c) Refined but without enzyme treatment and (d) Enzyme pre-treatment refined pulp were viewed at magnification 5 kx.

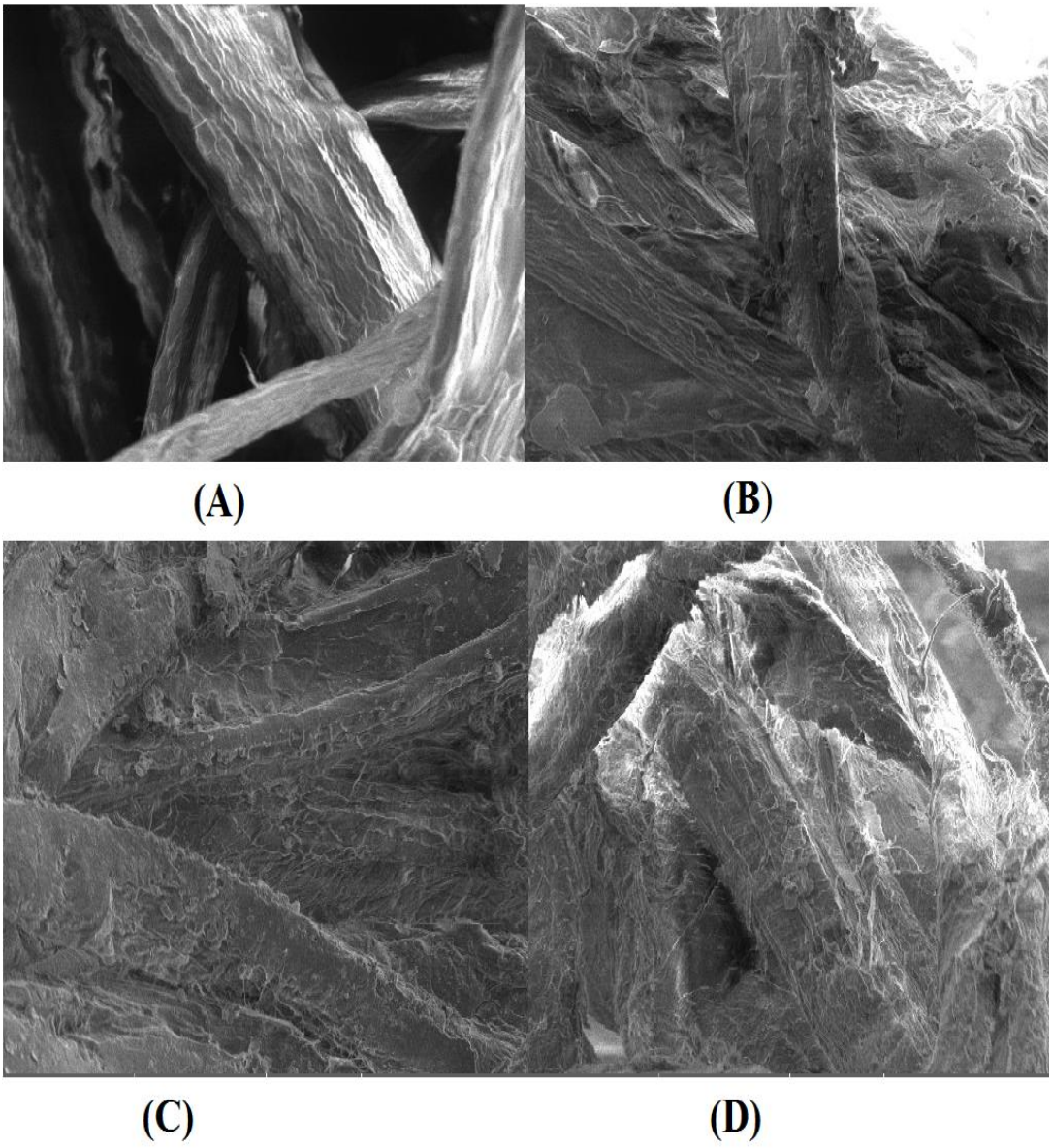


Figure 2: Scanning electron micrograph of (a) untreated, (b) Enzyme pre-treated, (c) Refined but no enzyme and (d) refined after enzyme pre-treatment, of mixed hardwood pulp

(SEM MAG: 5 kx; SEM HV: 3Kv; WD: 13.33 mm and View Field: 55.4 µm)

These micrographs shows the image (a) fibre surface having small particle with high affinity to water and large specific surface area. Image (b) is the fibre treated with xylanase and shows that the enzyme has effect mainly on external fibrillation or at outer surface and help water to penetrate into inner surface of fibre and enhance fibre swelling. Image (c) shows that fining impose high mechanical force on fibre so responsible for internal fibrillation and fibre shortening. And image (d) shows the combined effect of enzyme and refining so greater fibrillation and peeling effect was observed.

4.5. Fiber Analysis of pulp:

Fibre analysis of refined pulp after using enzyme pre-treatment with 'blend of enzymes' and enzyme from single source was done by "Morfi Fibre Analyser". Results of that analysis are summarized in the table below and different plots for fibre analysis obtained from Morfi fibre analyser are summarized in appendix.

Table 11: Values of different parameters obtained by Fibre analysis

Enzyme Dose (IU/g of OD pulp)		Revolutions & °SR		No. of fibers observed	Width	Coarse-ness	Broken edge (%)	Fine Elements	
		RPM	°SR					Avg. area (μm^2)	Avg. length (µm)
0		1300	35	5093	16.5	0.034	14.95	1165	48
Blend of enzymes (Xylanases)	150	1300	51	5110	16.6	0.048	19.6	984	44
	165	1300	52	5086	16.1	0.043	20.14	996	44
	180	1300	50	5150	16.8	0.042	18.47	941	42
Single sourced Xylanase	150	1300	43	5102	16.1	0.039	14.41	966	42
	165	1300	47	5001	16.5	0.037	16.11	1058	46
	180	1300	47	5039	16.2	0.037	15.69	1074	47
0		1400	46	5035	16.4	0.045	14.7	1083	45
150		1400	40	5062	16	0.049	17.32	1043	46

By studying the values from above table it can be observed that by using enzyme higher degree of pulp drainability was obtained because enzyme treatment open up fibre structure instead of cutting the fibre length. So the problem of fines generation can be minimised by giving enzyme treatment to the pulp during slushing step. With refining after enzyme pre-treatment the broken edges percentage increases because fibre structure loosened up by enzyme action and get swelled. The fibre coarseness increases when enzyme treatment is given because enzyme mainly effects external fibrillation cause the fibre surface rough, peeling action exposes more cellulose at the surface causing stronger hydrogen bonding in paper hence enhancing the strength properties.

4.6. Strength Properties Analysis:

By studying the above results it can be said that enzyme has enhanced the drainability of pulp but at the same time it should be kept in mind that enzyme must not have any negative impacts on strength properties. Hence it can be said that the too high drainability at the cost of strength properties or at very high enzymatic dose will not be economical. To study the effects of blend of enzymes on compensable level the tensile, tear and burst index of sheets were compared with those of untreated and treated pulp with enzyme from single source.

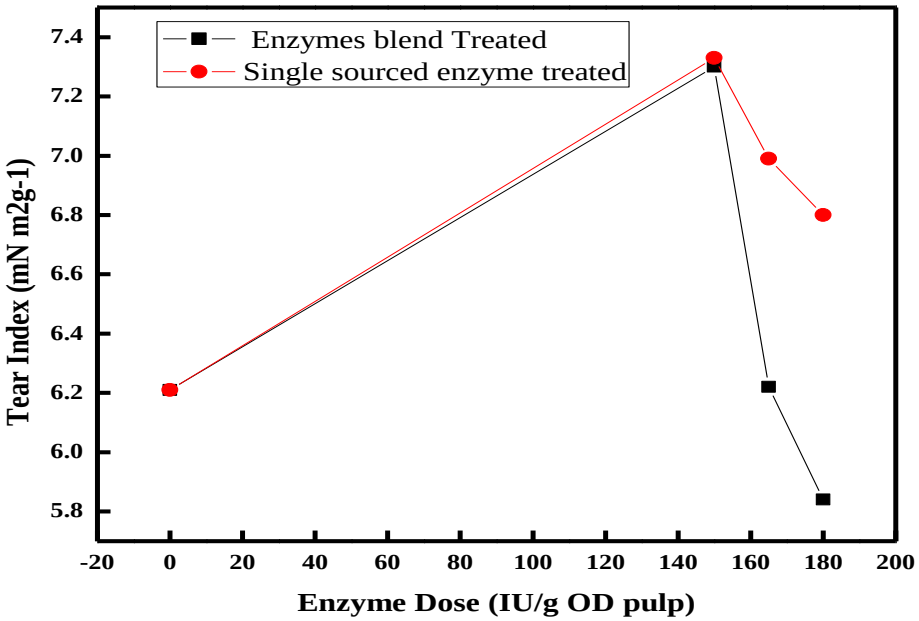
The results of this study are shown in table 12 and compared by plotting those results in graphs below.

Table 12: Showing the effects of enzyme treatment on strength properties of paper

Enzyme Dose (IU/g of OD Pulp)	Pulp Drainability (°SR)	Tear Index (mN m^2g^{-1})	Tensile Index (Nmg^{-1})	Burst Index (KPa $m^2 g^{-1}$)
0	35	6.21	60.76	4.9
Blend of enzymes				
150	51	7.3	65.33	5.25
165	52	6.22	66.64	5.30
180	50	5.84	64.67	5.25

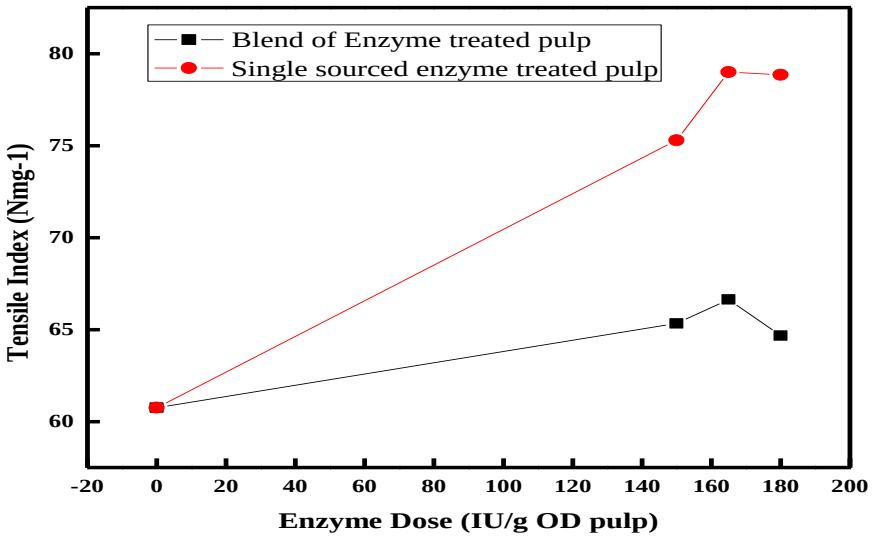
Enzyme from single source				
150	42	7.33	75.29	5.42
165	47	6.99	79.00	5.79
180	47	6.8	78.85	5.78
Initial °SR 10, 1300 PFI revolutions, pH 5.5, temp 50 consistency 4% and time 180 min				

As the fibre length decreases with excessive refining the tear index of paper decreases and tensile and burst index of paper increase. But here it can be observed that at same level of refining all the three properties are in increasing fashion or there is no negative effect so use of enzyme is beneficial in most of the cases.



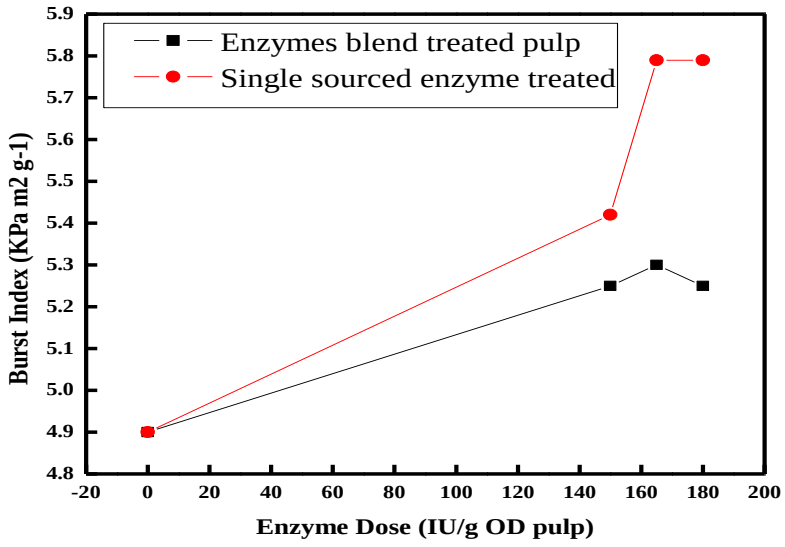
Graph 5: Effect of enzymes treatment on tear index of paper sheets

As the drainability increase excessively with excessive refining the tear index of fiber decreases and maintaining a good tear index after refining is a challenge. Here it can be observed that at enzyme charge 150 IU/g OD pulp, tear index is increasing with both enzymes treatment but enzyme from single source is more effective in maintaining a balance of tear index at higher draianability.



