REPORT

ON

PHASE-I ACTIVITIES

OF

THE PROJECT

USE OF XYLANASE AND ANCILLARY ENZYMES FOR PREBLEACHING OF KRAFT PULP

(IIT DELHI/CPPRI/TIFAC/IAPMA)

Sponsored by

Department of Science and Technology (TIFAC Division)

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PROJECT PROFILE

Title:	Use of xylanase and ancilliary enzymes for pre-Bleaching of Kraft pulp.
Key Objectives:	To evaluate the effectivity of xylanase enzymes produced from the fungus *Melancarpus albomyces* as pre-bleaching agent on Kraft pulp.
Duration:	May 99- October 2000
Amount Sanctioned: (initially)	Rs.3,50,000/- IIT-Delhi Rs. 1,50,000/- CPPRI Rs. 5,00,000/- Total
Additional amount released:	Rs. 44,400/- IIT-Delhi Rs. 44,400/- CPPRI Rs. 88,800/- Total
Investigators: (From IIT-Delhi)	Prof. S. Mishra, Principal Investigator and Project In-charge Prof. V.S. Bisaria, Co –PI Dr. T.R. Srikrishnan, Co-PI
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Associated Research Scholar	Ms. R. Chakrabarti (for Liquid-submerged fermentations)

1. INTRODUCTION

Changes in public policies, customer preference of environmentally benign products & new market demand have increased the interest in developing bleaching technologies with low AOX levels in the recipient. In response to these demands, various technologies are currently being studied and adopted to reduce or eliminate the use of chlorine and chlorine based chemicals during bleaching. However, the application of enzymatic pre-bleaching has proved to be an up-coming option before the paper industry which has received considerable attention during the recent years.

In the last few years there has been growing pressure for change in the way that chemical pulps are bleached. The traditional & effective approach of using chlorine as a bleaching agent is in less use for environmental concern since the process produces dioxin and other chlorinated compounds which contribute to the discharge of AOX (adsorbable organic halides) in the recipient streams. Gaseous chlorine (Cl_2) in particular, but also other chlorine containing bleaching chemicals such as chlorine dioxide (ClO_2) and hypo chlorites are blamed for the formation of chlorinated compounds.

Conventional bleaching of Kraft pulp has evolved over the years but there has been particular pressure to change to bleaching sequence like ECF & TCF in Europe & Scandinavian countries. A similar situation also exists in Asian countries particularly in India wherein the Pulp & Paper mills started switching over to partial ECF bleaching. Regardless of the pace of change, a market has been created for ECF and TCF pulps and alternatives have to be found to chlorine containing bleaching chemicals or new technologies have to be considered.

The options open to pulp mills considering a change to chlorine free bleaching are substitution of chlorine dioxide, oxygen delignification and extended delignification to reduce Kappa No. before bleaching, substitution of hydrogen peroxide and ozone to replace chlorine based chemicals. However, all the processes suffer from disadvantages like:

- High capital cost
- Risk of loss in pulp viscosity and strength
- High cost of bleaching chemicals.

This climate of change has provided an opportunity for enzymes as pre-bleaching agents with the basic aim of:

— Reduction in use of chlorine and chlorinated bleach chemicals
— Reduction in discharge of AOX in bleach effluents
— Gain in final brightness and improved pulp properties.

The enzymes used commercially in pulp bleaching are hemicellulases which selectively affect the accessible hemicellulose fraction of the pulps. Among various available hemicellulases, xylanases have been found to be more effective as pre-bleaching agents.

The use of hemicellulolytic enzymes particularly the xylan attacking enzymes-xylanases is widely reported in use in commercial bleaching sequence for production of bleached pulp from soft wood and certain species of hardwoods in the developed countries. In developed countries it has been possible to reduce chlorine demand to a level of 15-20% during bleaching with corresponding reduction in AOX level (20%) and improved pulp brightness to a level of 2-3% ISO. Since the effectiveness of a particular xylanase enzyme may vary in respect of this activity, purity (particularly in terms of cellulase freeness), enzyme pretreatment conditions and the type of pulps, evaluation studies for each type of enzyme is required to be carried out separately. This should be done to assess the response of the pulps being produced in paper industry and to develop tailor made enzymes for enzymatic pre-bleaching of pulp.

2. PRODUCTION OF XYLANASES AS PRE-BLEACHING AGENTS

The two main enzymes which depolymerise the hemicellulose backbone are endo-1,4-β-D-xylanase and endo-1,4-β-D-mannanase, referred to generally as xylanases and mannanases, respectively. Xylanases and mannases are produced by many species of bacteria and fungi. Xylanases are the enzymes applied in commercial bleaching and are available from several different sources.

Xylanase producers are found both among bacteria and fungi. Several criteria are essential for choosing micro-organisms to produce xylanases. In addition to giving the desired biobleaching effect, the enzyme must be produced in sufficiently huge quantity

and should be completely free of cellulase activity. Any cellulase activity will have serious economic implication in terms of cellulose loss, degraded pulp quality & increased effluent treatment cost. Now, xylanolytic preparations could be produced by recombinant DNA technology, selective inactivation or bulk scale precipitation. High productivity could be achieved by exhaustive screening, genetic engineering and growth optimisation programs.

3. STRUCTURE OF HEMICELLULOSES IN KRAFT PULPS

The native hemicellulose structure is heavily modified during pulping process. In the beginning of conventional sulphate i.e. Kraft cooking, xylan in wood is partly solubilized in the alkaline cooking liquid and many of the side groups and acetic acid residues are cleaved off. It has recently been observed that the majority of the 4-O-methylglucuronic acid side groups in xylan are converted to hexanuronic acid already in the early phases of the Kraft cook.

As the alkali concentration decreases towards the end of the Kraft cook, dissolved xylan tends to reabsorb on the surface of cellulose microfibrils. It has recently been suggested that pine Kraft xylan reprecipitates evenly over the accessible surface to the fibre wall. In addition to xylan chains, dissolved lignin and covalently bound lignin and xylan have been suggested to reprecipitate on fibre surfaces during cooking, resulting in relatively high amounts of lignin deposits on the fibre surfaces. The amount of xylan readsorbed during cooking depends on the wood species used in pulping. High amounts of xylan have been found to be present on the surface of birch Kraft fibres, probably partly due to readsorption, whereas in pine Kraft fibres the concentration of xylan on the fibre surfaces has not been observed to be higher than in the whole fibres. A large part of wood glucomannan is also dissolved in the beginning of the Kraft cook, but due to their instability in alkali, the solubilized polymers are completely degraded in the pulping liquor.

As a result of solubilization of hemicelluloses during cooking, the distribution and content of xylan and glucomannan in Kraft pulp fibres differs from that in the native wood fibres. In softwood Kraft fibres, the xylan concentration is generally higher in outer layers, and glucomannan is more concentrated in the middle layers of the fibre. However, due to different methods of analysis, variations in the distribution of polysaccharides in

softwood Kraft fibres have been reported, although there is general agreement that the outer surface layer of hardwood Kraft fibres is rich in xylan.

Recently, several modified Kraft pulping methods as well as totally new sulphate pulping methods have been introduced. In the pulps produced by these methods no or little reprecipitation of xylan and lignin is expected to occur due to the relatively constant alkali concentration throughout the cooking process. Consequently, the composition of outer surfaces of pulp fibres is probably different from that of the conventional Kraft pulp fibres. In sulphite cooking, hemicellulose is extensively solubilized to mono and oligomeric compounds and no reprecipitation occurs. Thus, the distribution of hemicellulose is relatively constant across the pulp fibres.

4. PROPOSED MECHANISM OF ENZYME ACTION

Hemicelluloses are polysaccharides associated with cellulose and lignin in plants. The two most common hemicelluloses are xylans and mannans. Not only do the relative amounts of these two polysaccharides vary in hardwoods and softwoods, but their chemical composition varies as well.

Extensive modification of hemicelluloses takes place during pulping processes. During the initial heating period in Kraft pulping, when the alkali concentration is comparatively high, the xylan is partially depolymerized and stripped of substituents such as acetyl-and arabinosyl-groups. As pulping proceeds, the alkali concentration decreases and degraded, short-chain xylans precipitate in a more or less crystalline form on the surface of cellulose microfibrils. Overall, xylan constitutes over 90% of the hemicelluloses in hardwood Kraft pulp and 50% in softwood pulp. Because of the reprecipitation, hemicelluloses are concentrated on the fiber surfaces of microfibrils although a part remains at its original location in the fibers.

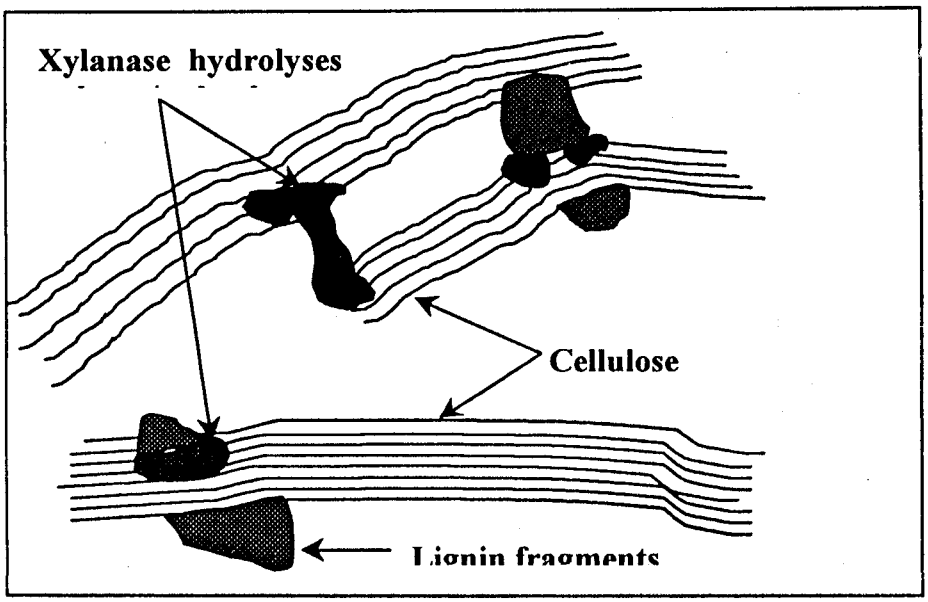


Fig. 1: Proposed mechanism of action of xylanases

One of the predominating hypotheses for the mechanism of xylanase activity in bleaching is that these enzymes catalyze the hydrolysis of reprecipitated xylan on the surface of the pulp fibers (Fig. 1) making the lignin fragments in and on the fiber easier to remove in the following bleaching and alkaline extraction stages. This hypothesis is not supported by the results of experiments in which pulp was delignified under high alkalinity conditions which precluded xylan deposition. In that situation, xylanase treatment lead to a reduction in the amount of chemical required to reach a final brightness equivalent to that obtained for a conventional pulp in which the xylan was reprecipitated on the pulp surface. Another hypothesis is that xylanases, by catalyzing the depolymerization of xylan in the cell walls allow entrapped lignin to diffuse more easily out of the fiber.

5. FACTORS AFFECTING THE PERFORMANCE OF ENZYMES

The key factors affecting the performance of enzyme are pH, temperature & adequate mixing besides the doses of the enzyme which will be effected by the residence time during enzyme treatment before the chlorination stages. During the course of studies, it has been observed that pretreatment of pulp with higher doses of xylanase could result in loss of viscosity of pulp from 540 cm^3/g to 390 cm^3/g & accordingly strength properties were also reduced drastically. Therefore, it was of utmost importance to optimize the doses of enzyme under normal residence time being practiced in the mills in high density storage tank before chlorination stages.

In order to assess the suitability of xylanases produced by the fungus *Melanocarpus albomyces*, (enzyme production work carried out at IIT-Delhi) studies were carried out at CPPRI to evaluate the response of xylanase enzyme as prebleaching agents towards pulps procured from the Indian paper mills using various fibrous raw materials. Under the project the scope of studies covers the evaluation of xylanase enzymes for treatmet of Kraft pulp from wood & non wood based mills. Initially the studies were carried out on wood Kraft pulp procured from integrated paper mill employing eucalyptus & bamboo as raw material so that the data generated could be compared with studies carried out in developed countries with compatible woody raw materials. Most of the work on enzymatic treatment of pulp carried out in the developed countries is on wood Kraft pulp. Studies were also carried out on assessment of the xylanase enzyme produced from *M. albomyces* using wood Kraft pulp & its comparison with commercially available xylanases having similar pH optima. This was expected to give an indication about the suitability of the enzyme produced from this particular strain of *M. albomyces*. Having evaluated the xylanase enzyme response on wood Kraft pulp, the response of non wood based pulp was also investigated. The details of the pulps procured from mills using various fibrous raw materials and its characterization with respect to Kappa no. is detailed in Table 1.

6. EXPERIMENTAL:

6.1 Organism:

Melanocarpus albomyces was used as the producer of xylanase enzymes. The strain was routinely cultured on Yeast Phosphate Soluble Starch (YPSS) agar slants containing in g/l : Yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; soluble starch, 15.0; agar, 20.0. The pH was kept at 7.0. The slants were incubated at 45^0C for 4-5 days and then stored at 4^0C. The cultures were sub-cultured once a month.

6.2 Production of enzyme:

Production of enzyme was carried out at three levels: shake-flask, liquid submerged fermentation and solid-state cultivation. The preliminary media optimisation studies were carried out in shake-flasks.

(i) Shake-flask studies: The medium composition for inoculum preparation was glucose (10 g/l), KH_2PO_4 (0.6 g/l), K_2HPO_4 (0.4 g/l), $MgSO_4.7H_2O$ (0.5 g/l), urea (0.5 g/l), yeast extract (0.1 g/l). *M. albomyces* was grown in 250 ml Erlenmeyer flask containing 50 ml of the above medium for approximately 36 h at 45^0C and 220 rpm in an incubator shaker. The contents of each flask were then transferred into two 500 ml flasks containing 100 ml of production medium. In the production medium glucose was replaced by wheat straw (1%, w/v). The wheat straw was obtained from local market and sieved though sieves of variable range (250 μm to 1000 μm) and used in the experimental set up. The flasks were shaken at 220 rpm for around 6 days at 45^0C. Samples were removed at appropriate time intervals, as indicated in the Results section, and enzyme activities measured.

Various agricultural residues rich in xylan were also evaluated for their ability to induce xylanases in this fungus. The composition of these materials is given in Table 2. Bark pith (the pith material obtained after extraction of the juice from bagasse (1-3

% w/v), wheat bran boiled (1-3% w/v), wheat bran unboiled and rice husk were used as carbon source in place of wheat straw.

Using 3% wheat bran as carbon source, different organic nitrogen sources were evaluated for their effect on xylanase production. The effect of varying inorganic nitrogen source, urea, in combination with a fixed level of yeast extract was also studied on enzyme production.

Crude enzyme preparations were obtained by centrifuging (under cold) the mycelial suspension at 8000 x g for 10 min. The clear culture broth obtained was used as the source of enzyme preparation and stored at 4^0C until required. For long- term storage, sodium azide (0.01%, w/v) and phenylmethylsulphonyl fluoride (PMSF) at 1 mM final concentration were added to the enzyme preparations. This was stored at -20^0C until required.

(ii) Fermenter studies: The large-scale fermentations were carried out at two scales. First, a 14l (Chemap AG) fermenter was used. It was equipped with pH, temperature and dissolved oxygen monitoring controls. The working media was 10l. The composition of the medium was the same as employed for shake flask studies. Wheat straw (mesh size 850 μm) was used as the substrate for xylanase production. The seed culture of 1l was prepared containing the same medium as for final fermentation. Wheat straw was substituted with glucose (1%). The entire contents were inoculated into the fermenter under aseptic conditions. The initial pH of the medium was set at 6.0 and left uncontrolled during the course of the fermentation. The fermentation was carried out at 45^0C at a moderate rpm of 100. Samples were removed periodically and crude enzyme preparations were obtained as described above. The samples were assayed for xylanase activity as described under assay conditions (Section 6.3).

The second set of fermenter runs was carried out in a 6L (Bioengg) fermenter containing 4l of total medium. Based on our shake flask data, wheat bran was used as the carbon source. The seed culture (600 ml) was prepared by inoculating 100 ml medium (contained in a 500 ml flask) with a freshly grown slant of the fungus (x 6). Glucose (1 %) was used as the carbon source and the flasks were grown at 45^0C with

constant agitation at 200 rpm. The flasks were grown for 48h until the pelleted mycelia grew. The contents of the flasks were pooled aseptically into an inoculation bottle which was used to inoculate the fermenter. The air flow was adjusted to 0.5 vvm. The initial pH was set at 6.0 and left uncontrolled during the runs. The fungus was grown at 45^0C with a slow agitation of 120 rpm. The pH and dissolved oxygen were monitored continuously. The first sample was removed after 48h and the subsequent ones after every 24h and analysed for xylanase activity as described.

(iii) Solid-state cultivation: The solid-state cultivation was carried out as per the protocols outlined in Fig. 2 on next stage. The composition of media used to supplement wheat straw is also outlined in the following table.

A number of parameters affect the enzyme production in solid substrate cultivation techniques. The following parameters were studied for their possible effect on enzyme production:

a. inoculum age and percentage
b. medium composition (medium C)
c. extraction conditions of the enzyme (variation of extraction pH and surfactant)

6.3 Enzyme assays:

Xylanase activity was estimated by determining the release of reducing sugars (measured as xylose equivalents) from oat-spelt xylan. The substrate preparation and the assay method are described below:

Xylanase activity (Biely et al., 1992):

The substrate solution was prepared by adding the finely powdered oat-spelt xylan (1% w/v) to sodium phosphate buffer (0.05 M, pH 6.0) (preheated at 60^0C for 10 min) and stirring the mixture at 25-35^0C for 10 min. Oat spelt xylan formed a suspension which was stirred uniformly to distribute the xylan in the buffer,

Xylanase activity was measured by adding 0.2 ml of appropriately diluted enzyme solution to 1.8 ml preheated oat-spelt xylan suspension in sodium phosphate buffer (0.05 M, pH 6.0). The reaction mixture, having a final substrate concentration of 9.0 mg/ml, was incubated for 5 min at 70^0C thermostat water bath with constant shaking (60 rpm). The reaction was terminated by adding 3 ml of DNS reagent and boiling the reaction mixture for 5 min in a boiling water bath, and immediately cooling it in the melting ice. The undegraded xylan was removed by centrifuging the samples at 200 rpm at room temperature for 5 min. The reducing sugars generated in the supernatant, by xylanase action, were estimated by deducting the A_{540} value of enzyme blank (heat inactivated enzyme subjected to the same treatment, as above) from the absorbance value of enzyme assigned sample.

The xylanase activity is reported as International units (IU). One IU of xylanase was defined as one μmole of xylose equivalents produced by 1 ml undiluted enzyme in 1 min. The μmoles of xylose produced by xylanase, were deduced from xylose standard plot.

Endoglucanase activity (IUPAC, 1982):

The substrate was prepared by adding carboxymethylcellulose (CMC) (2% w/v) to sodium phosphate buffer (0.05 M, pH 6.0). The solution was autoclaved and used as the substrate.

The endoglucanase activity was measured by adding 0.5 ml of appropriately diluted enzyme to 0.5 ml of CMC solution (as prepared above) and incubating for 30 min at 50^0C in a thermostat water bath at 40 rpm. The reaction was terminated by adding 3 ml DNS reagent and boiling it for 5 min in boiling water bath. The sugars released were estimated at 540 nm using appropriate enzyme blank. Enzyme activity is reported as the μmoles of glucose released per ml per min wherein one μmoles refers to 1 IU. Glucose equivalents were deduced from the standard graphs.

FPA activity (IUPAC, 1984):

The FPA activity was determined by adding 0.5 ml of appropriately diluted enzyme to 1.0 ml of sodium phosphate buffer (0.05 M, pH 6.0) containing 50 mg Whatman filter paper (1 cm x 6 cm strip) and incubating this reaction mixture at 50^0C in a thermostat water bath at 40 rpm. The rest of the procedure was same as in endoglucanase assay method.

6.4 Differential precipitation of enzymes:

Low activities of endoglucanase and filter paper activity were detected in the culture supernatant. Thus, ammonium sulphate precipitation technique was used to precipitate the xylanase enzymes and to investigate if the enzyme preparations could be obtained which were nearly free of these cellulase activities. Four differential cut-offs (0-20%, 20-40%, 40-60%, 60-80%) were chosen. The amount of ammonium sulphate for these cut off values was calculated by the following formula.

$G= 533 (S_2-S_1)/ 100-0.3S_2$

where,

G= g of NH_2SO_4 to be added/ 1 supernatant

S_2; final saturation

S_2; initial saturation

Co-precipitation of cellulase activity (measured in terms of endoglucanase and FPA activity) was also monitored. The samples were dialyzed extensively against the buffer to remove (NH_4) SO_4 that may interfere with enzyme activities. Comparisons were also made between dialyzed and undialyzed fractions.

6.5 Characterization of the enzyme:

The xylanase enzymes were characterized in terms of pH and temperature optima. The acetate buffer was used for the pH range of 4-6, sodium phosphate buffer for pH 6-8 and Tris-Cl buffer for the pH 8-10. The thermal stability of xylanases was monitored at temperatures ranging from 50^0C to 70^0C at 5^0C interval.

6.6 Enzyme treatment of pulp

Xylanase treatment of the pulp samples procured from forest based mills (Eucalyptus & Bamboo, Bagasse), was carried out on 300 gm pulp batches. The pulp was adjusted to pH 6.2 by addition of 1 M H_2SO_4 solution. Enzyme was properly mixed by kneading mechanism. Temperature was maintained at 50-60°C for 2 h. An optimized dose of enzyme charge of 10-15 IU/gm of pulp was applied at pulp consistency of 8-10%. Control pulps were prepared identically to the enzyme treated pulps with enzyme being replaced with water. Table 3 shows the conditions of enzyme treatment of pulp.

Optimisation of enzyme doses

Enzyme doses were optimized so that minimum quantity of enzyme is required under normal pulp residence time of nearly 90-120 minutes. Enzyme doses were optimized at optimized temperature and pH optimum by measuring the release of lignin & chromophores in pulp filtrate besides determining the reduction in Kappa No. of enzyme treated pulp, if any against control pulp sample.

6.7 Determination of the Kappa no. of pulp

Kappa No. of the pulp procured from the mills, control pulp & enzyme treated pulp i.e. just after xylanase treatment, after chlorination & extraction stage (CE) were determined following standard Tappi procedure T-236-0S-76.

6.8 Bleaching of pulp

Conventional bleaching sequence i.e. chlorination, alkali extraction followed by hypochlorite (CEH) being practiced in most of the Indian mills, has been employed during the experiment which involved optimization of the doses of the elemental chlorine, caustic and hypochlorite and respective stage of both the control pulps & enzyme treated pulp. Each stage of bleaching was optimised by small scale treatment using 20 gm OD pulps which required at least 500 gm OD pulp to provide a sample of (200gm OD basis) pulp for evaluation studies.

6.9 Evaluation of bleached pulp characteristics

(a) Determination of pulp brightness

Brightness of the pulp samples was measured at following the procedure given in ISO DIS 3688.

(b) Determination of strength properties

Strength properties of both enzyme treated & untreated pulp samples were determined by beating the pulp in PFI mill to various degree of freeness under standard conditions as per ISO DP 5264 i.e.:

—	Beating pressure	—	17.7 N/Cm
—	Relative speed	—	6.0 m/s
—	Beating consistency	—	10% on weight basis
—	CSF measurement	—	ISO DP 5267

The temperature of the stock was recorded immediately after beating. Hand sheets was made as per ISO DP 5269 & dried on plates in stack under the standard conditions for tropical countries (27°C, 65%RH). Physical testing of the hand sheets was made according to the following standards:

—	Tensile index	—	ISO 1924
—	Tear index	—	ISO 1974
—	Burst index	—	ISO 2758

(c) Determination of intrinsic viscosity

Viscosity of the pulp was measured as per standard procedure SCAN C-15:62.

7. RESULTS & DISCUSSION:

7.1 Production of xylanase enzymes by *Melanocarpus albomyces* in shake –flasks, liquid submerged fermentation and solid state cultivation

7.1.1 Production of enzyme in shake flask:

Melanocarpus albomyces produces inducible extracellular xylanase activity when grown on xylan at 45^0C for a period of over 5 days. Initial studies made on evaluation of a number of inducers for xylanase production indicated that wheat straw was the best extracellular enzyme inducer. Little or nearly no endoglucanase activity could be detected in the enzyme preparations induced on wheat straw. This substrate was thus used for enzyme production in shake flask studies. The wheat straw was sieved through mesh of different sizes (250 μm, 500 μm, 850 μm, 1000 μm) and permeates obtained were used as carbon source at 1% (w/v) level in separate experiments. Fig.3 shows the profile of extracellular xylanase production on wheat straw of different mesh sizes. The maximum enzyme production occurred between 96 and 160 h on all samples and highest activities of 120 IU/ml were obtained on wheat straw of mesh size below 250 μm. The maximum enzyme activities were attained faster on this substrate. High enzyme activities were obtained on the next particle size studied but these values were obtained around 144-160 h. Thus higher productivities were obtained on wheat straw of small particle size. The larger surface are provided by small particle size is assumed to be responsible for such differences. Table 4 gives the cellulase activities obtained in the enzyme preparations obtained on particle size of less than 500 μm harvested at 144 h. As indicated, very low cellulase activities were observed.

Effect of different carbon sources:

The composition of different agricultural residues used for production of xylanase enzymes is given in Table 1. The cellulose in these materials did not vary much and was from 30-36 (% dry weight). The hemicellulose content was also relatively constant (24-30% dry weight) except for rice husk which was 15% hemicellulose.

The ash, as expected, was also high in this material compared to other carbon sources. Of the different materials studied, wheat bran was found to be the best material for production of enzyme giving 134 IU/ml (at the end of 96h) Table 5. Although bark pith at 3% (w/v) also gave similar high values but it was at the end of 192 h. Thus high productivities were obtained on wheat bran. Wheat bran (unboiled) gave low activities possibly due to repression exerted by associatedsugars. Removal of this by boiling improved remarkably enzyme production. Very low activity of 15 IU/ml was obtained on rice husk (data not shown).