Graph 6: Effect of refining on tensile index of paper.

In general the tensile strength of paper get increased with refining. But at higher dose of enzyme this comes not true because of degradation of hemicellulose and hence the degradation of fibres (Nuno Gil et. al, 2009). Due to fiber degradation the physic-mechanical properties of pulp deteriorate. This deterioration of pulp properties was not observed until the enzyme dose 165 IU/g of OD pulp where the tensile strength was improved but beyond that dose tensile strength start deteriorate.



Graph 7: Effect of refining on burst index of enzyme treated pulp

Burst index of sheets follow the same pattern with that of tensile index. So the burst index of sheets also increases up to the enzyme charge 165 IU/g OD pulp and after that it starts decreasing like the tensile strength.

4.7. Comparison of strength properties at optimized conditions:

After optimisation of operational conditions for enzyme and analysing & comparing fibre and sheets properties on those condition, final experiment was carried out with optimised condition on pulp C with initial drainability 8. The pulp was refined up to equal no. of PFI revolutions and then fibre and sheet properties were compared with that of untreated pulp. The results of fibre analysis and sheet properties analysis in comparison with that of untreated pulp is shown below in the tables 12 and 13.

Table 13: Values of different parameters obtained by Fibre analysis

Enzyme Dose (IU/g of OD pulp)	Revolutions & °SR		No. of fibers observed	Width	Coarse-ness	Broken edge (%)	Fine Elements	
	RPM	°SR					Avg. area (μm^2)	Avg. length (µm)
0	1400	46	5035	16.4	0.045	14.7	1083	45
150	1400	40	5062	16	0.049	17.32	1043	46

Although the tensile and burst strength of paper were better at enzyme charges 165 IU/g OD pulp than at 150 IU/g OD pulp but beyond the 150 IU/g OD pulp, the tear strength of paper started deteriorating. The loss in tear strength was not compensable in comparison to increase in tensile and burst strength at 165 IU/g OD pulp enzyme dose. So the dose was optimised at 150 IU/g OD pulp.

Table 14: comparison of strength properties at optimised condition with that of untreated pulp

Enzyme Dose (IU/g of OD Pulp)	Pulp Drainability (°SR)	Tear Index (mN m^2g^{-1})	Tensile Index (Nmg^{-1})	Burst Index (KPa m^2 g^{-1})
0	40	7.76	75.13	6.47
150	46	8.17	79.7	6.26
Initial drainability 8, 1400 PFI revolutions, pH 5.5, temp 50 °C, pulp consistency 4%				

By comparing the properties of final experiment it can be proposed that at the same level of refining enzyme treated pulp showed higher pulp drainability and better strength properties than the untreated pulp. So the application enzyme treatment was beneficial when all parameters were considered.

CHAPTER 5

5. CONCLUSION

It can be concluded from the results achieved during this experiment that biotechnology can positively contribute to the pulp and paper industry. Applying the blend of enzymes from GM microbes (Xylanase in nature) on unbleached mixed hardwood pulp, revealed very interesting results. Different enzyme charges, different pH values showed the different impacts. The noticeable impacts, this enzyme induced are:

- Duration of refining can be minimized so the refining energy can be reduced to obtain same level drainability of pulp.
- The effects of refining can be enhanced when the washed pulp after pretreatment step was kept for some 10-12 hrs before refining.
- After enzyme treatment fibers were more swelled, hydrated and fibrillated to large extend.
- In getting the higher degree of drainability without enzyme, the fiber cutting and fines generation was a problem but by using enzyme refining intensity can be reduced to constrain the intense fiber cutting because the same degree of drainability can be obtained at lesser PFI revolutions on enzyme pre-treated pulp.
- The most interesting results were obtained at enzyme dose 150 IU/g of OD pulp. This charge presented the best compromise between paper properties development, fiber quality development, energy conservation and the treatment cost of enzyme. By using this dose the tear index, which reduces with fiber cutting, can be enhanced or maintained at the same value by using enzyme pre-treatment with compensable loss of tensile and burst indexes.

But treatment of unbleached mixed hardwood pulp with the xylanase from single source showed the comparative efficiency of blend of enzymes. It revealed that blend of enzymes is efficient in enhancing the effect of refining but the synergistic effect of enzymes in blend is not higher than the effect of xylanase from single source. Drainability of pulp is higher in case blend of enzymes but when strength properties were observed the enzyme from single source showed better results.

CHAPTER 6

6. REFERENCES

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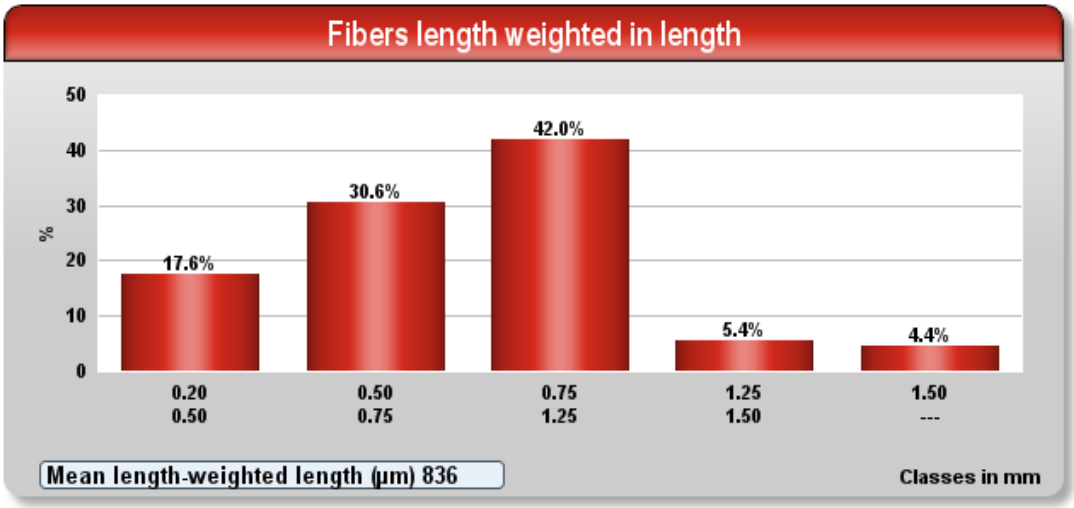
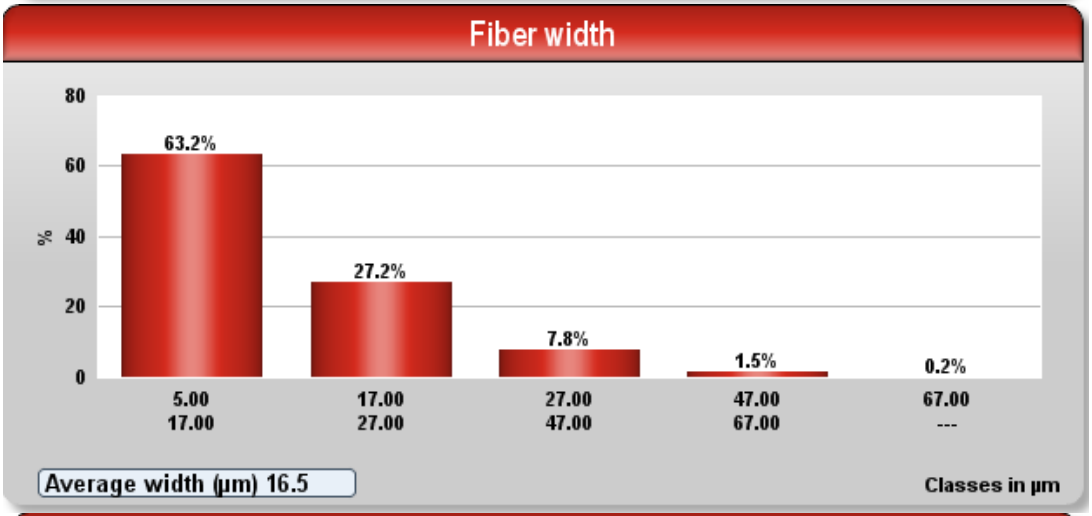
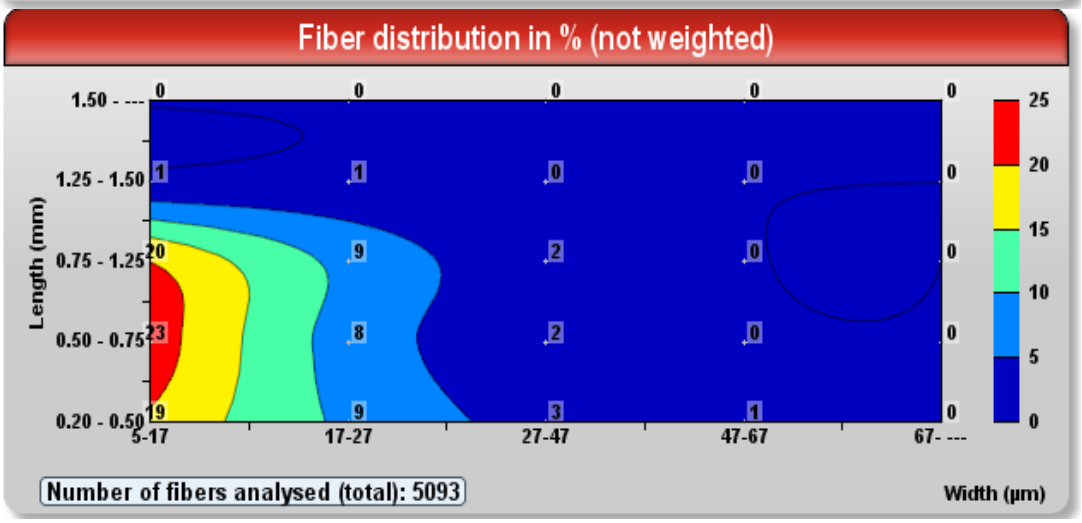
APPENDIX

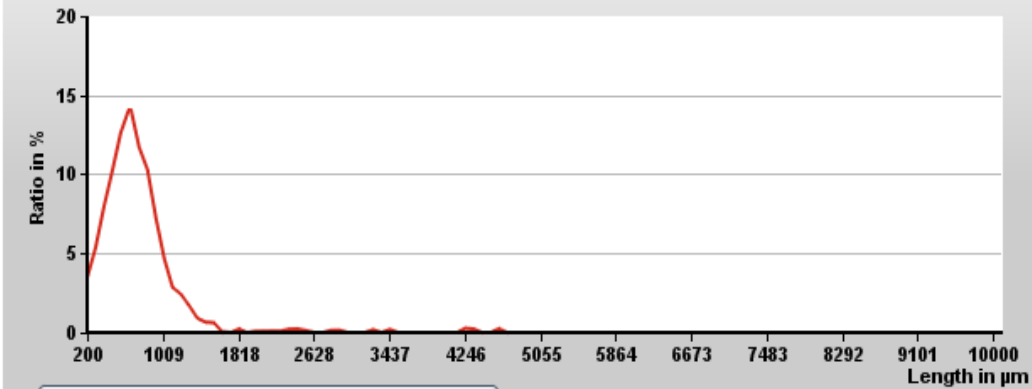
(1) Fiber analysis of mixed hardwood pulp without enzyme pre-treatment refined at 1300 PFI revolution without pH control