Nitrogen Source:

The optimization of nitrogen in microbial fermentation is equally important and was studied for its possible effect on xylanase production. Using 3% wheat bran as carbon source, different organic nitrogen sources were evaluated for their effect on enzyme production and the results are shown in Table 6. The organic sources did not result in an increase in enzyme production. In fact slightly lowered levels were obtained over the initial combination of nitrogen used in the study (urea 0.05 + yeast extract 0.01 %). The maximum productivity of 1400 U/l/h was obtained on this nitrogen combination. A number of studies have reported favorable effect of tryptone or soya bean meal but these had no favourable effect in the present study.

Since urea was found to effect positively enzyme production, concentration of urea was varied in combination with yeast extract. The carbon source was kept constant at 3% wheat bran in all experiments. A detailed profile of enzyme production is shown in Fig. 4. As observed, both 1% and 2% levels of urea in combination with 0.01% yeast extract resulted in considerable increase in xylanase production. About 200 IU/ml of xylanase activity was obtained at 72 h giving very high productivities of 3000 IU/ml/h.

7.1.2 Production of enzyme in fermenter:

In the first study carried out in 14l fermenter containing 10l working volume, enzyme activity of 80 IU/ml was obtained at the end of 72 h using wheat straw as carbon source. The data obtained in shake-flasks with respect to optimization of carbon

source, which was wheat bran, was translated to fermenter studies. The results carried out at 6l level, containing 4l working volume, and using 2% wheat bran (boiled) as carbon source are shown in Fig. 5. As observed, enzyme activities of 70 IU/ml were obtained at the end of 144h. The pH was left uncontrolled in these studies and increased from initial 6.0 to 7.5 at the end of 144 h. Good growth was observed in the fermenter and it is expected that media optimization studies will lead to considerable increase in enzyme production.

7.1.3 Production of enzyme by solid-substrate cultivation:

A number of parameters were optimized for solid-substrate cultivation of the fungus and the details are reported in Narang, Bisaria and Sahai (2001). Various combinations of inoculum age, moisture content and media concentrations were employed to study their effect on enzyme production. Keeping all the variables at the following optimised level, enzyme production was studied as a function of time and the results are shown in Fig. 6.

Variables (optimised values):

Media C:	1.6 ml/g
Surfactant:	1% (v/w)
Urea:	3% (w/w)
Moisture content:	80%
Inoculum percentage:	80%
Inoculum age:	40h
$MgSO_4$:	1.67%
Extraction pH:	7.0

The results in Fig. 6 indicate maximum enzyme production of 6830 IU/g wheat straw at the end of 98 h. Based on the conclusions of these studies, the software design expert^{TM} version 5.0 of Stat-Erase, Inc. was used to optimize fermentation conditions. Optimization was carried out using Response Surface Methodology. Out of the 10 solutions obtained (Table 7) only two solutions 8 and 10 fell in the area where the Design-Expert could precisely (95% confidence level) predict the response. The actual value of solution 8 was 7580 IU/g vis a vis predicted value of 7831 IU/g and

for solution 10 it was 7365 IU/g vis a vis the predicted value of 7643 IU/g. Thus very high enzyme levels were also obtained by solid substrate cultivation and this data was also used for sensitivity analysis.

7.2 Characterization of enzymes

The pH optima of the xylanase enzyme was determined by measuring the enzyme activities between pH 5.0 and 8.5. The results are shown in Fig. 7 and indicated the optimum at pH 6.0. Between pH 5.5 and 7, the enzyme retained over 60% of the enzyme activity. Increase in the pH above 7.0 resulted in loss of enzyme activity. Thus, the enzyme can be considered to be operating optimally around neutral pH with more tolerant activity at lower pH.

Measurement of cellulase activities was also performed at these pH values and the results in Fig. 7 indicate stable cellulase activities from pH 5 to 8. More than 50% of the cellulase activity was stable in this pH range. Although low cellulase activities were obtained on wheat straw, it would be interesting to study the cellulase production on other cellulase inducers in this system.

The temperature optima of the xylanase enzyme was determined by measuring the enzyme activity at temperature ranging from 30° C to 80°C. The results are shown in Fig. 8 which indicated that temperature optimum for xylanase was 70°C. The enzyme retained more than 80% activity in the temperature range from 60-70°C. Any further increase in temperature resulted in drastic drop in activity. Thus, the enzyme is compatible with the operating conditions of 50-55 ° C used at the mill site for enzymatic treatment of the pulp.

The optimum for endoglucanase activity was 60°C and, interestingly, the enzyme retained more than 80% activity in 40-70°C range. Hence, the endoglucanase produced in the fungus may be a broad temperature range enzyme. The FPA activity operated maximally at 50-55°C and was relatively less stable compared to the endoglucanase activity.

Some early results indicated slight loss in pulp properties due to enzyme treatment. Thus, attempts were made to use the ammonium sulfate selective precipitation technique to obtain, if possible, cellulase free xylanase preparations. The results of this study indicated that greater than 60% of the xylanase activity was precipitated by 40% ammonium sulfate levels while relatively little (less than 35%) endoglucanase and FPA activity were precipitated under these conditions (Fig. 9). Maximum cellulase activity was precipitated at greater than 40% saturation levels. It was indicated thereby that cellulase activities could be further reduced in the xylanase preparations. A detailed analysis of xylanase activities was carried out at different salt cut-off concentrations and the results are presented in Fig. 10. The measurement of xylanase activity in the pellet indicated less than what was expected based on the results of the supernatant studies (Fig. 9). Thus, some inactivation and loss of yield by the salt was indicated. A cut off of 40% can thus be used to selectively precipitate the xylanase enzymes. Between the undialysed and dialysed fractions of enzymes, little difference in enzyme activity was noticed. Hence, extensive dialysis to remove residual salt may not be necessary.

During the enzyme treatment of the pulp, the temperature as well as the time of treatment are critical. Maintaining the enzyme in the intact form is a necessary prerequisite under the process conditions. Thus, the thermal stability of xylanase enzyme preparation was evaluated at different temperatures. The results are given in Figs. 11 and 12. The results indicated that the enzyme was highly stable at 50^0C retaining about 90% of the original activity for 90 min. At 55^0C, the enzyme retained around 70% activity for about an hour. Further incubation for additional 30 min reduced the enzyme activity only marginally. The enzyme was inactivated at temperature of 60^0C and above. The ln % residual activity is shown in Fig. 12 and the linear part of the slopes were used to calculate the apparent pseudo first order rate constants for deactivation. The results indicated the strong stability of the enzyme preparations around 50-55^0C. Thus, from these studies, it was recommended to use the enzyme in the temperature range of 50-55^0C. The enzymes obtained on wheat bran were also evaluated for thermal stability and indicated these enzymes to be slightly more stable than the enzyme preparations obtained on wheat straw.

7.3 Effect of enzyme treatment on Kraft processed pulp

Various sets of experiments were conducted and finally the results of enzymatic pretreatment of pulp (eucalyptus + bamboo and bagasse) procured from the mills as detailed in Table 1, followed by bleaching of pulps using conventional CEH bleaching sequence are shown in Tables 8-11. The different parameters such as Kappa no., pulp filtrate analysis, viscosity, savings in bleach chemicals, strength and optical properties, and environmental impact were obtained from both category of pulps and the results are discussed below.

7.3.1 Effect of enzyme treatment on Kraft processed wood pulp

a) Kappa no.

From the results shown in Table 8a, it was clearly evident that there was reduction in Kappa no. of unbleached Kraft wood pulp after enzyme treatment which was decreased by 1.5 points i.e. from 18.0-16.5 with gain in pulp brightness of 1.0 point i.e. from 24.0 to 25% ISO.

(b) Analysis of pulp filtrates

The extractability of the dissolved lignin and the chromophores in pulp filtrates after xylanase treatment and the control pulp sample were studied and the solubilised lignin and chromphores were measured by UV at 280nm and in visible range at 465nm respectively. The filtrate after enzyme treatment was found to be more coloured as compared to the control pulp samples. The determination of the lignin at 280nm showed extraction of higher amount of lignin e.e. 1.03 kg/tp as against 0.39 kg/tp in control pulp. The chromophores released were also high i.e. 2.72 kg/tp after xylanase treatment as against 0.93 kg/tp in control pulp indicating the effectivity of the xylanase enzyme for wood Kraft pulp (Table 8b). The reduction in Kappa No. of the enzyme treated sample from 18.0-16.5 could be attributed to the release of lignin and chromophores. The brightness of the unbleached pulp could also be improved to 1.0 point i.e. from 24-25% ISO. (Table 8a)due to this.

(c) Viscosity of pulp

Not much appreciable reduction in viscosity of the pulp after enzyme treatment was found when the pulp was pre-treated with optimized dose of enzyme. However, in one of the final set of experiments, at optimised enzyme dose of 10 IU/gm, a little increase in CED viscosity was observed which was from 690 cm^3/g (control pulp) to 740 cm^3/gm (enzyme treated pulp; Table 8a). As expected this little increase in viscosity could be due to the fact that the xylan has a molar mass that is generally lower than that of cellulose. So its removal leaves behind cellulose that has a higher viscosity. Therefore, the conventional view that higher pulp strength is observed with higher pulp viscosity might not hold for xylanase treated pulp.

(d) Savings in bleaching chemicals

One of the main objectives of the study was to evaluate the response of xylanases as prebleaching agent & to reduce the requirement of chlorine as bleaching chemical by maintaining targeted brightness level. This could ultimately reduce the release of AOX in bleach effluents. From the results shown in Table 9a, it was indicated that by xylanase treatment of wood Kraft pulp there was reduction in chlorine requirement which was reduced from 4.1 to (41 kg/tp) to 3.5% (35 kg/tp) after xylanase treatment. The alkali during extraction stage was also reduced to a little extent as reflected by consumption of alkali, as percent alkali consumed was reduced to 60.1% as against 68.4% in control pulp.

(e) Strength & optical properties of enzyme treated pulp

Strength and optical properties of the untreated and treated pulp, shown in Table 9b were found to be almost at par in terms of tensile, burst which were 64.0 & 62.0 Nm/g and 4.40 & 4.20 $Kpa.m^2/g$ respectively. Little drop in tear could be noticed after enzyme treatment which was dropped from 6.04 to 5.8 Mnm^2/g. Such a drop can be arrested by reducing the treatment time and/or enzyme dose. Final brightness of the pulp was improved by 3 points i.e. from 80 to 83% ISO (Table 9a).

(f) Environmental impact of enzyme treatment

Characterisation of the effluents after enzyme treatment showed an improved environmental status in terms of AOX & Colour and COD. The AOX level in bleach effluents was reduced by more than 20% i.e. 2.12 kg/tp to 1.8 kg/tp in enzyme treated

bleach effluent (Table 10). There was an indirect improvement in terms of COD after enzyme treatment. This number was reduced from 32 kg/tp to 27.0 kg/tp.

Comparative studies were carried out with commercially available xylanase (Bleachzyme F) from Biocon. This enzyme formulation had similar pH optimum as the enzyme produced from *M. albomyces*. The results presented in Tables 11 & 12 indicated that enzymes from the test organism had very similar effectivity as the commercial enzyme. Savings in chlorine were of the order of 15% *M. albomyces*) and 16% (commercial enzyme) and the data is shown in Table 11. Slight improvement in final brightness of the pulp was observed with the fungal enzyme which was higher by 3%. The strength properties are detailed in Table 12 and indicate similar values for both the *redanocarpus* as well as commercial enzyme (Table 12).

7.3.2 Effect of enzyme treatment on agro based pulps

Having evaluated the effectivity and positive response of xylanases of *Melanocarpus sp.* on wood samples of Kraft pulp, studies were continued on pulps collected from agro based mills. Bagasse pulp was procured from an identified mill by IAPMA. Pulps were characterized with respect to their Kappa no. and wide variation was observed in Kappa no. of the two samples of the pulp obtained on two different occasions. Kappa no of the second batch of bagasse obtained from M/S Seshasayee paper mills, Erode was 27 (Table 1).

The initial studies on enzymatic pre-treatment of bagasse pulp included optimisation of enzyme dosages/g of pulp. Bleaching of the enzymatically treated pulp was carried using CEH sequence as per standard procedures and conditions were followed/as optimised for wood Kraft pulp. Kappa no of both the control & enzyme treated pulp was determined following Tappi standard procedures, as detailed in the previous section.

(a) Effect on Kappa no

The magnitude of the effect of enzymatic treatment on the Kappa no. was not significant. However, it has been observed that the effect of xylanases may vary in their ability to decrease the Kappa no of Kraft pulps. In depends on the nature & type

of the pulp. As seen from the data shown in Tables 13a & 15a, the Kappa No of enzyme treated Kraft bagasse pulp was reduced by 0.5 – 1.0 i. e. from 7.7 to 7.0 & (Table 13a) 26.5 to 25.5 (Table 15a). However, this reduction can not be taken as a reliable measure but this drop in Kappa No. has consistently been observed in the pulp after enzyme treatment which could be supported from the improved brightness of unbleached Kraft bagasse pulp which was improved form 55.5% ISO to 57.3% ISO (Table 13a) and from 30.0% ISO to 31.0% ISO (Table 15a) respectively.