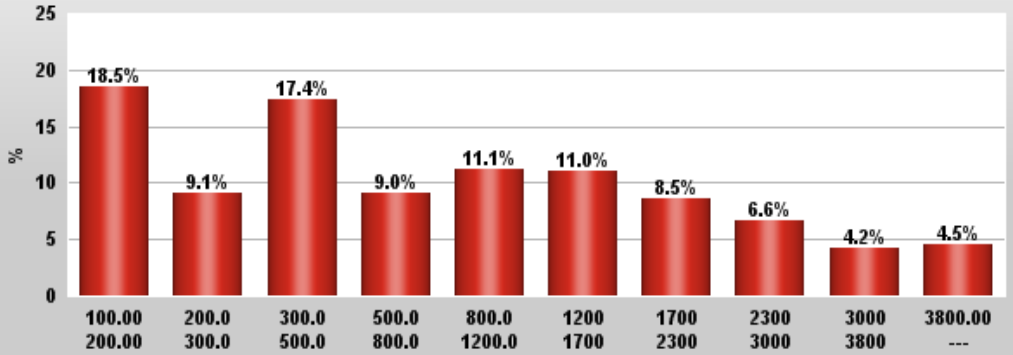


Fiber distribution



Mean length-weighted length (µm) 836

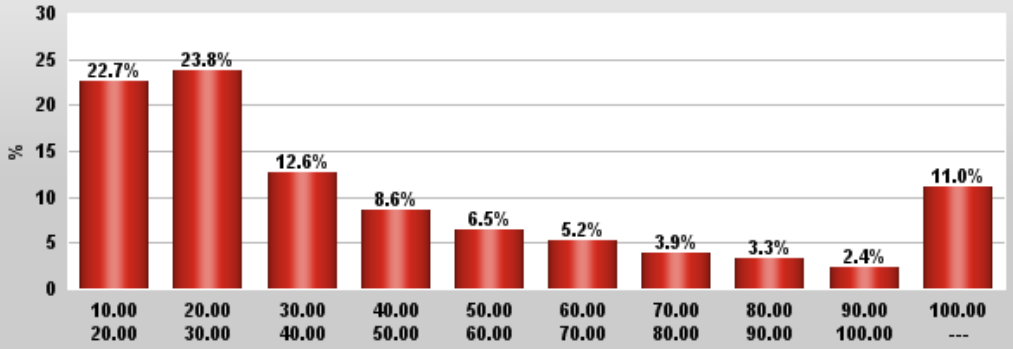
Fines area



Average area (µm²) 1165

Classes in µm²

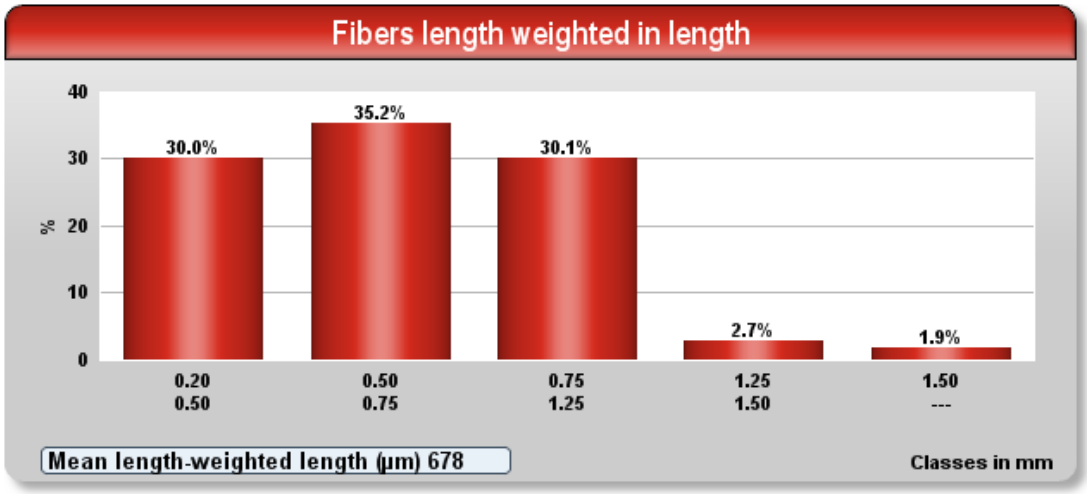
Fines length

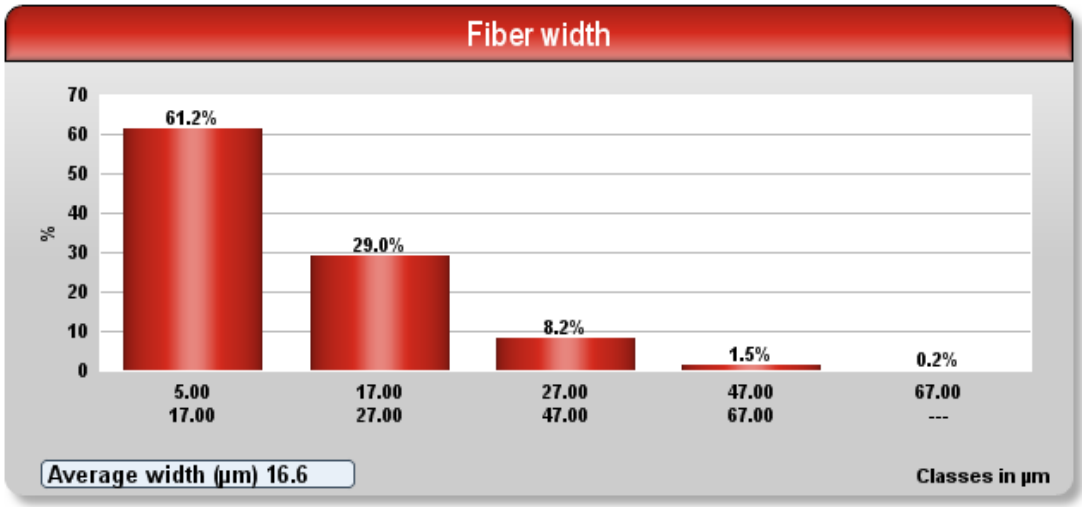


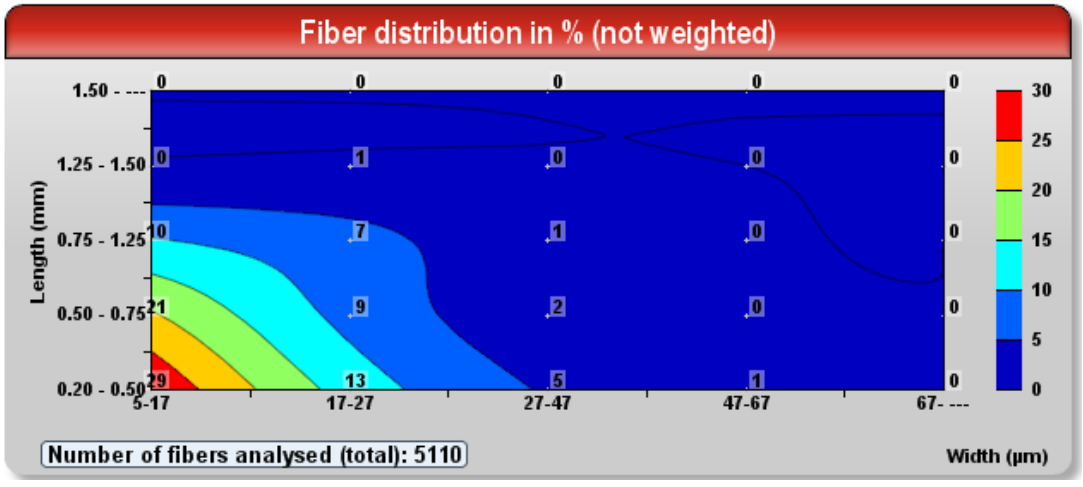
Average length (µm) 48

Classes in µm

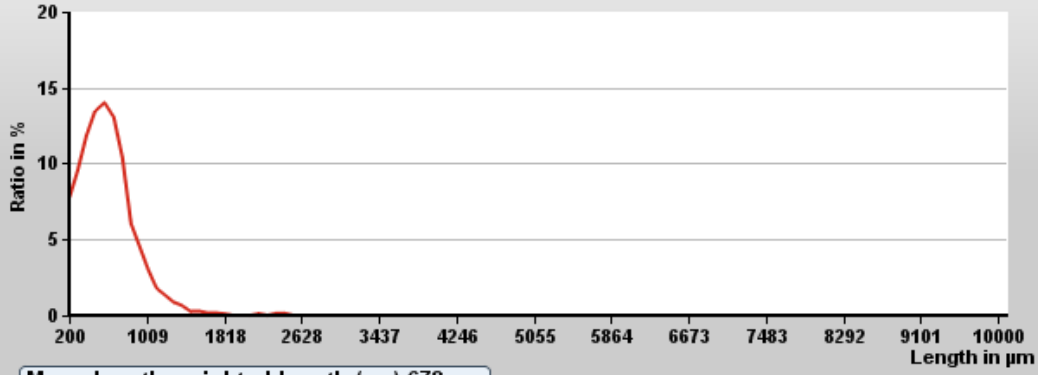
(2) Fiber analysis of Blend of enzyme pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 150 IU/g OD pulp, pH 5.5)





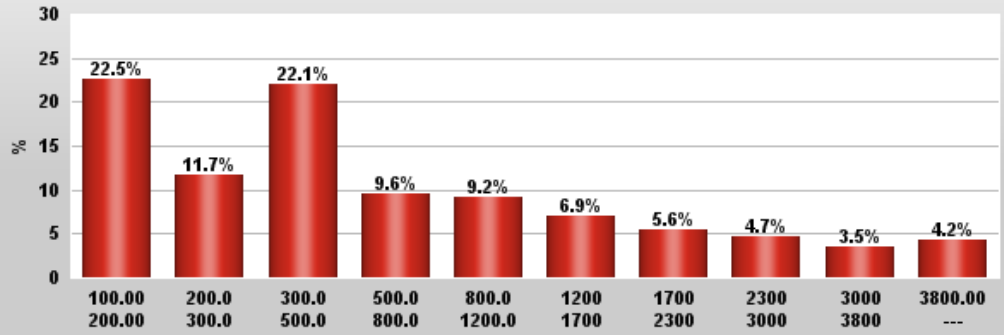


Fiber distribution



Mean length-weighted length (µm) 678

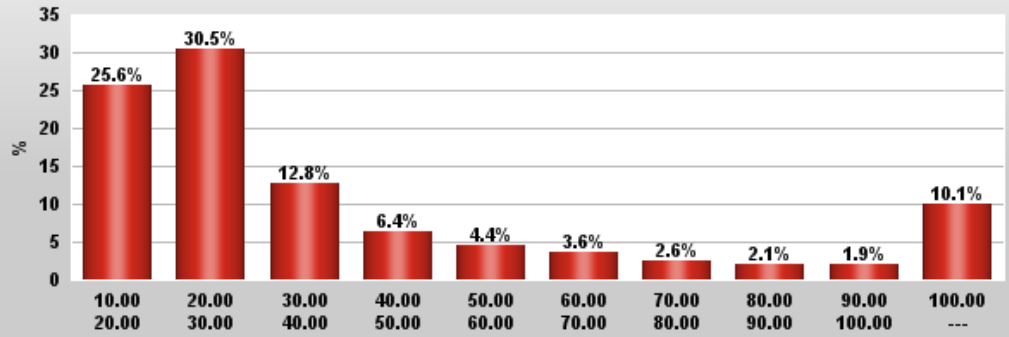
Fines area



Average area (µm²) 984

Classes in µm²

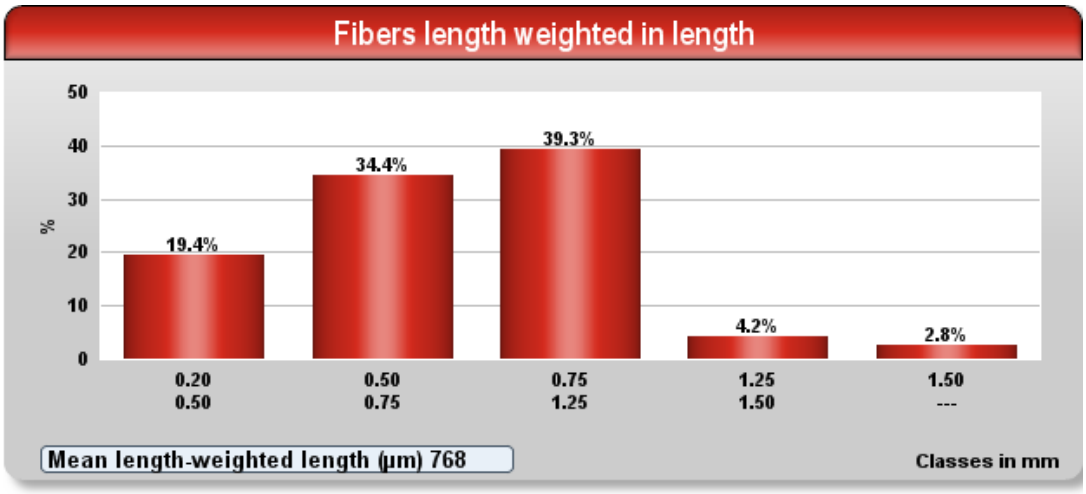
Fines length

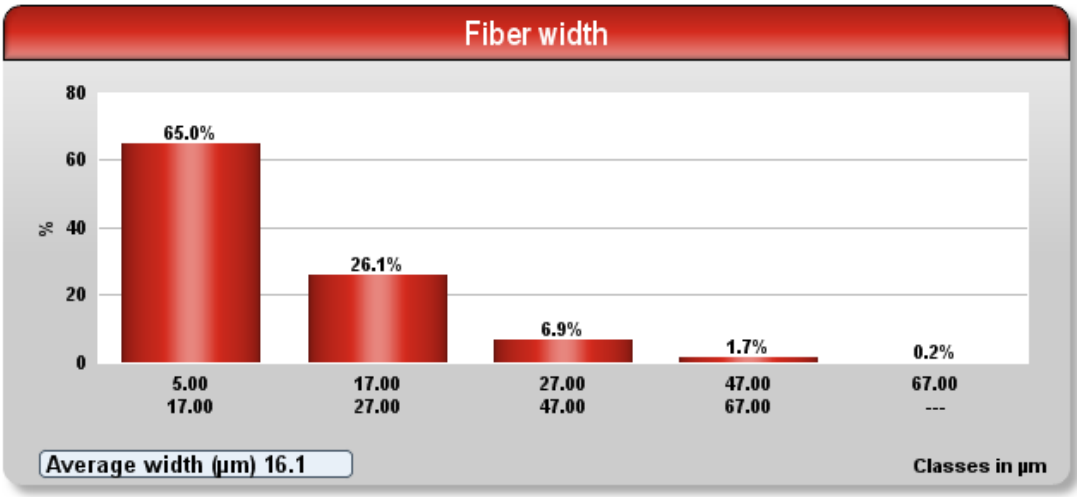


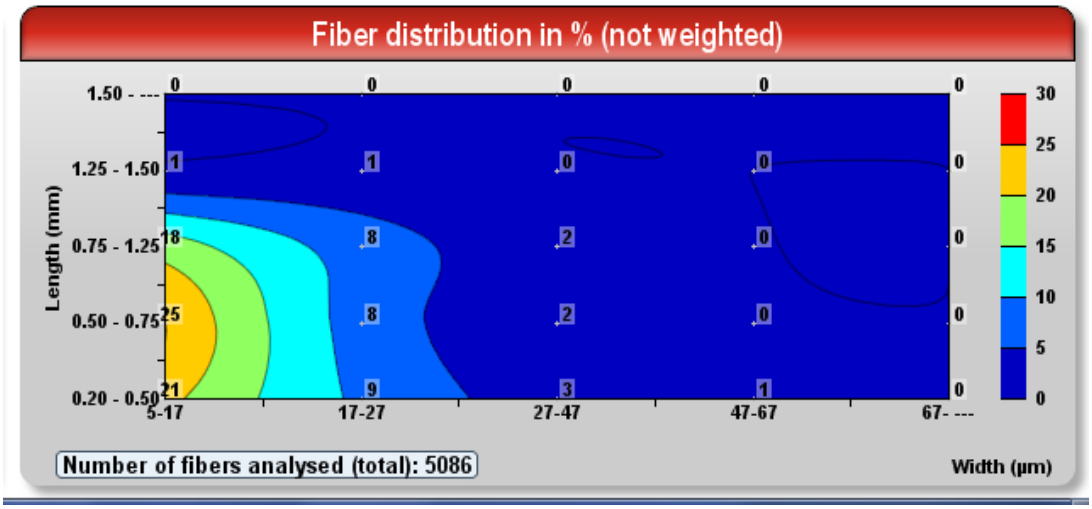
Average length (µm) 44

Classes in µm

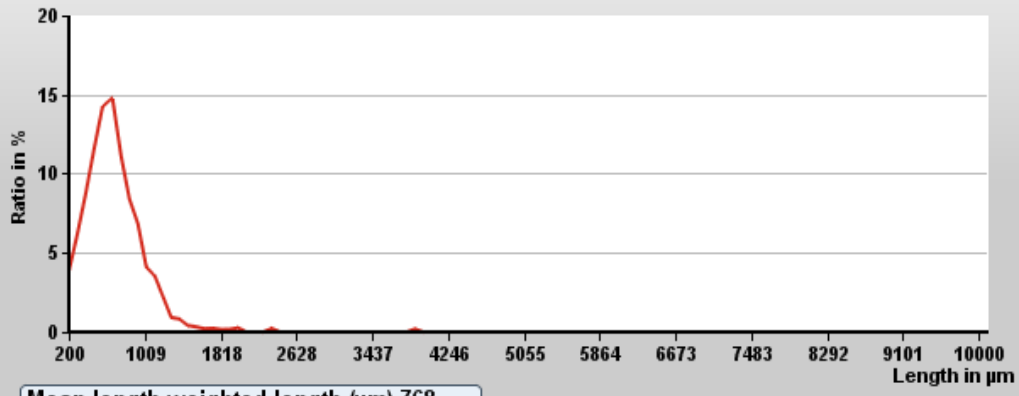
(3) Fiber analysis of Blend of enzymes pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 165 IU/g OD pulp, pH 5.5)