(b) Analysis of pulp filtrates

The analysis of the pulp filtrates after enzyme treatment indicated it to be more coloured as compared to the control sample which presumably was be due to solubilisation of the xylan derived chromophores leading to the extraction of some lignin components associated with the xylan i.e lignin – carbohydrate complexes. This could be confirmed from the results shown in Table 13b & 15b. From the table it is indicated that lignin at 280 nm in the enzyme treated filtrate showed higher amount of lignin i.e. 0.44 kg/tp as against 0.08 kg/tp (Table 13b) and 0.64 kg/tp as against 0.15 kg/tp in control pulp (Table 15b), similarly the chromophore release was also high i.e 1.8 kg/tp (Table 13b) as against 0.33 kg/tp and 4 kg/tp as against 1.0 kg/tp indicating the effectivity of the enzyme on Kraft bagasse pulp.

(c) Viscosity of pulp

As observed from Table 15a, the viscosity of the pulp was about the same, 520 cm^3/g as against the control value of 535 cm^3/g.

(d) Savings in bleach chemicals

During conventional CEH bleaching of the Kraft bagasse pulp reduction in requirement of elemental Cl_2 was observed which was reduced from 5.6 kg/tp to 4.8 kg/tp after xylanase treatment where as during alkali extraction state when we analyse the percent alkali consumed, there was an indication of less consumption of alkali as shown from the data in Table 161. At similar doses of elemental Cl_2 & alkali the percent consumed was 76% as opposed to 88% in control (Table 16). Similarly the alkali consumption was 61.2% in enzyme pre-treated pulp as against 74.2% in the

control pulp clearly showing savings in the alkali requirement during enzymatic pre-bleaching.

(e) Strength & Optical properties of the pulp

Extensive testing of the xylanase enzyme treated pulps showed that the treated pulps needed slightly more refining energy to refine the pulp at same freeness level than control pulp. However, it showed the strength properties almost at par with the control (untreated pulp)in terms of tensile & burst which was found to be 43.0 NM/gm & 2.60 Kpa M2/g of the enzyme treated pulp as against 45.5 NM/gm & 2.55 K pa M2/g in case of control pulp(Table 17). Slight improvement in the tear index could be noticed in enzyme treated pulp, which was improved from 4.05 MNm2/g to 4.20 MNm2/g. The results are shown in Table 17. Brightness of the pulp was improved by 2 points i.e from 83.9 to 85.1 ISO (Table 16).

(f) Environmental impact of enzyme treatment

Characterization of the effluents after enzyme treatment showed an improved environmental status in terms of AOX, colour and COD. The results shown in Table 18 revealed that the AOX level in bleach effluents was reduced 25% from i.e 4.13 kg/tp to 3.13 kg/tp in enzyme treated bleach effluent. There was an indirect improvement in terms of colour & COD as the colour in the bleach effluent after enzyme treatment was reduced from 30. 18 kg/tp to 17.07 kg/tp. The biodegradability of effluent was also improved as reflected from COD: BOD ratio of 1: 4 compared to 1:3 in case of enzyme treatment.

REFERENCES:

1. Biely, P., Vrsanska, M., and Kucar, S. (1992). In Xylans and Xylanases (Visser, J., Beldman, G., Kusters-van Someran, M.A. and Voragen, A.G.S. eds.) Elsevier Amsterdam. Pp. 81-95.
2. IUPAC (1984). Measurement of Cellulase Activities. Recommendations of the Commission of Biotechnology, International Union of Pure and Applied Chemistry. New-Delhi: Indian Institute of Technology, Delhi.
3. Narang, S., Bisaria, V.S. and sahai, V. (2001) Biotechnology and Bioprocess Engg. (In press).

8. ECONOMIC ANALYSIS

INTRODUCTION:

In the last few years there has been growing pressure for change in the way pulp is bleached. It is traditionally done by chemicals like chlorine, chlorine oxide, hypochlorites, and hydrogen peroxide. The effluent from such bleaching is very toxic (AOX: Adsorbable Organic Halides) and very difficult to treat. Companies are trying to shift to cleaner technologies such as ECF (Elemental Chlorine Free) and TCF (Total Chlorine Free) process.

An alternative solution to it was bleaching with enzyme, which can prebleach the pulp and thus reduce the amount of chemicals required for complete bleaching. The effluent thus produced would be less toxic and would be easily degraded by waste treatment techniques.

In the present work, effort has been put to design and do economic analysis for all the processes to achieve bleaching for 50 TPD of Paper Pulp such that figures of estimation for economic feasibility can be obtained.

Following is the Sequence of further Analysis:

Complete flow chart showing the lay-out of the plan proposed.

Summary of

- Mechanical design of mixer where bleaching takes place.
- Mechanical design of fermenter used for production of enzyme that is further used for bleaching.
- Mechanical design of pre-fermenter which is used as inoculation unit for the main fermenter.

Preliminary calculations for evaluation of design parameters.

Calculations for mixer.

Calculations for fermenter.

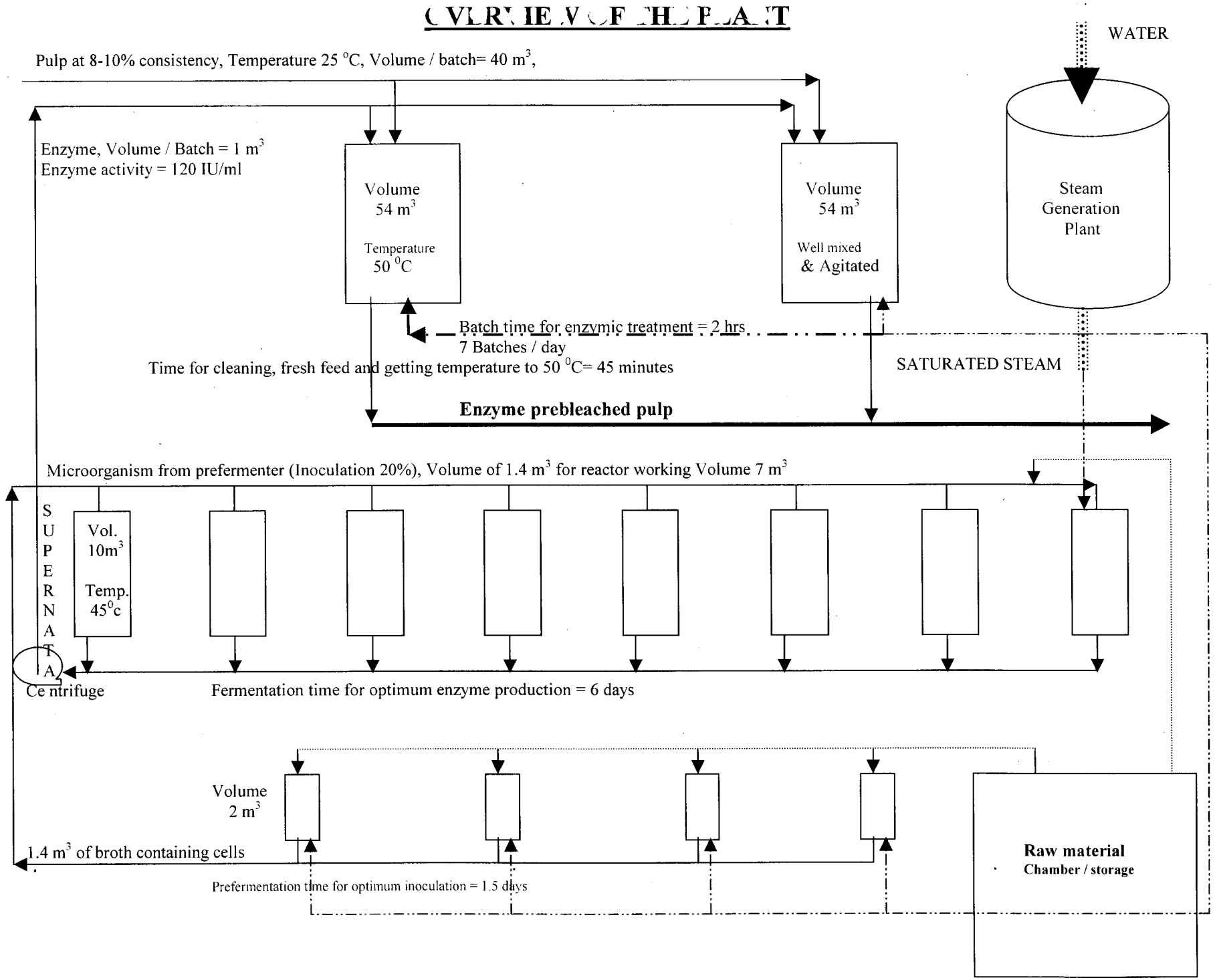
Calculations for pre-fermenter.

Economic analysis for the plant.

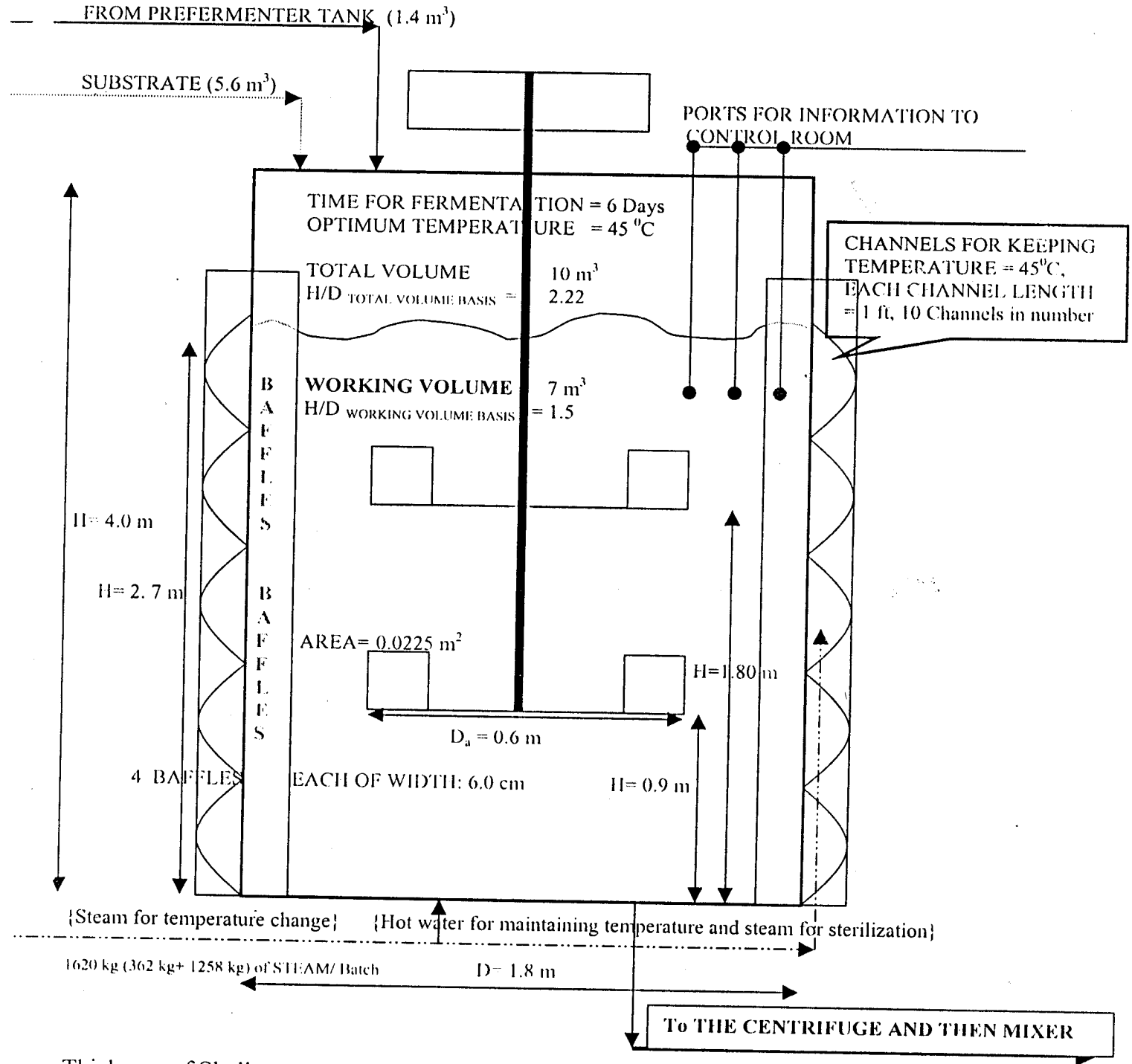
Salient features and shortcomings of design.

This project requires substantial investment at start but one should go for it as some of capital is recovered from spending less on Waste treatment Process. Also, we will look for a Pollution free World.

OVERVIEW OF THE PLANT

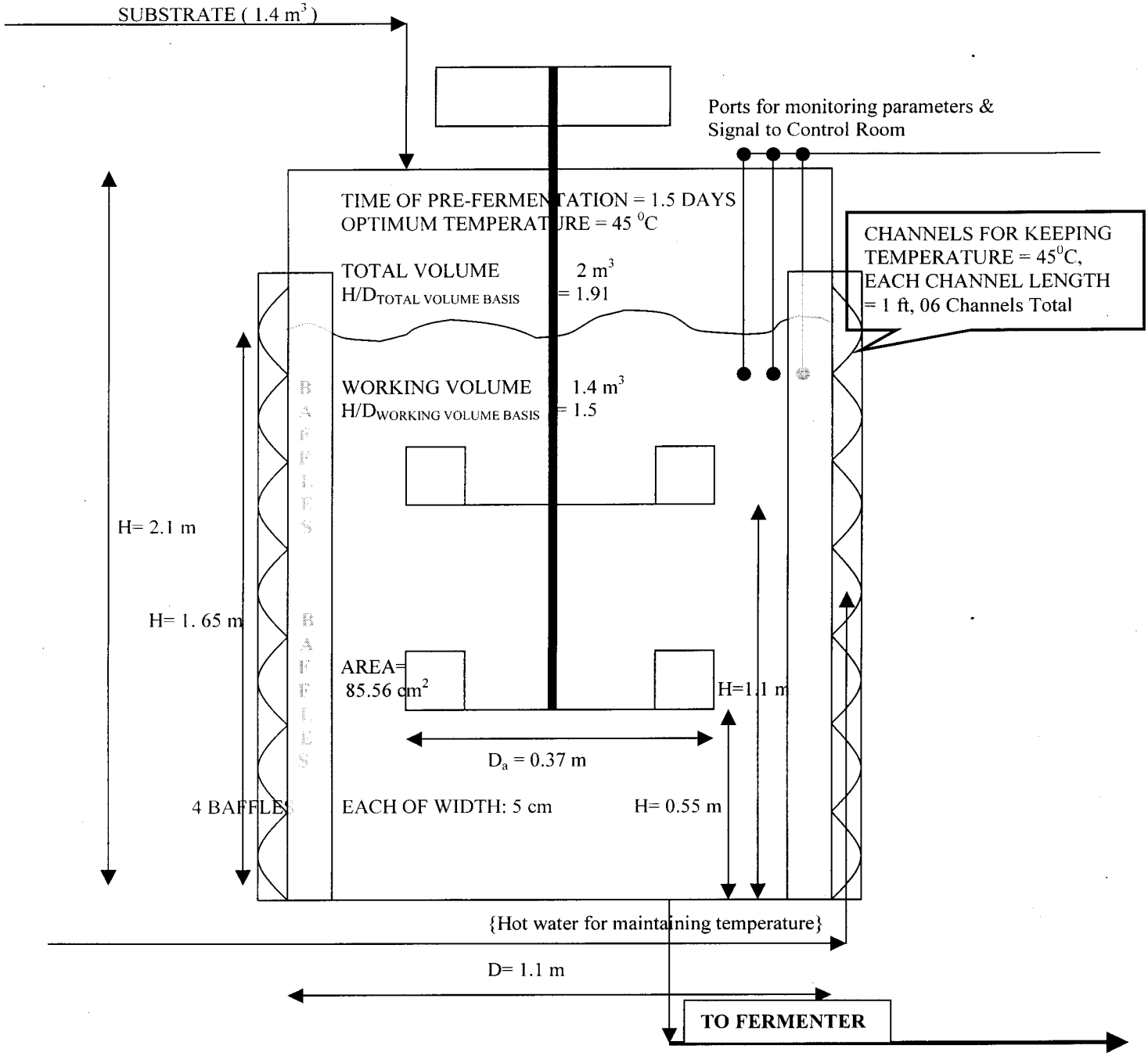


DETAILS OF FERMENTER (SECTIONAL VIEW)



Thickness of Shell	:0.75 inches
Material Used for Construction	: SA- 240 Steel Plates which have f = 130 N/mm² @ T= 95 °C
Internal Pressure that can be handled	:15 atm. @ T= 121 °C (Sterilization Conditions)
Thickness of Bottom Plates	:0.75 inches
Thickness of Jacket	:0.75 inches

DETAILS: PRE-FERMENTER (SECTIONAL VIEW)



Thickness of Shell	:0.50 inches
Material Used for Construction	: SA- 240 Steel Plates which have f = 130 N/mm^2 @ T= 95 ^{0}C
Internal Pressure that can be handled	:15 atmosphere @ Temperature = 121 ^{0}C
Thickness of Bottom Plates	:0.5 inches
Thickness of Jacket	:0.75 inches

PRELIMINARY CALCULATIONS:

Working for **50 TPD (50,000 kg / Day)** paper plant @ consistency of **9% (90 kg / m^3)**
Thus, Volume to be treated by Enzyme is 50000 / 90 = **556 m^3.**
Proposal to handle 556 m^3 by Two reactors handling 40 m^3 each of Paper pulp. We propose operation of **7 Batches a Day taking 2 hours each. Cleaning** of each Mixer will take **45 minutes** after each batch operation is completed.
Thus total time taken = 7 x 2 hrs, 45 min. =**19 hrs, 15 min**. which can be done in two shifts of ten (10) hours each by labor.

The enzyme activity from fermentation tank is 120 IU/ml and we require 15 IU/ gm of pulp for effective bleaching of Pulp.
Amount of Enzyme required in liters / kg of Pulp would be (15000 / 120000) = 0.125
To treat 40 m^3 pulp with consistency factor of 9% = Treatment of 3600 kg of Pulp
Thus, Enzyme required / Batch = 3600 x 0.125 liters = 450 liters =0.45 m^3
Enzyme required on per day Basis: 0.45 m^3 x 2 Reactors x 7 Batches / Day = 6.3 m^3.
Enzyme required on per Year Basis: 6.3 m^3 / Day x 365 Days = 2300 m^3.

CALCULATION FOR FERMENTER:

Working Volume = 7 m^3 (Provide for 30 % Head Space)

$(H/D)_{Working\ Volume}$ = 1.5 gives D= 1.8 m

Thus, Total Volume = 10 m^3, and H= 4.0 m

$(H/D)_{Actual}$ = 2.22

Material Used for construction of Vessel: SA-240 Steel Plates

f (Strength): 120 N/mm^2 @ Temperature = 121 ^0C

Thickness of Shell: $t = pD_i / (2fJ-p)$

where {p= 1.672 N/mm^2, D_i= 180 cm, J= 0.85, f= 120 N/mm^2}

=0.58 inches

Thickness for Safe Design =0.75 inches

Thickness of Bottom Plates = 0.75 inches (As the Pressure encountered by it is not more than 15 atmosphere and as it is we are over designing for the shell)

Minimum Thickness of Channel Jacket = $\mathbf{t_c = d \times (k_2\ p / f)^{1/2}}$

where k_2 = 0.12, p = 1.672 N/mm^2, f = 120 N/mm^2 and d (distance of 1 Channel)= 1 ft

t_c = 1.227 cm

Thickness of Jacket channel for Safe Design = 0.75 inches

Calculation for Steam Requirement:

We take Saturated Steam @ 100 ^0C with λ = 970.3 Btu/lb

Enthalpy of Water @ 100 ^0C = 180.16 Btu/lb = H_1

Enthalpy of Water @ 50 ^0C = 88.00 Btu/lb = H_2

Enthalpy of Water @ 25 ^0C = 48.09 Btu/lb = H_3

Inlet Temperature of the pulp = 25 ^0C, All properties taken of that Water due to inadequate Data.

Mass of Broth ; m_b= 7 m^3 x 1200 kg / m^3 = 8400 kg = 18502 lb

Heat Lost by Steam = $m_s (\lambda + H_1 - H_2)$

Heat Gained by Water / Broth = $m_b (H_2 - H_3)$

By Conservation of Energy we get m_s = 315 kg

Assume 15 % Losses, Thus Actual m_s = 362.25 kg

CALCULATION FOR PRE-FERMENTER:

Working Volume = 1.4 m^3 (Provide for 30 % Head Space)

$(H/D)_{Working\ Volume}$ = 1.5 gives D= 1.1 m

Thus, Total Volume = 2 m^3, and H= 2.1 m

$(H/D)_{Actual}$ = 1.91

Material Used for construction of Vessel: SA-240 Steel Plates

f (Strength): 120 N/mm^2 @ Temperature = 121 ^0C

Thickness of Shell: $t = pD_I / (2fJ-p)$

{p= 1.672 N/mm^2, D_I= 110 cm, J= 0.85, f= 120 N/mm^2}

=0.355 inches

Thickness for Safe Design =0.5 inches

Thickness of Bottom Plates = 0.5 inches (As the Pressure encountered by it is not more than 15 atmosphere and as it is we are over designing for the shell)

Minimum Thickness of Channel Jacket = $\mathbf{t_c = d \times (k_2\ p / f)^{1/2}}$

where k_2 = 0.12, p = 1.672 N/mm^2, f = 120 N/mm^2 and d (distance of 1 Channel)= 1 ft

t_c = 1.227 cm

Thickness of Jacket channel for Safe Design = 0.75 inches

ECONOMIC ANALYSIS

FIXED COST

Cost of Fermenter	8 x Rs. 20,00,000	1,60,00,000
Cost of Inoculation Fermenter	4 x Rs. 08,00,000	32,00,000

Total Fixed Cost	**Rs. 1,92,00,000**

VARIABLE COST on YEARLY BASIS

Personnel Cost			**Rs.**
Mixer + Fermeter + Pre-fermenter			
	Skilled	1 x Rs. 1,50,000	1,50,000
	Semiskilled	2 x Rs. 1,00,000	2,00,000
Cost / Shift	(3 Shifts / Day of 8 hours each)		3,50,000
Cost / Year			10,50,000

Total Personnel Cost	**Rs. 10,50,000**

Raw material	**Amount (kg)**	**Price (Rs./kg)**	**Amount (Rs.)**
Glucose	4900	35	1,71,500
KH_2PO_4	1470	36	52,920
K_2HPO_4	980	25	24,500
$MgSO_4$	1225	60	73,500
Urea	1225	4	4,900
Corn steep liquor			
Wheat Straw	20440	2.5	51,100

Total Raw Material Cost	**Rs. 3,78,420**

Steam Requirement	**No Boiler Cost , Rate of Steam Production = Rs. 700 / Ton**		
Mixer	Kg / Batch	Kg/ Day	Rs./ Year
For Temperature Control	414	5796	14,20,020
Fermenter			
For Temperature Control	362	362	91,980
For Sterilization Purpose	1258	1258	3,21,419
Pre-fermenter			
For Sterilization Purpose	250	250	63,875

Total Steam Cost	**Rs. 18,97,294**

TOTAL VARIABLE COST	**Rs. 33,25,714**

YEARLY PAYMENT FOR THE FIXED COST

- No inflation is taken into account.
- We get a tax-advantage of 35 % on interest and thus effective rate of interest = 0.65 x 15 % = 10 % (approximately). This is applicable if the total project is funded by long term loans. However, this is never true and there is balance of equity, preference share, long term loans and debentures to fund a project. The cost of capital is normally 15 % but largely depends upon the ratios of funding sources.
- We would also get tax advantage due to the depriciation
- Assuming 15 % capital cost, following would be the yearly payment structure

Year	Installment Paid (Lakhs)	Capital for interest payment (Lakhs)	Interest (Lakhs @ 15 %)	Total (Lakhs)
Year 1	Rs. 10.00	Rs. 182.0	Rs. 27.30	Rs. 37.30
Year 2	Rs. 10.00	Rs. 172.0	Rs. 25.80	Rs. 35.80
Year 3	Rs. 10.00	Rs. 162.0	Rs. 24.30	Rs. 34.30
Year 4	Rs. 20.00	Rs. 142.0	Rs. 21.30	Rs. 41.30
Year 5	Rs. 20.00	Rs. 122.0	Rs. 18.30	Rs. 38.30
Year 6	Rs. 20.00	Rs. 102.0	Rs. 15.30	Rs. 35.30
Year 7	Rs. 25.00	Rs. 77.0	Rs. 11.55	Rs. 36.55
Year 8	Rs. 25.00	Rs. 52.0	Rs. 7.80	Rs. 32.80
Year 9	Rs. 25.00	Rs. 27.0	Rs. 4.05	Rs. 29.05
Year 10	Rs. 27.00	Rs. 0.0	Rs. 0.00	Rs. 27.00
Sub Total	**Rs. 192.00**		**Rs. 155.70**	
Grand Total				**Rs. 347.70**

COST TO COMPANY FOR FOUR YEARS

Year	Total Fixed Cost (Lakhs)	Total Variable Cost (Lakhs)	Total Cost (Lakhs)
Year 1	Rs. 37.30	Rs. 33.25	Rs. 70.55
Year 2	Rs. 35.80	Rs. 33.25	Rs. 69.05
Year 3	Rs. 34.30	Rs. 33.25	Rs. 67.55
Year 4	Rs. 41.30	Rs. 33.25	Rs. 74.55

COST SAVED ON ACCOUNT OF DECREASE IN USE OF CHLORINE

According to Data, We save 6 kg chlorine / ton of paper	
Days saving on production of 50 ton / Day	300 kg of chlorine
Yearly Saving	109500 kg of chlorine
Cost of chlorine	Rs. 10 / kg
Savings	Rs. 10,95,000
Savings / Day / ton of pulp	Rs. 60 = US $ 1.25

COST OF PROCESS ON VARIABLE COST BASIS

Total variable expenditure (Yearly):	Rs. 33,25,714
Total variable expenditure (Daily):	Rs. 9,502
Expenditure per ton of pulp:	Rs. 190 = US $ 4