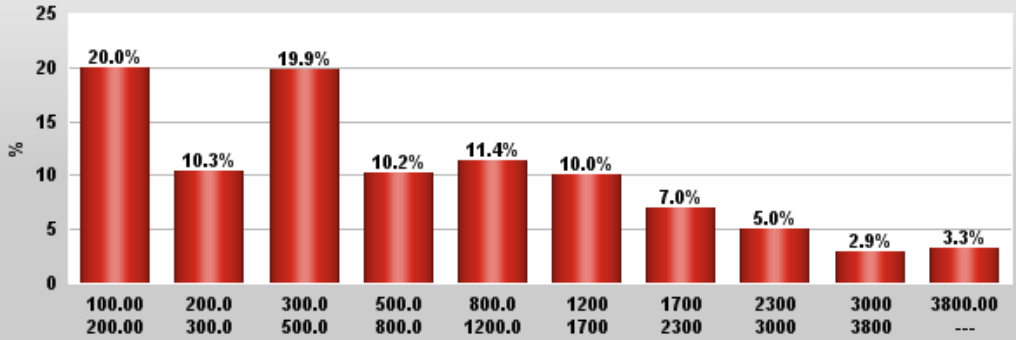


Fiber distribution



Mean length-weighted length (µm) 768

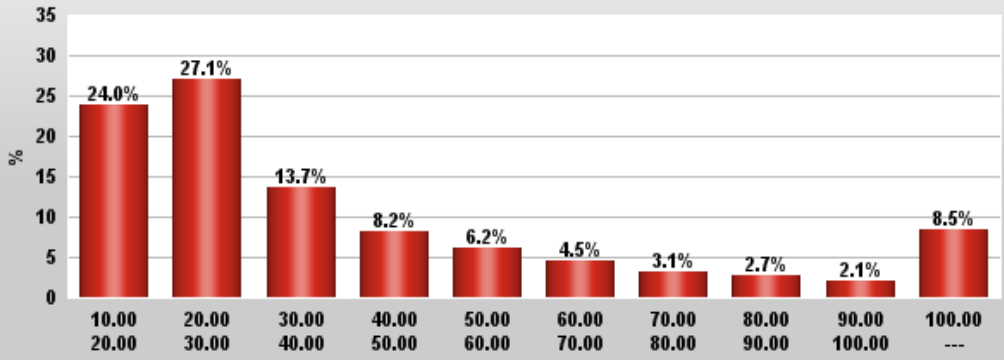
Fines area



Average area (µm²) 996

Classes in µm²

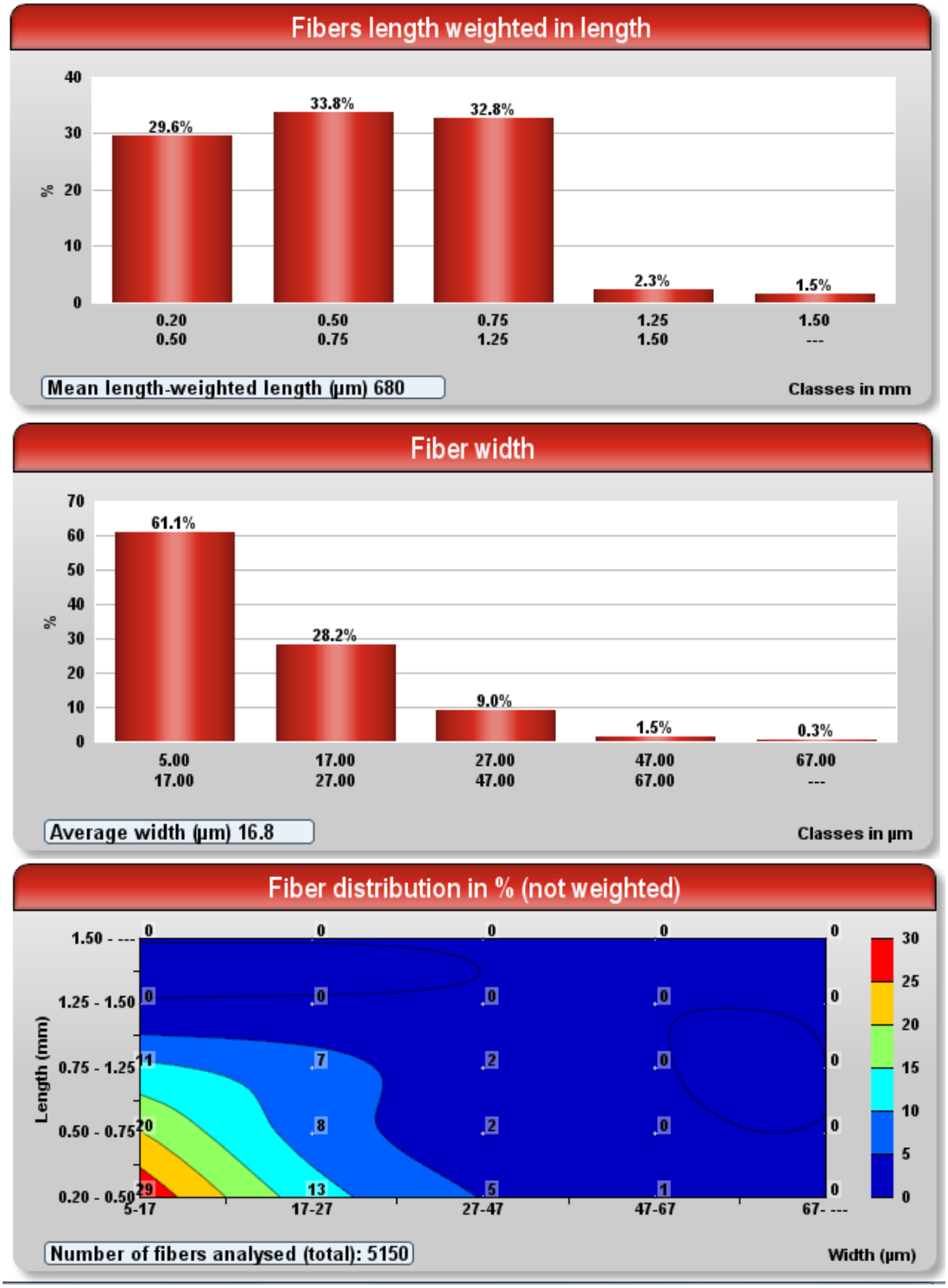
Fines length

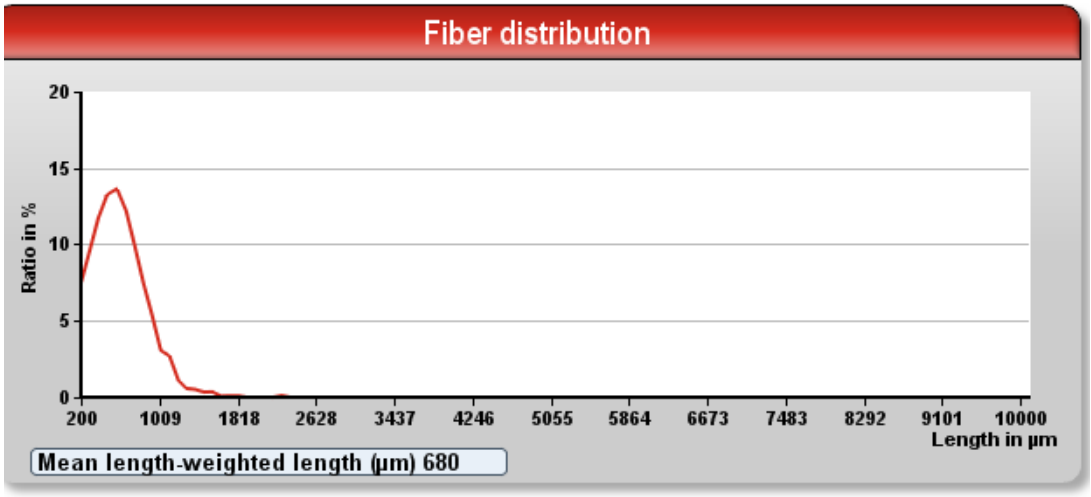


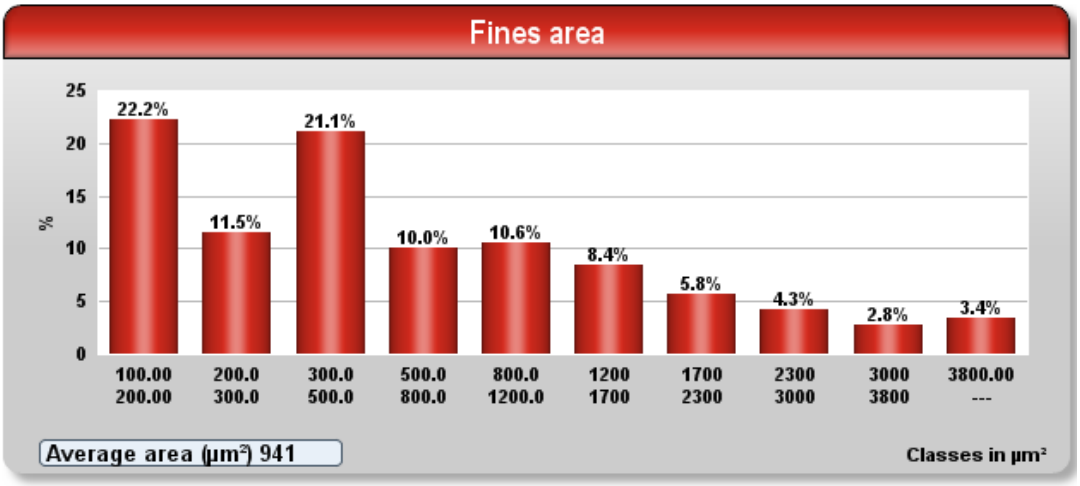
Average length (µm) 44

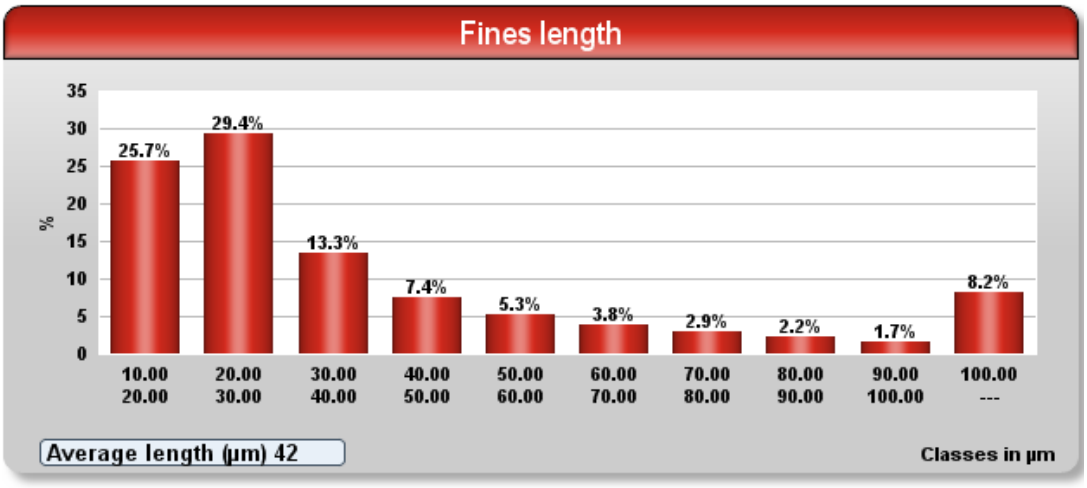
Classes in µm

(4) Fiber analysis of Blend of enzymes pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 180 IU/g OD pulp, pH 5.5)

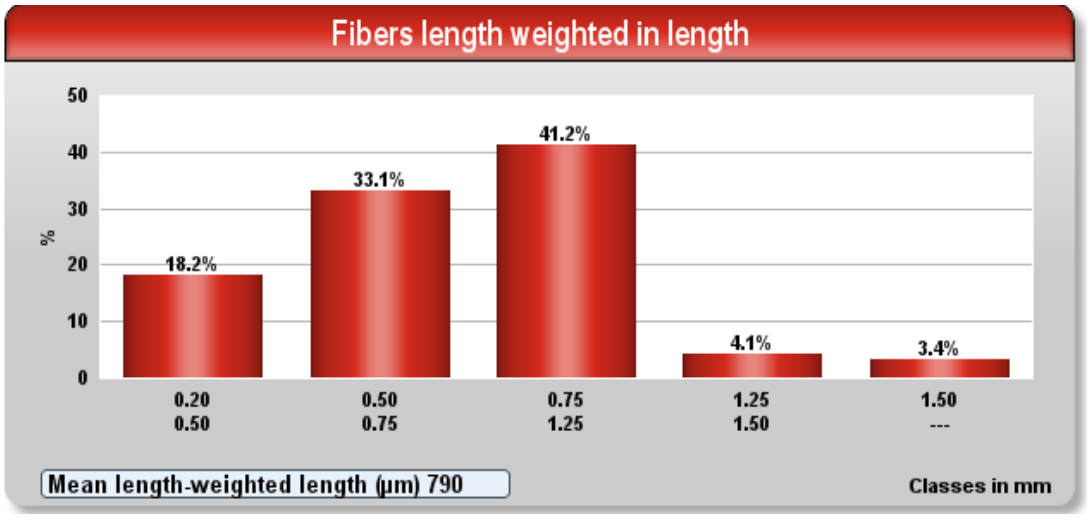


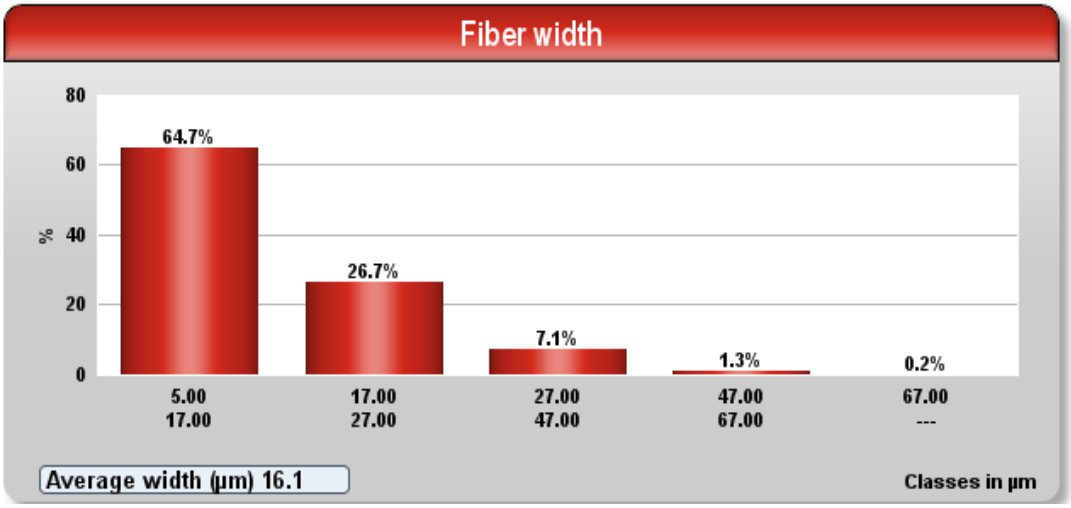

Fiber distribution
Ratio in %
20
15
10
5
0
200
1009
1818
2628
3437
4246
5055
5864
6673
7483
8292
9101
10000
Length in µm
Mean length-weighted length (µm) 680

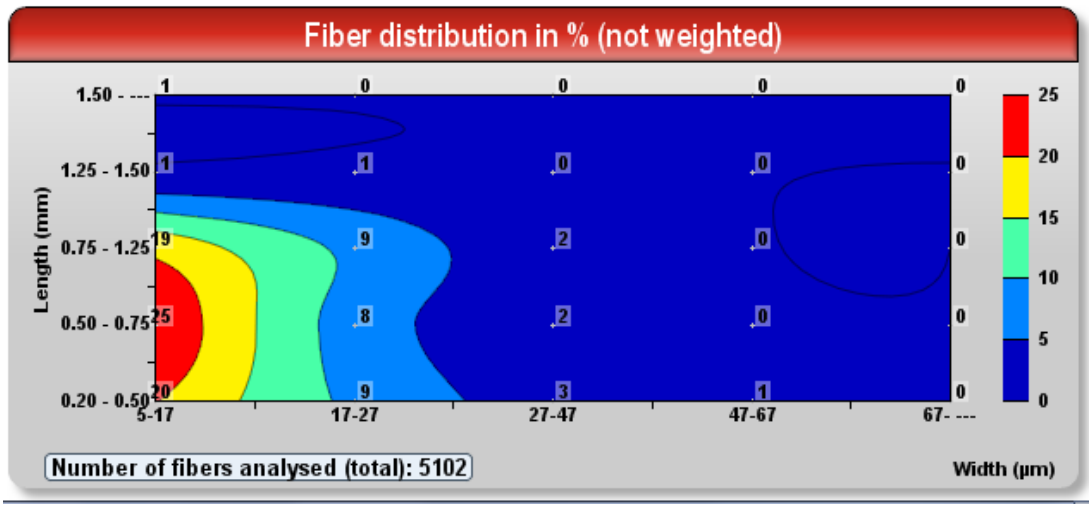

Fines area
%
25
20
15
10
5
0
22.2%
11.5%
21.1%
10.0%
10.6%
8.4%
5.8%
4.3%
2.8%
3.4%
100.00 200.00
200.0 300.0
300.0 500.0
500.0 800.0
800.0 1200.0
1200 1700
1700 2300
2300 3000
3000 3800
3800.00 ---
Average area (µm²) 941
Classes in µm²


Fines length
%
35
30
25
20
15
10
5
0
25.7%
29.4%
13.3%
7.4%
5.3%
3.8%
2.9%
2.2%
1.7%
8.2%
10.00 20.00
20.00 30.00
30.00 40.00
40.00 50.00
50.00 60.00
60.00 70.00
70.00 80.00
80.00 90.00
90.00 100.00
100.00 ---
Average length (µm) 42
Classes in µm

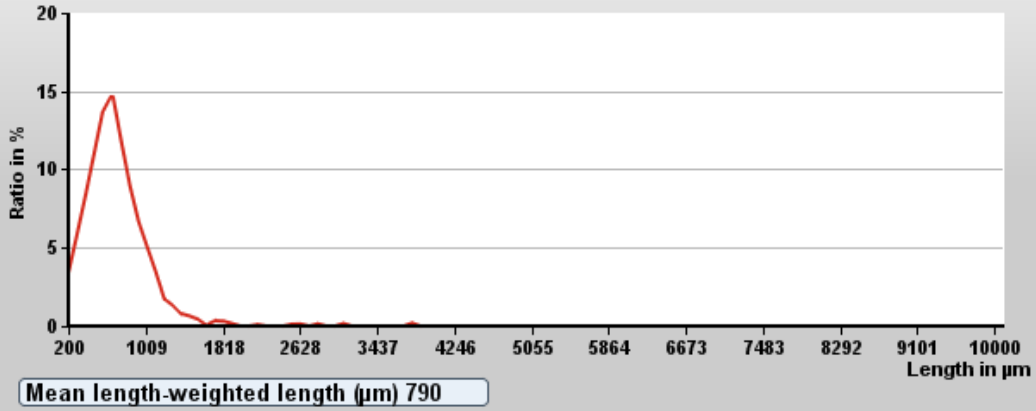
(5) Fiber analysis of Single sourced Xylanase pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 150 IU/g OD pulp, pH 5.5)





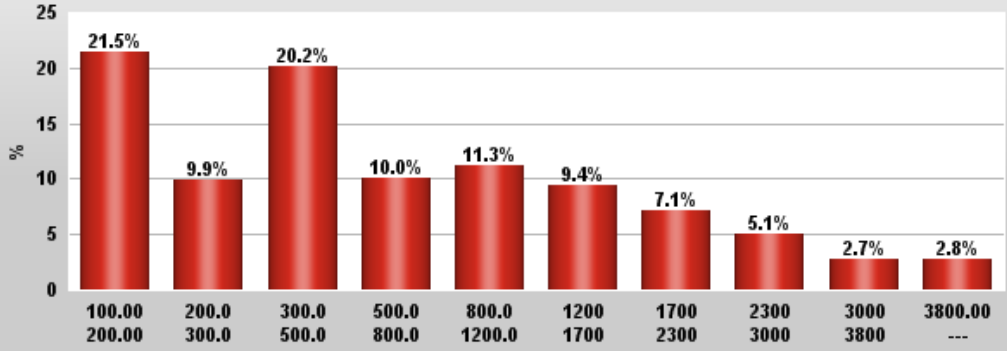


Fiber distribution



Mean length-weighted length (µm) 790

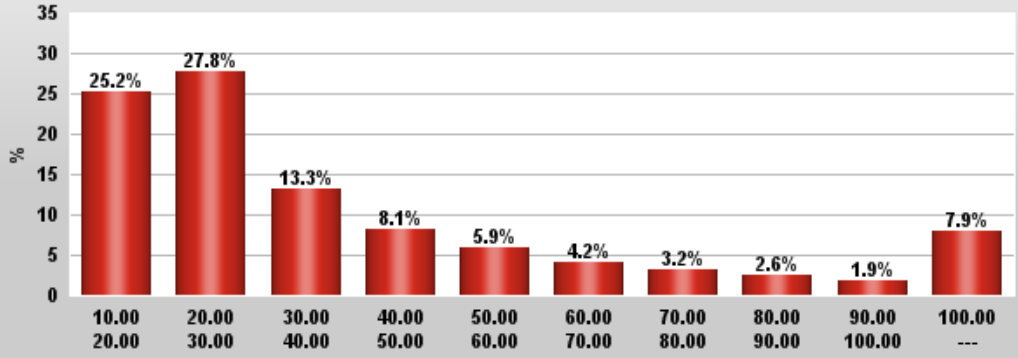
Fines area



Average area (µm²) 966

Classes in µm²

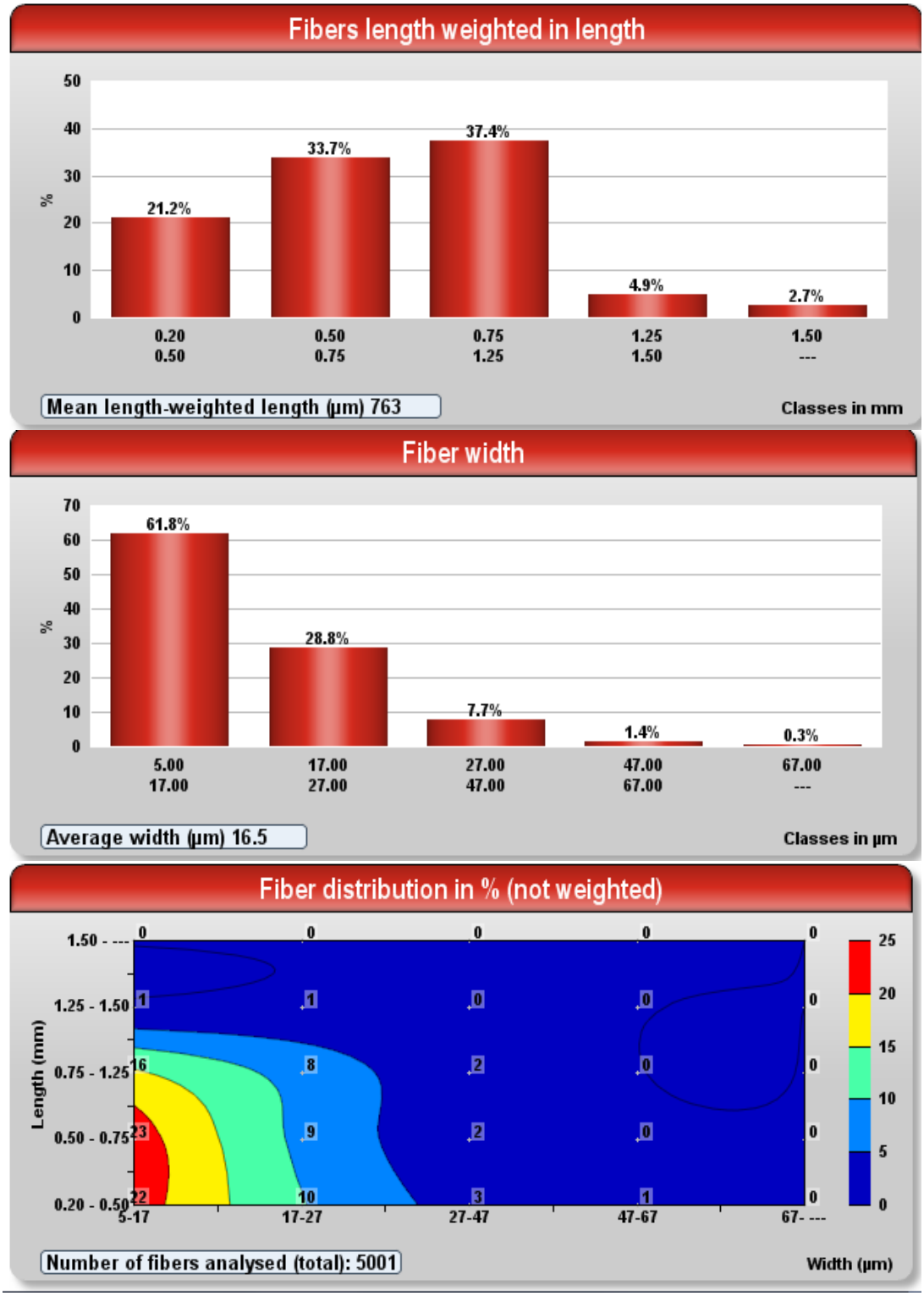
Fines length



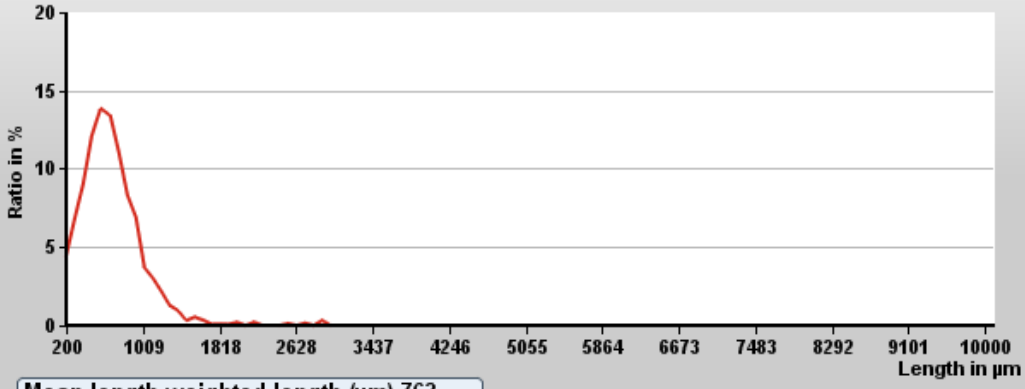
Average length (µm) 42

Classes in µm

(6) Fiber analysis of Single sourced Xylanase pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 165 IU/g OD pulp, pH 5.5)