Raw Material as Basis for Variable costing

Variable expenditure (Yearly):	Rs. 3,78,420
Total variable expenditure (Daily):	Rs. 1,036
Expenditure per ton of pulp:	Rs. 21 = US $ 0.45

***Costing after savings from chlorine*:**

Variable expenditure (Yearly):	Rs. 22,30,714
Variable expenditure (Daily):	Rs. 6373
Expenditure per ton of pulp:	Rs. 125 = US $ 2.75

COST WORKING FOR STEAM

MIXER

- Steam requirement only for getting temperature to 50 degree Celsius

Steam required / Batch = 0.414 Tons

Rate / Ton of steam = Rs. 700

Cost of steam / Batch = Rs. 700 /Ton x 0.414 Tons = Rs. 290

Cost of steam / Day = Rs. 290 / Batch x 2 Reactors x 7 Batches / Day = Rs. 4060

Cost of steam / Year = Rs. 4060 / Day x 350 Days =**Rs. 14,20,020**

FERMENTER

- Steam requirement for getting temperature from 45 to 50 degree celsius

Steam required / Batch = 362.25 kg

Rate / Ton of steam = Rs. 700

Cost of steam / Batch = Rs. 700 /Ton x 0.36 Tons = Rs. 252

Cost of steam / Year = Rs. 252 / Day x 365 Days = **Rs. 91,980**

- Steam requirement for sterlization

Steam required / Batch = 1.258 ton

Cost of steam / Batch = Rs. 700 / ton x 1.258 ton = Rs. 880.6

Cost of steam / Year = Rs. 880.6 / Day x 365 Days = **Rs. 3,21,419**

PREFERMENTER

- Steam requirement for sterlization

Steam required / Batch = 0.25 ton

Cost of Steam / Batch = Rs. 700 / ton x 0.25 ton = Rs. 175

Cost of Steam / Year = Rs. 175 / Day x 365 Days =**Rs. 63,875**

ECONOMIC ANALYSIS

(Solid State Fermentation)

FIXED COST

Trays for fermentation	750 x Rs. 2,000	15,00,000
Accessories		15,00,000
Inoculation fermenter	2 x Rs. 10,00,000	20,00,000
Extraction Unit	1 x Rs. 10,00,000	10,00,000

Total Fixed Cost	**Rs. 60,00,000**

VARIABLE COST on *YEARLY BASIS*

Personnel Cost			**Rs.**
Inoculation + Main fermenter + Extraction unit			
	Skilled	1 x Rs. 1,50,000	1,50,000
	Semiskilled	4 x Rs. 1,00,000	4,00,000
Cost / Shift	(3 Shifts / Day of 8 hours each)		5,50,000
Cost / Year			16,50,000

Total Personnel Cost	**Rs. 16,50,000**

Raw material	**Amount (kg)**	**Price (Rs./kg)**	**Amount (Rs.)**
Inoculum requirements			
Yeast extract	140	400	56,000
KH_2PO_4	840	36	30,240
K_2HPO_4	560	25	14,000
$MgSO_4$	700	60	42,000
Urea	700	4	2,800
Sub total Cost for Inoculum			**1,45,040**
Tray fermentation requirement			
Surfactant	262.5	100	26,250
Urea	787.5	4	3,150
$MgSO_4$	437.5	60	26,250
Wheat Straw	26250	2.5	65,625
Sub total Cost for Tray fermentation			**1,21,275**

Total Raw Material Cost	**Rs. 2,66,315**

Steam Requirement	No Boiler Cost , Rate of Steam Production = Rs. 700 / Ton		
	Ton/ Day	Ton / Year	Rs./ Year
Requirement for temperature maintenance	5.8	2030	14,00,000
Sterlization of 120 litre Media and 75 kg Wheat straw per day	2	700	5,00,000

Total Steam Cost	**Rs. 19,00,000**

TOTAL VARIABLE COST	**Rs. 38,16,315**

YEARLY PAYMENT FOR THE FIXED COST

- No inflation is taken into account.
- We get a tax-advantage of 35 % on interest and thus effective rate of interest = 0.65 x 15 % = 10 % (approximately). This is applicable if the total project is funded by long-term loans. However, this is never true and there is balance of equity, preference share, long-term loans and debentures to fund a project. The cost of capital is normally 15 % but largely depends upon the ratios of funding sources.
- Assuming 15 % capital cost, following would be the yearly payment structure

Year	Installment Paid (Lakhs)	Capital for interest payment (Lakhs)	Interest (Lakhs @ 15 %)	Total (Lakhs)
Year 1	Rs. 5.00	Rs. 55.0	Rs. 8.25	Rs. 13.25
Year 2	Rs. 5.00	Rs. 50.0	Rs. 7.50	Rs. 12.50
Year 3	Rs. 5.00	Rs. 45.0	Rs. 6.75	Rs. 11.75
Year 4	Rs. 15.00	Rs. 30.0	Rs. 4.50	Rs. 19.50
Year 5	Rs. 15.00	Rs. 15.0	Rs. 2.25	Rs. 17.25
Year 6	Rs. 15.00	Rs. 00.0	Rs. 0.00	Rs. 15.00
Sub Total	**Rs. 60.00**	**Rs. 29.25**		
Grand Total				**Rs. 89.25**

COST TO COMPANY FOR FIRST THREE YEARS

Year	Total Fixed Cost (Lakhs)	Total Variable Cost (Lakhs)	Total Cost (Lakhs)
Year 1	Rs. 13.25	Rs. 38.16	Rs. 51.41
Year 2	Rs. 12.50	Rs. 38.16	Rs. 50.66
Year 3	Rs. 11.75	Rs. 38.16	Rs. 49.91

COST SAVED ON ACCOUNT OF DECREASE IN USE OF CHLORINE

According to Data, We save kg chlorine / ton of paper	
Days saving on production of 50 ton / Day	kg of chlorine
Yearly Saving	kg of chlorine
Cost of chlorine	Rs. 10 / kg
Savings	Rs.
Savings / Day / ton of pulp	Rs. = US $

COST OF PROCESS ON VARIABLE COST BASIS

Total variable expenditure (Yearly):	Rs. 38,16,315
Total variable expenditure (Daily):	Rs. 10,903
Expenditure per ton of pulp:	Rs. 218 = US $ 4.6

Raw Material as Basis for Variable costing

Variable expenditure (Yearly):	Rs. 2,66,315
Total variable expenditure (Daily):	Rs. 761
Expenditure per ton of pulp:	Rs. 15 = US $ 0.3

SALIENT FEATURES OF DESIGN:

- Complete design parameter of the vessels is specified.
- Working Capital Cost is taken in to account.
- Personnel Cost, Steam Cost has been included for better Variable Cost Analysis.
- The figures for the capital cost of the vessel manufacture and material are latest and are taken from the net.
- Total estimated cost is taken as 85% of the actual cost which is good estimate.

SHORTCOMINGS OF DESIGN:

- Power requirement arising from driving the motor for the agitator is not there.
- Land cost, if additional in nature has not been accounted for.
- Boiler, if additional required is not accounted for.
- Steam requirement for Sterilization is not accounted for the main fermenter and pre-fermenter. Also, Heat required to maintain the temperature of the Mixer, fermenter, and Pre-fermenter is not taken.
- Cost like charges for Electricity, and Water are not taken into account which may contribute substantially for the Variable Cost Analysis.
- Cost arising for making the fermenter, prefermenter, and mixer automated in nature are not taken into account.
- Air requirement for Sparging is not taken into account, as the shake flask studies have not been scaled to Pilot Plant Scale.

9. CONCLUSION

1. Xylanases produced from *Melanocarpus albomyces* were found to be satisfactory in terms of activity and its cellulase freeness, which was indicated by determination of CMC and filter paper activity as it was found to be negligible as compared to xylanase activity.

2. Studies on evaluation of xylanase as pre-bleaching agent on wood Kraft pulp showed that the enzyme was effective in reducing the chlorine demand during CEH bleaching of enzyme treated Kraft pulp to the tune of more than 15%. There was also a gain in pulp brightness by 2-3 points. Further, the toxicity of the bleach effluents (CE stage) could be reduced by nearly 20% in terms of AOX.

3. Effectiveness of the xylanases from *Melanocarpous albomyces*, on Kraft bagasse pulp was found to be satisfactory in terms of reducing the demand of elemental Cl_2 by 15% during conventional CEH bleaching sequence with gain in pulp brightness of 2.0 points. Toxicity (AOX) of the bleach effluent was also reduced by 25%.

4. Further, the comparative studies on enzymatic pre-bleaching of wood Kraft pulp using xylanases from *Melanocarpus albomyces* with commercially available xylanases having similar pH optimum indicated compatibility of both the enzymes towards pre-bleaching of Kraft hard wood pulp.

5. The proposed diagrammatic flow sheet for implementation of enzymatic pretreatment in a mill is shown in Fig. 13.

6. The economic analysis of the process is presented in detail for enzyme produced by submerged and solid-state cultivation methods.

ACKNOWLEDGEMENTS

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Saroj Mishra

(Saroj Mishra)
Principal Investigator
and Project In-charge

Table-1. Details of Pulp Samples Procured From Indian Paper Mills Using Various Raw Materials

Name of the Mill	Raw material	Nature of Pulp	Kappa No. Of Pulp
M/s Star paper mill, Saharanpur	Eucalyptus & Bamboo	Kraft	Sample(A)-18.0 Sample(B)-18.5
M/s Seshasayee paper mills, Erode. M/s Sri Vindhya paper mills, Jalagan,Maharastrha.	Bagasse	Kraft	Bagasse Sample(c) – 8.0 Sample(D) – 27

Table 2: Composition of different agricultural residues

Residue	Composition, % dry weight			
	Cellulose	Hemicellulose	Lignin	Ash
Wheat straw	30	24	18	10
Wheat bran	30	27.2	20.8	7.5
Rice straw	32	24	13	12
Rice husk	36	15	19	20
Bagasse	33	30	29	4
Bark pith	Not available			

Table 3a

Table- . Conditions for Enzyme Pretreatment of Pulp with Xylanase from *Melanocarpus albomyces*

Parameters	Values
Dose of Enzyme, IU/gm Pulp	10.0
Consistency of Pulp, %	8.0 – 10.0
Retention Time, min	120
pH at 25^{0}C	6.2
Temperature, ^{0}C	50-60

Table 3b

Table- . Process Conditions Used During Bleaching of Pulps

Particulars	Chlorination stage	Alkali Extraction Stage	Hypo stage
Temperature, ^{0}C	Ambient	60	40
Pulp Consistency, %	3.0	8.0	8.0
Retention Time, min	30	60	120
Final pH	1.8-2.0	>10.5	>9.0

TABLE - 4

Table-3: Xylanase and Cellulase Activities on Wheat Straw

Particulars	Xylanase Activity(IU/ml)	Endoglucanase Activity(IU/ml)	Filter Paper Activity(IU/ml)
Wheat Straw<500 μ partical size(144 hr)	108.0	1.77	0.92

TABLE-5: EFFECT OF DIFFERENT CARBON SOURCES ON XYLANASE PROUDCTION

Carbon Source	Xylanase activity (IU/ml)
Wheat Straw (1%) (mesh size < S.O.u)	110.0 (144 h)
Bark Pith (1%)	76.0 (144 h)
Bark Pith (2%)	95.0 (168 h)
Bark Pith (3%)	134.0 (192 h)
Wheat bran (boiled) 1%	44.0 (72 h)
Wheat bran (boiled) 2%	62.0 (72h)
Wheat bran (boiled) 3%	134.0 (96 h)
Wheat bran (unboiled)	30.0 (72 h)

TABLE-6: EFFECT OF DIFFERENT NITROGEN SOURCES ON XYLANSE ACTIVITY WITH 3% WHEAT BRAN (BOILED)

Nitrogen Source	Xylanase activity (IU/ml)
Urea (0.05%) + YE (0.01%)	134.0*
Tryptone (1%)	78.0
Corn Steap Liquor (1%)	100.0
Soyabean meal (1%)	76.0
Yeast Extract (1%)	68.0

*Productivity (U/l/h) = 1400 U/l/h

TABLE 7.

Soln	Factor A Media C ml/gm	Factor B Surfactant %	Factor C Urea %	Factor D Moisture ml/gm	Factor E Inoculum Age hrs.	Factor F Harvesting Age hrs.	Response Xylanase IU/gm
1	2.40	1.90	1.51	4.66	43.60	84.00	8456.54
2	2.40	1.90	1.68	5.48	44.44	84.00	8363.30
3	2.40	1.90	1.50	5.01	45.26	86.33	8306.11
4	2.40	1.81	2.20	4.81	40.75	84.00	8164.76
5	1.38	0.12	3.57	6.90	43.02	108.00	7848.20
6	1.34	0.10	3.70	7.00	42.24	108.00	7845.11
7	2.40	1.90	2.50	3.91	40.32	84.00	7834.82
8	2.40	1.38	1.50	5.66	45.02	91.00	7831.02
9	1.20	0.22	3.71	6.61	42.38	108.00	7763.96
10	1.91	0.56	2.74	6.83	45.37	108.00	7643.30

TABLES 8a and 8b

Table- . Results of Enzymatic Pretreatment of Wood Pulp (Eucalyptus + Bamboo) using Xylanases from *Melanocarpus albomyces*

Table-8a. Characterization of Unbleached Pulp, Before & After Enzyme Treatment

Particulars	Control Pulp	Enzyme Treated Pulp
Kappa No. of Pulp	18.0	16.5
Brightness, % ISO	24.0	25.0
CED Viscosity, cm^3/g	690	740

Table-8b. Analysis of Pulp Filtrates Before & After Enzyme Treatment

Particulars	Control Pulp	Enzyme Treated Pulp
$Lignin_{280}$ Kg/tp	0.39	1.03
Reducing sugars as xylose, Kg/tp	1.21	4.08
$Colour_{465}$ PCU Kg/tp	0.93	2.72

Tables 9a and 9b

Table- Enzymatic prebleaching of Wood Pulp(Eucalyptus + Bamboo) using xylanases from *Melanocarpus albomyces*

Table-9a Bleaching of Pulp using Conventional CEH Sequence Before & After Enzyme Treatment

Particulars	Control Pulp	Enzyme Treated Pulp
Chlorination Stage		
%, Chlorine Applied	4.1	3.5
%, Chlorine Consumed	98	91
Saving in Chlorine, %	---	15
Alkali Extraction		
NaOH, %	1.5	1.5
%, NaOH Consumed	68.4	60.1
Final pH	11.0	11.2
Kappa No.	4.6	3.3
Hypo Stage		
%, Applied	2.0	2.0
%, Consumed	98	93
Final Brightness of the pulp, % ISO	80	83

Table-9b Strength & Optical Properties of Wood Pulp (Eucalyptus + Bamboo) Before & After Enzyme Treatment

Particulars	Control Pulp	Enzyme Treated Pulp
Strength properties		
Revolution, PFI	3000	3000
Freeness, CSF	295	270
Apparent density, g/m^3	0.78	0.81
Burst Index, $Kpa.m^2/g$	4.40	4.20
Tensile Index, Nm/g	64.0	62.0
Tear Index Mnm^2/g	6.04	5.80
Optical Properties		
Brightness, % ISO	69.3	72.6
Opacity, %	82.1	79.0

Table 10.

Table-6. Characterisation of Bleach Effluent (C+E Stage) Before & After Enzyme Treatment of Pulp from Wood based Mills

Particulars	Control Pulp	Enzyme Treated pulp
COD , Kg./tp	32.0	27.0
AOX, Kg./tp	2.12	1.8
AOX Reduction, %	-----	20.0

Table II.

Table- Comparative Studies on Enzymatic Prebleaching of Wood Pulp (Eucalyptus + Bamboo) using Xylanases from *Melanocarpus albomyces* with that of a Commercial Xylanase Enzyme

Parameters	Xylanase Enzyme from *Melanocarpus albomyces*		Xylanase Enzyme from M/s Biocon India Ltd.	
	Control Pulp	Enzyme Treated Pulp	Control Pulp	Enzyme Treated Pulp
Enzyme Dose	-----	10 IU/g	-----	0.05%
Consistency, %	8.0	8.0	8.0	8.0
Time, min.	120	120	120	120
Temp. ^{0}C	55-60	55-60	55-60	55-60
PH	6.2	6.2	6.2	6.2
Brightness of unbleached Pulp	24.0	25.0	24.0	25.0
Chlorination Stage				
5, Chlorine Applied	4.1	3.5	4.4	3.7
%, Chlorine Consumed	98	91	92	93
Savings in Chlorine, %	-----	15	-----	16
Alkali Extraction Stage				
NaOH, %	1.5	1.5	1.5	1.5
Final pH	11.0	11.2	11.0	10.9
Hypo stage				
%, Applied	2.0	2.0	2.5	1.8
%, Consumed	98.0	93.0	-----	-----
Final Brightness of Pulp, % ISO	80.0	83.0	77.5	80.0

Table 12

Table- Strength & Optical Properties of Wood Pulp (Eucalyptus + Bamboo) Before & After Enzyme Treatment using Xylanases from *Melanocarpus albomyces* with that of a Commercial Enzyme

Parameters	Enzyme from *Melanocarpus albomyces*		Enzyme from M/s Biocon India Ltd.	
	Control Pulp	Enzyme Treated Pulp	Control Pulp	Enzyme Treated Pulp
Strength Properties				
Revolution, PFI	3000	3000	3000	3000
Freeness, CSF	295	270	320	345
Apparent Density, gm/cm^2	0.78	0.81	0.79	0.76
Burst Index Kpa m^2/g	4.4	4.2	4.6	4.3
Tensile Index Nm/g	64.0	62.0	64.8	63.4
Tear Index Mnm^2/g	6.04	5.8	5.6	5.7
Optical Properties				
Brightness of Pulp, % ISO	77.0	80.0	77.5	80.0
Opacity, %	82.1	79.0	82.4	79.5

Tables 13a and 13b

Table- .Results of enzymatic pre-treatment of Kraft bagasse pulp using xylanase from *Melanocarpous albomyces*		
Enzyme pre-treatment conditions		
Particulars	**Control pulp**	**Enzyme treated pulp**
Enzyme dose , IU/g	0	10
Pulp consistency, %	10	10
Temperature°C	50	50
Treatment time, (hrs)	2	2
PH	6.0-6.5	6.0-6.5
Table 13a Characterization of unbleached pulp before & after enzyme treatment		
Particulars	**Control pulp**	**Enzyme treated pulp**
Kappa no. of pulp	7.7	7.0
Brightness,%ISo	55.5	57.3
Table 13b Analysis of pulp filtrates before & after enzyme treatment		
Particulars	**Control pulp**	**Enzyme treated pulp**
$Lignin_{280}$ Kg/tp	0.08	0.44
$Colour_{465}$ Kg/tp	0.33	1.80
Reducing sugars (as Xylose),Kg/tp	0.0	1.99

Table 14.

Enzymatic Prebleaching of Kraft bagasse Pulp using Xylanase

Table-14 Bleaching of Pulp (Sample-C) using Conventional CEH Sequence Before & After Enzyme Treatment

Particulars	Control	Enzyme Treated
Chlorination		
% Chlorine Applied	1.40	1.40
%, Chlorine Consumed	90.0	80.0
Alkali Extraction		
% Alkali Applied	1.50	1.50
% Alkali Consumed	26.0	27.0
Final pH	12.10	12.05
CE Brightness %	60.0	67.9
Hypo stage		
% Hypo Applied	0.50	0.50
% Hypo Consumed	98.0	86.0
Brightness, % ISO	84.5	86.6
Brightness Gained, %	----	2.1

Tables 15a and 15b

Table Results of enzymatic pre-treatment of Bagasse kraft pulp (Sample-D) using xylanase

Enzyme pre-treatment conditions		
Particulars	**Control pulp**	**Enzyme treated pulp**
Enzyme dose , IU/g	0	10
Pulp consistency, %	10	10
Temperature°C	50	50
Treatment time, (hrs)	2.0	2.0
PH	6.0	6.0
15a. Characterization of unbleached pulp before & after enzyme treatment		
Particulars	**Control pulp**	**Enzyme treated pulp**
Kappa no. of pulp	26.25	25.85
Brightness, % ISO	30.0	30.0
CED viscosity , cm^3/g	535	520
15b. Analysis of pulp filtrates before & after enzyme treatment		
Particulars	**Control pulp**	**Enzyme treated pulp**
$Lignin_{280}$ Kg/tp	0.15	0.64
$Colour_{465}$ Kg/tp	1.02	4.01
Reducing sugars (as Xylose),Kg/tp	0.03	6.80

1 %

1 %

Table 16

Enzymatic Prebleaching of Kraft Bagasse Pulp using Xylanase

Table-16 Bleaching of Pulp using Conventional CEH Sequence Before & After Enzyme Treatment

Particulars	Control	Enzyme Treated	
Chlorination			
% Chlorine Applied	5.6	5.6	4.8
%, Chlorine Consumed	88	76	90
% , chlorine savings	--	--	(15)
Alkali Extraction			
% NaOH Applied	2.0	2.0	2.0
% NaOH Consumed	74.2	61.2	73.8
Final pH	10.24	11.55	10.25
Hypo stage			
% Hypo Applied	2.0	2.0	2.0
% Hypo Consumed	80.0	80.0	74.0
Brightness% ISO	83.92	85.09	84.34

Table 17

Table Strength & Optical properties of enzyme treated & untreated Kraft bagasse pulps

Particulars	Control Pulp	Enzyme treated Pulp
Revolution, PFI	500	500
Freeness, CSF	335	355
Apparent density, gm/m^2	0.66	0.72
Burst index, Kpa m^2/g	2.60	2.55
Tensile index, Nm/g	45.5	43.0
Tear index, MNm^2/g	4.05	4.20
Fold Kohler Molin (log)	1.23	1.07

Table 18

Table Characteristics of bleach effluent (CEH stage) of enzyme pretreated & untreated Bagasse kraft pulp.		
Particulars	**Untreated pulp effluent**	**Enzyme treated pulp effluent**
Colour, Kg/tp	30.18	17.07
Lignin, Kg/tp	0.008	0.006
COD , Kg/tp	34.6	50.7
BOD , Kg/tp	8.5	15.0
AOX, Kg/tp	4.13	3.13