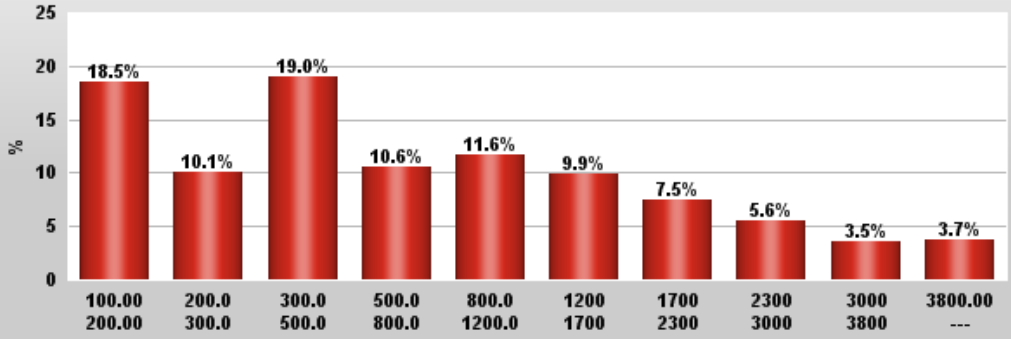


Fiber distribution



Mean length-weighted length (µm) 763

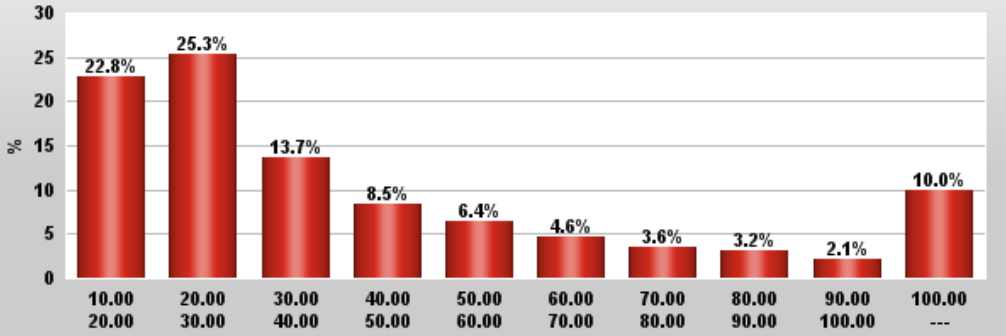
Fines area



Average area (µm²) 1058

Classes in µm²

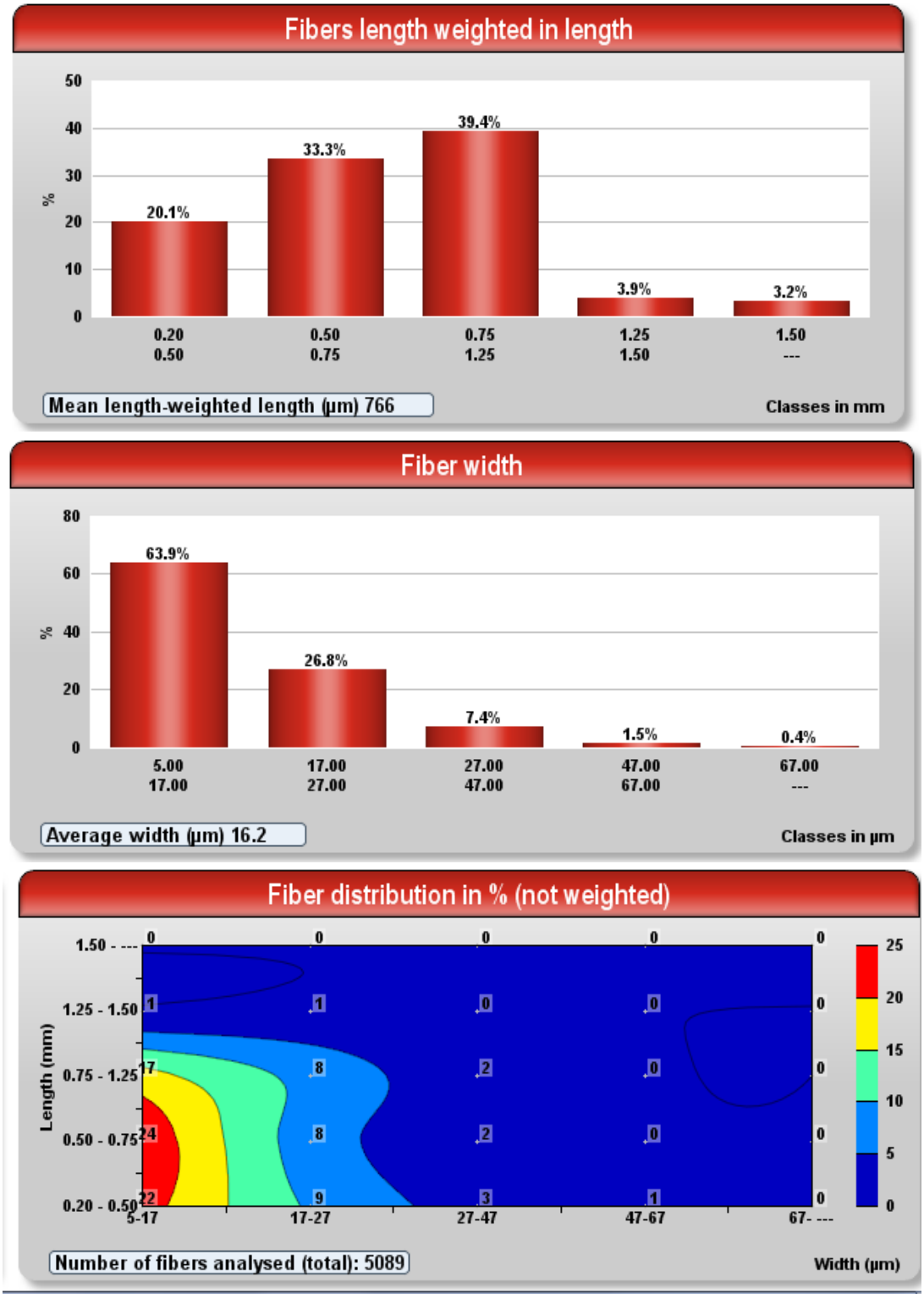
Fines length



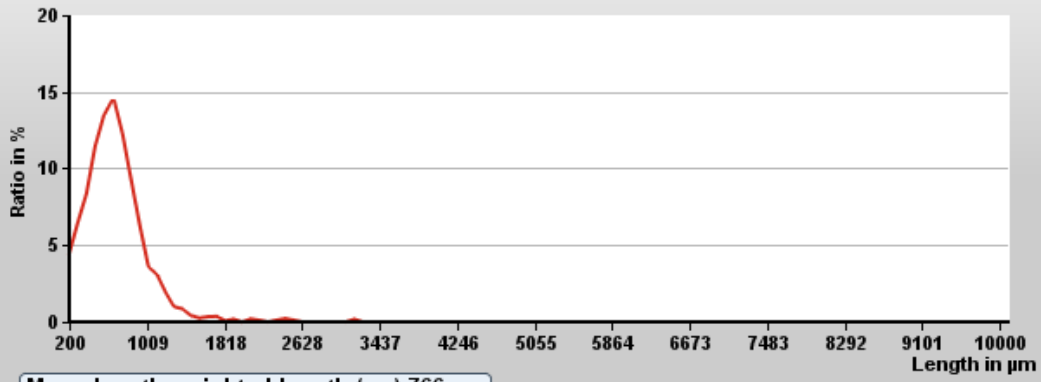
Average length (µm) 46

Classes in µm

(7) Fiber analysis of Single sourced Xylanase pre-treatment mixed hardwood pulp, refined at 1300 PFI revolution (Enzyme dose 180 IU/g OD pulp, pH 5.5)

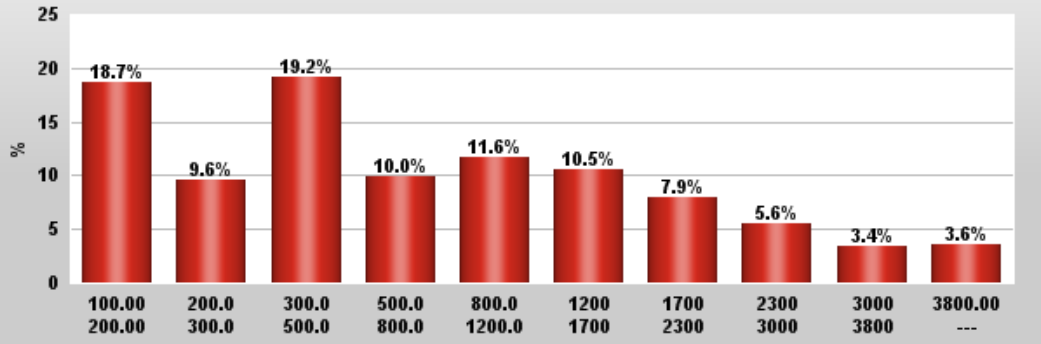


Fiber distribution



Mean length-weighted length (µm) 766

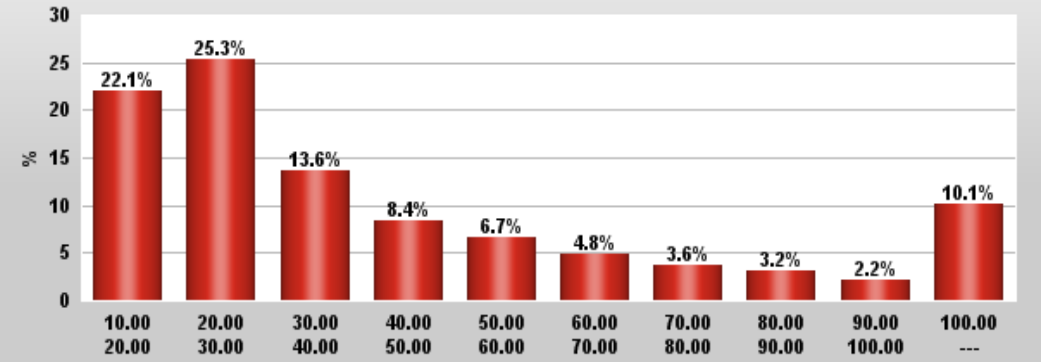
Fines area



Average area (µm²) 1074

Classes in µm²

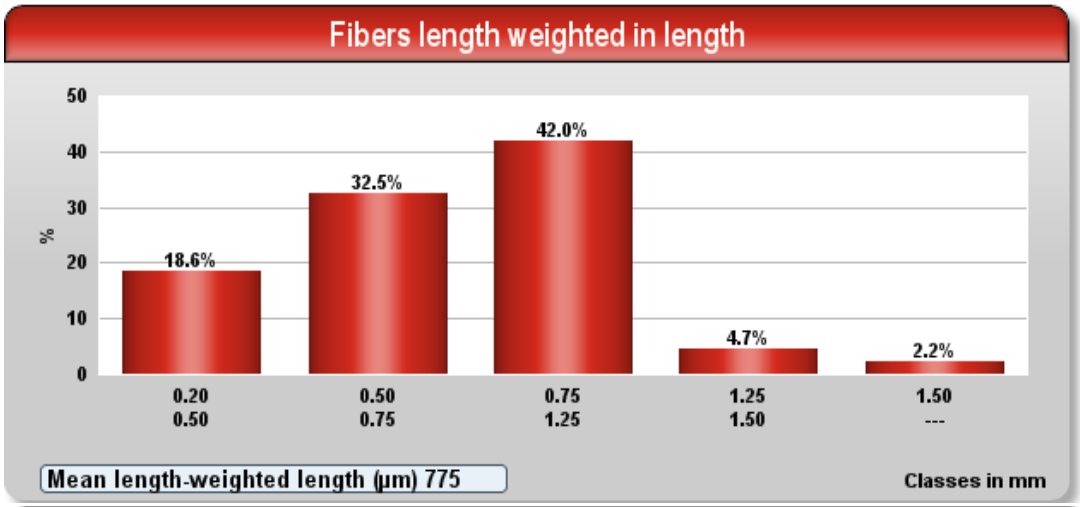
Fines length

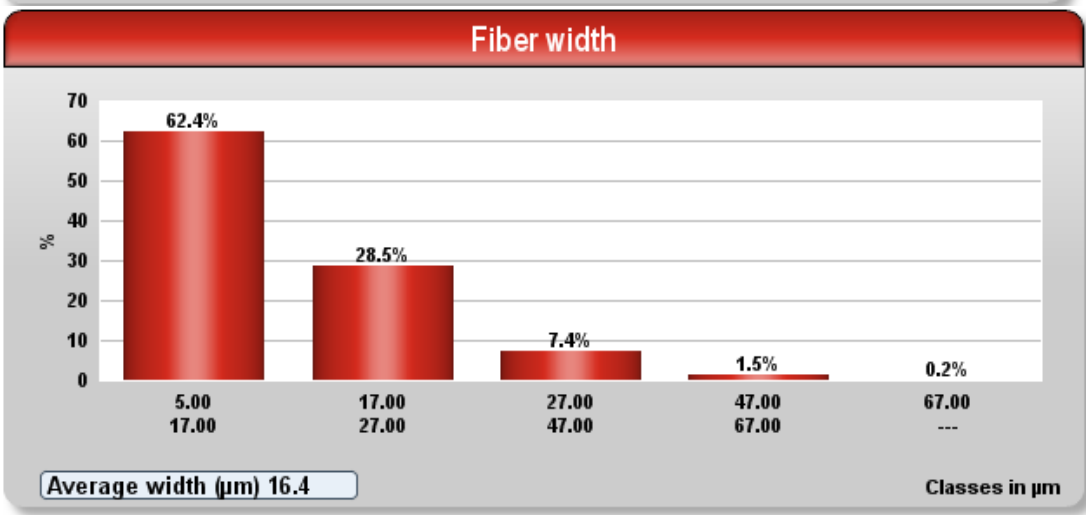


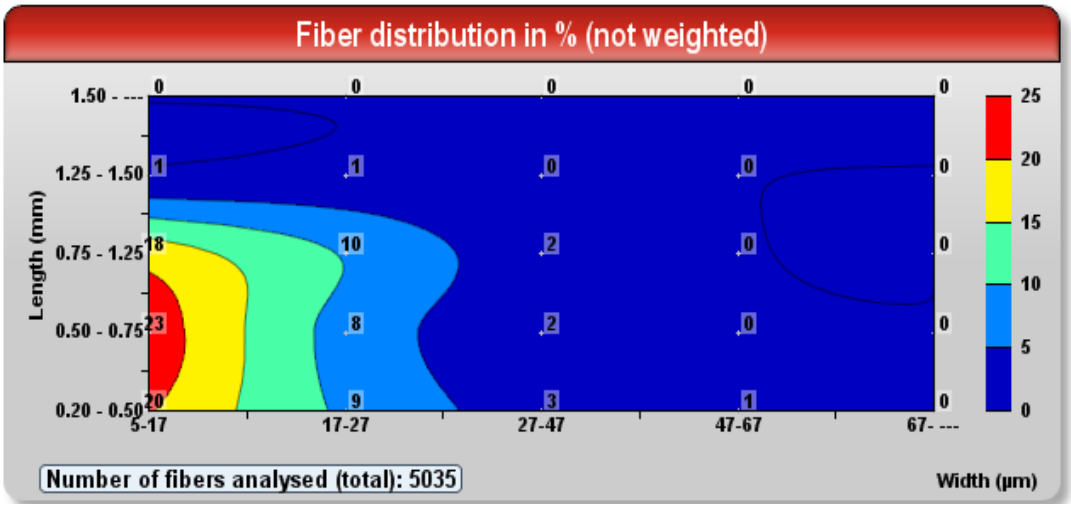
Average length (µm) 47

Classes in µm

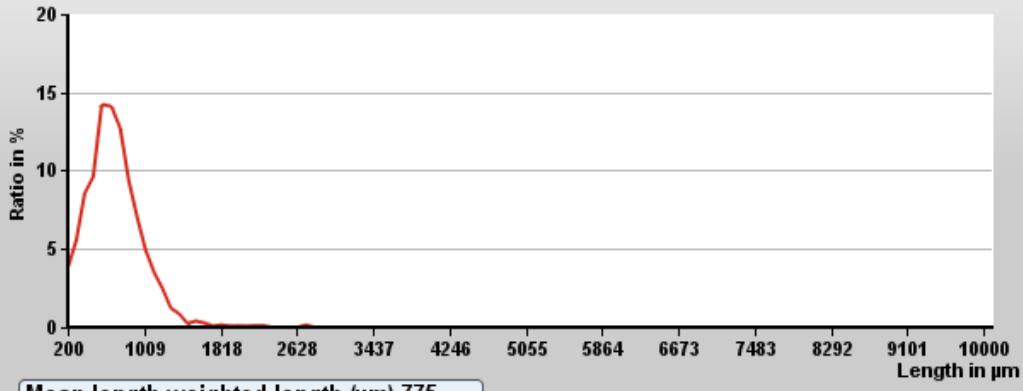
(A) Fiber analysis of without enzyme pre-treatment mixed hardwood pulp, refined at 1400 PFI revolution (for comparison with enzyme pre-treated pulp at same refining level)





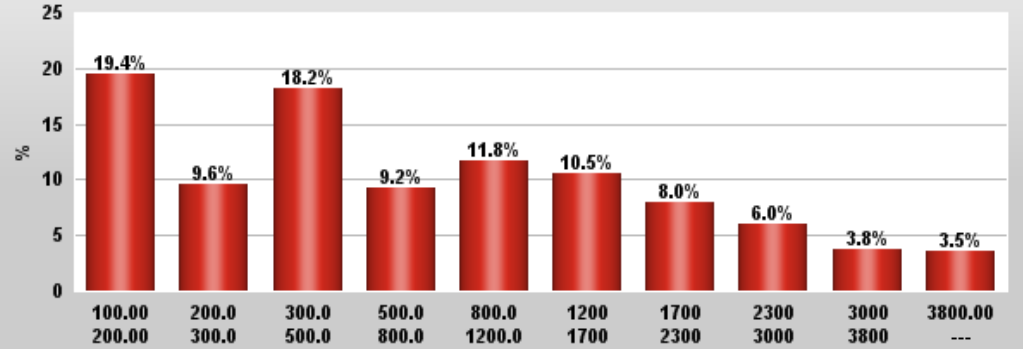


Fiber distribution



Mean length-weighted length (µm) 775

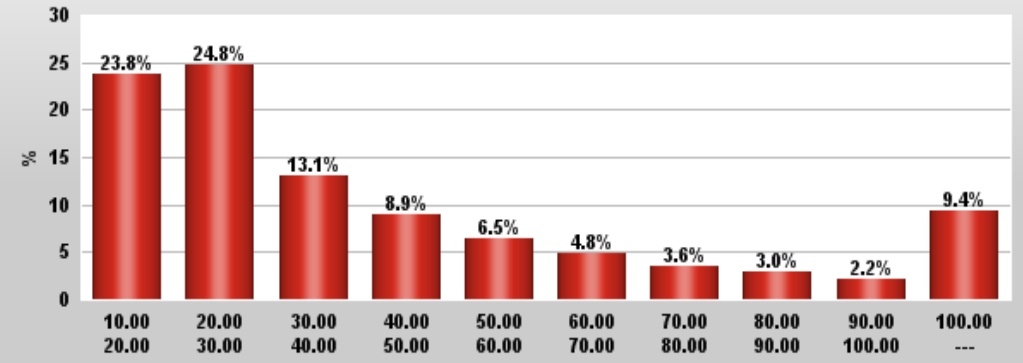
Fines area



Average area (µm²) 1083

Classes in µm²

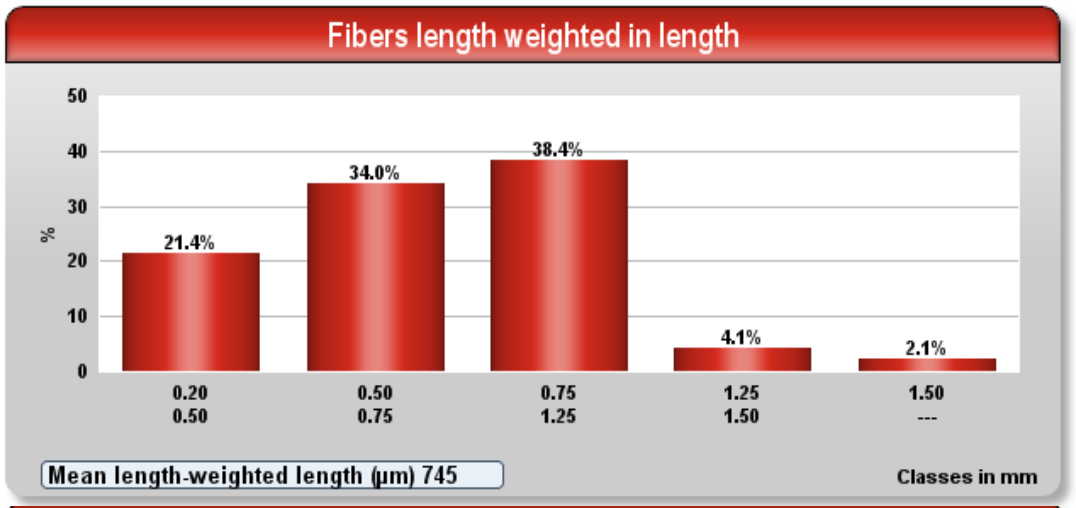
Fines length

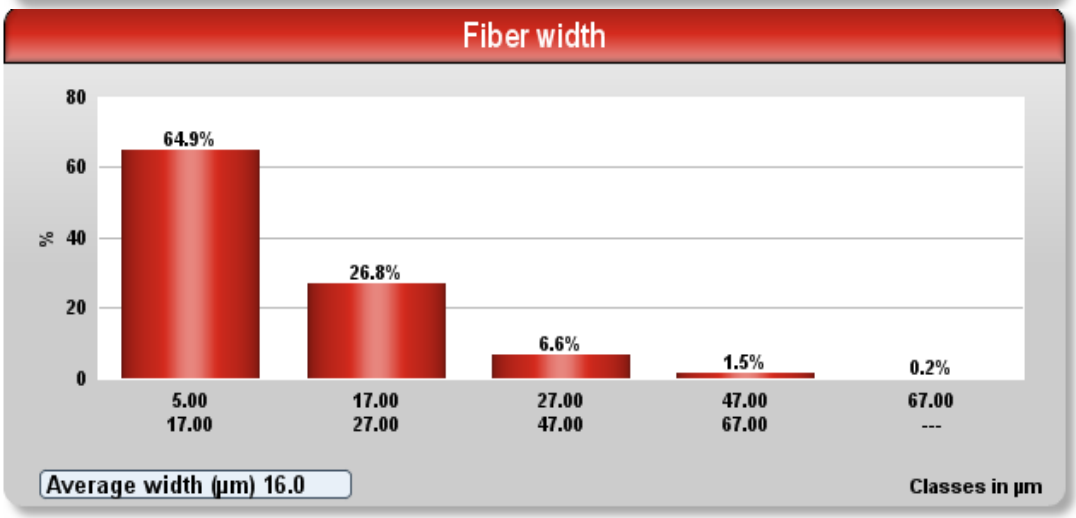


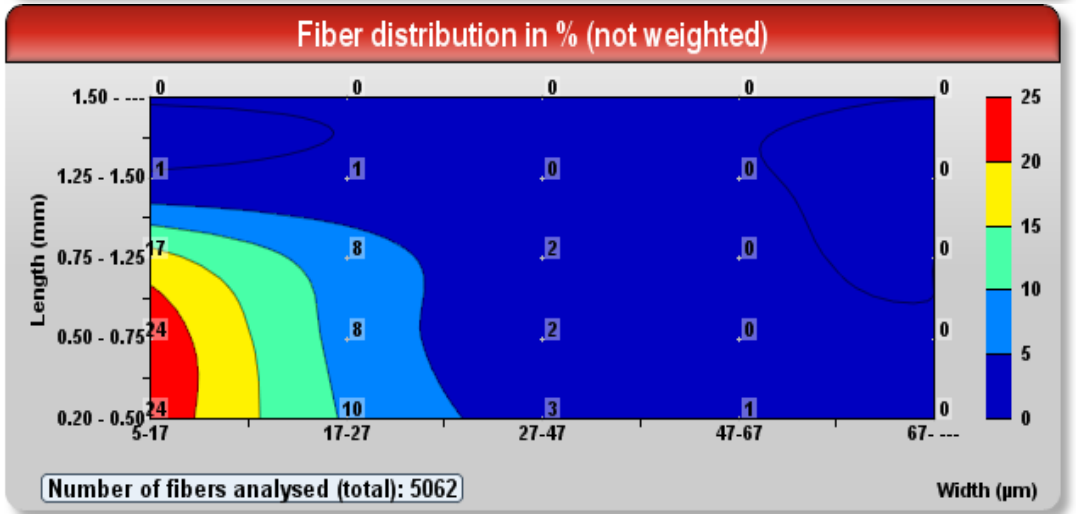
Average length (µm) 45

Classes in µm

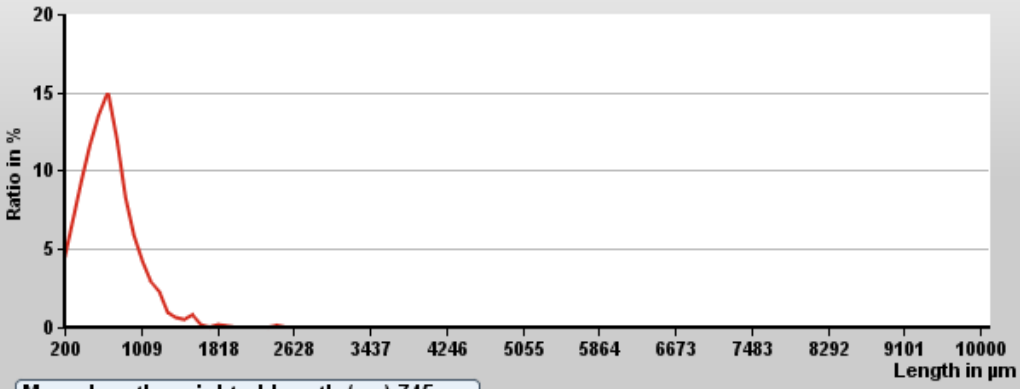
(B) Fiber analysis of Enzymes blend pre-treatment mixed hardwood pulp, refined at 1400 PFI revolution (Enzyme dose 150 IU/g OD pulp, pH 5.5)





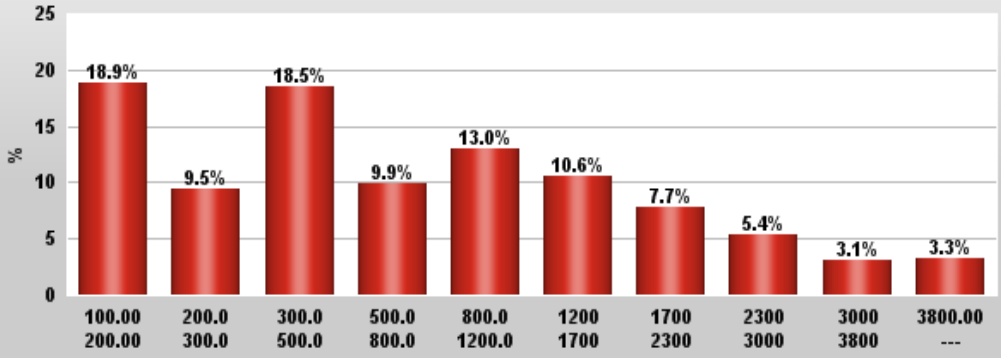


Fiber distribution



Mean length-weighted length (µm) 745

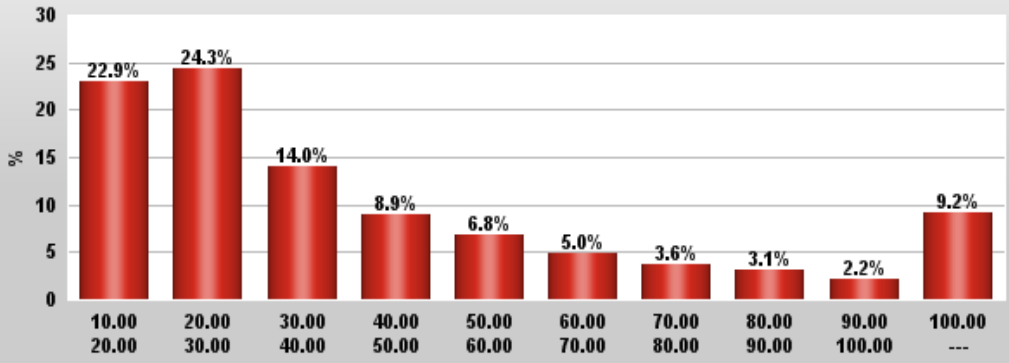
Fines area



Average area (µm²) 1043

Classes in µm²

Fines length



Average length (µm) 46

Classes in µm