Fig. 2 Scheme of solid-state cultivation

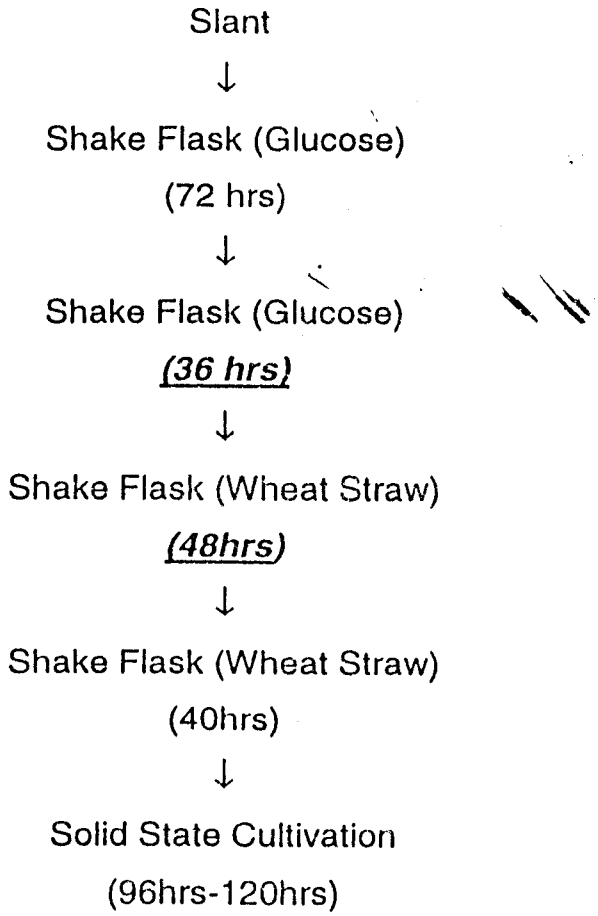


Fig.3 Xylanase Activity on Wheat Straw of different mesh sizes

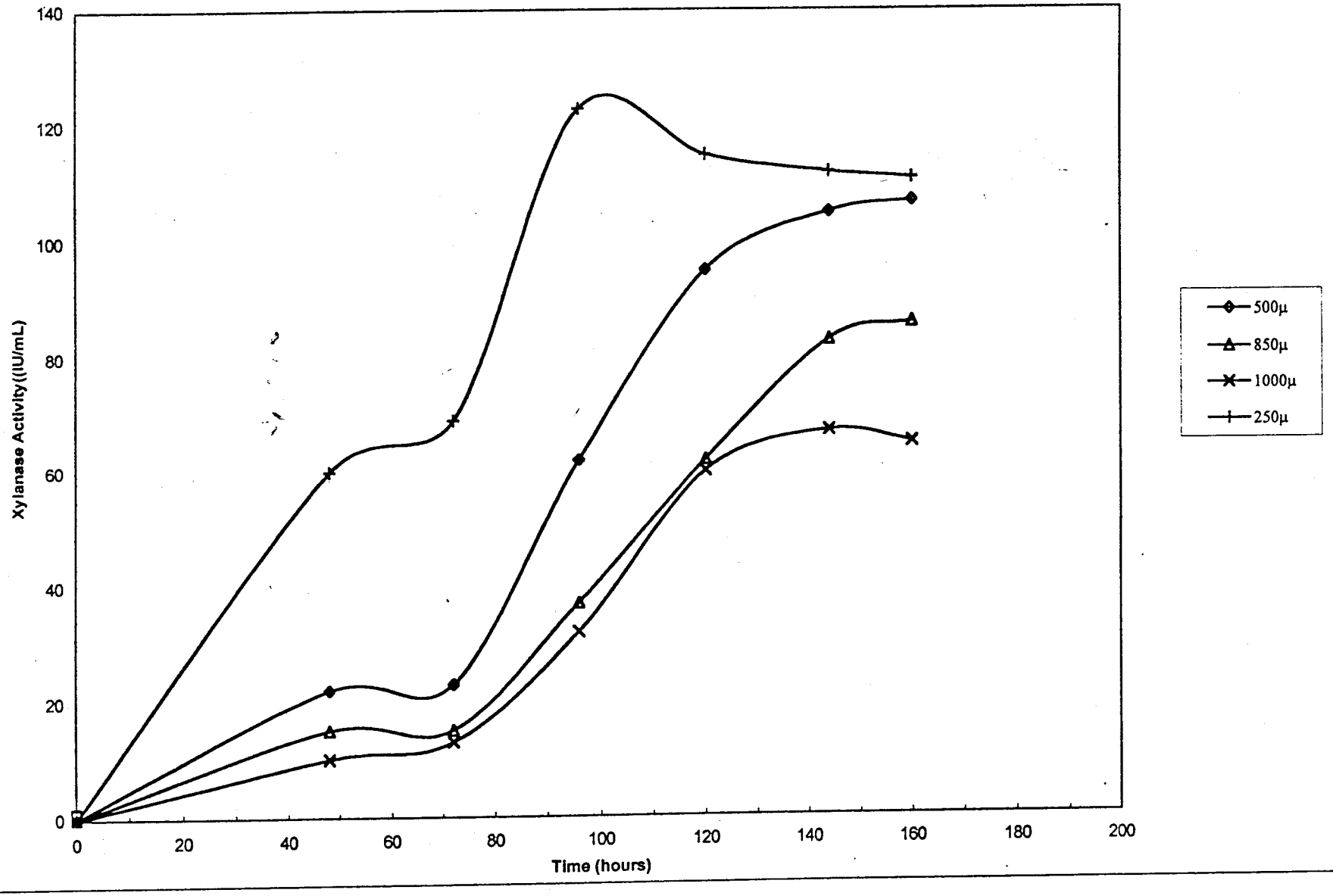


Fig. 4 **Effect of urea composition on Xylanase Activity**

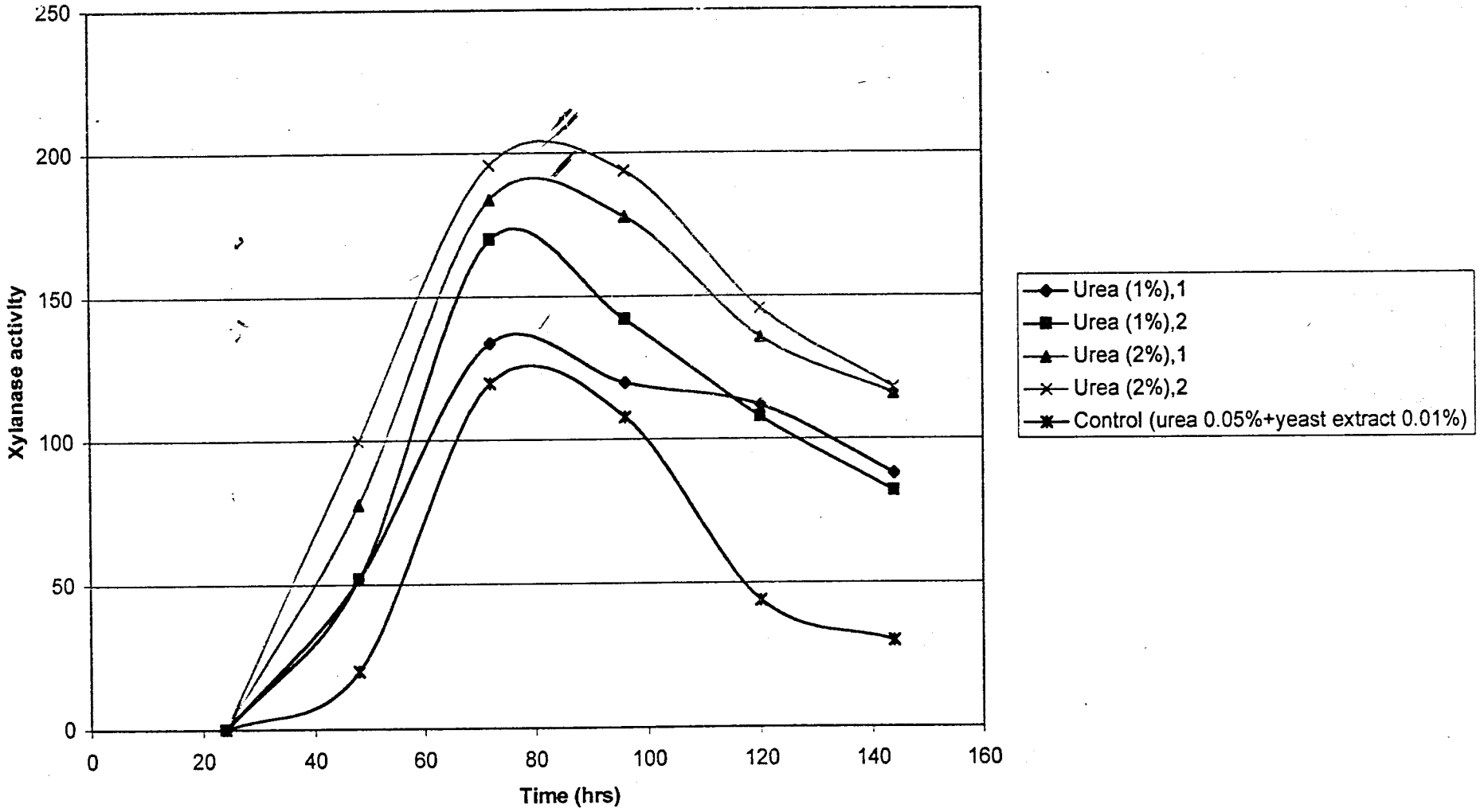


Fig. 6 Xylanase Production w.r.t Harvesting Time

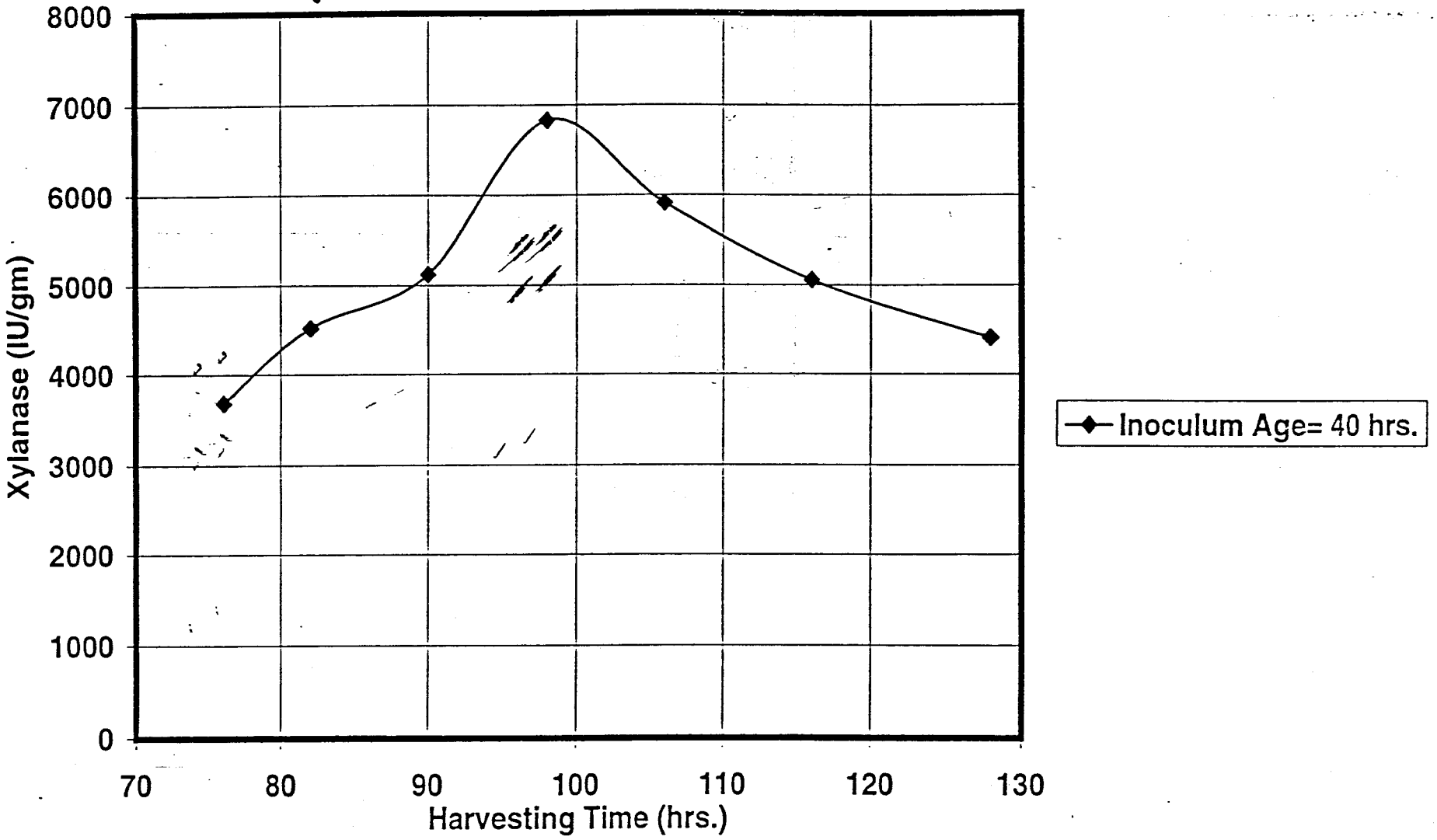


Fig. 5

Fig. 7. Effect of pH on Xylanase, Endoglucanase and Filter Paper Activity

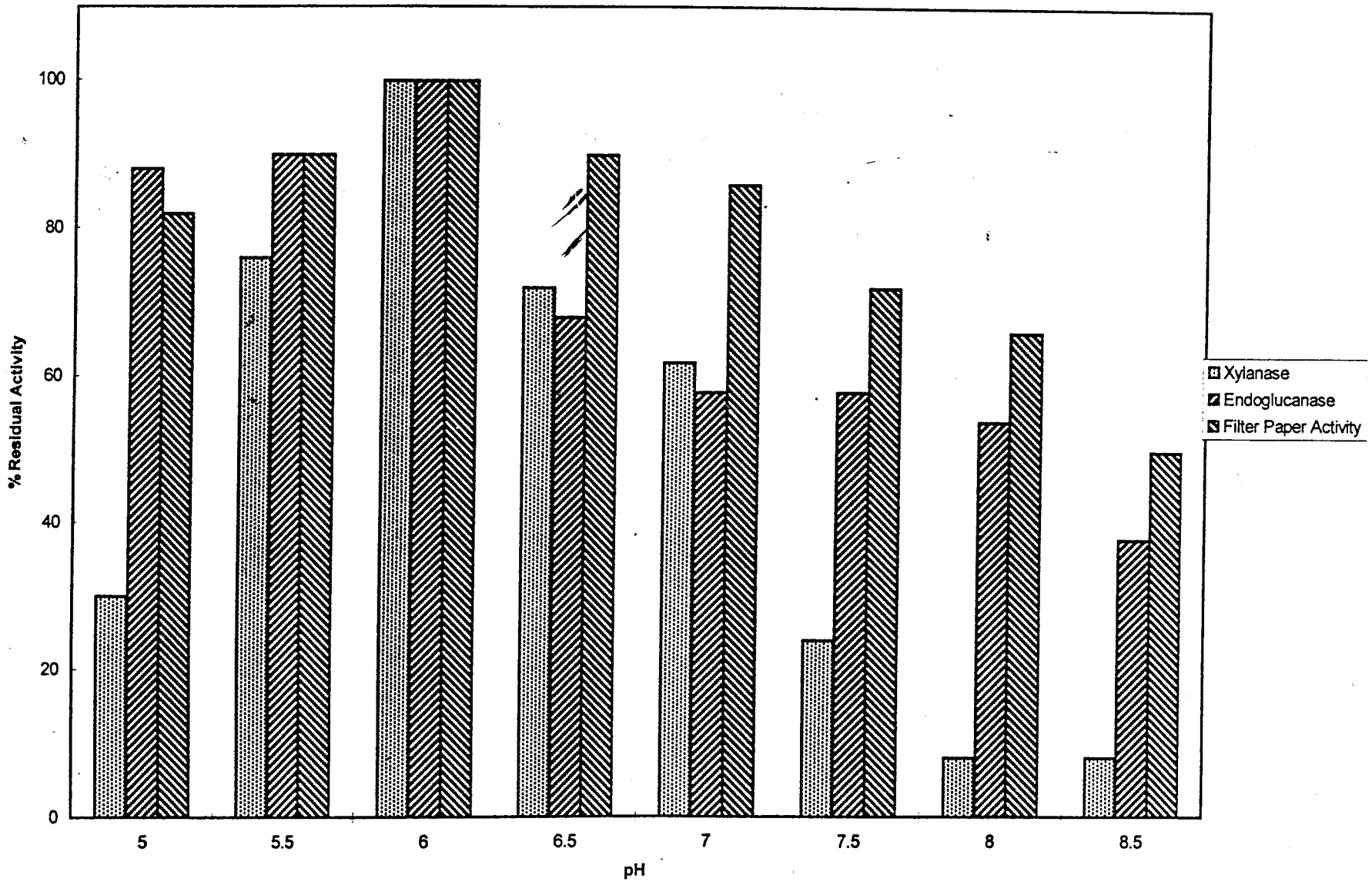


Fig.8. **Effect of Temperature on Xylanase, Endoglucanase and Filter Paper Activity**

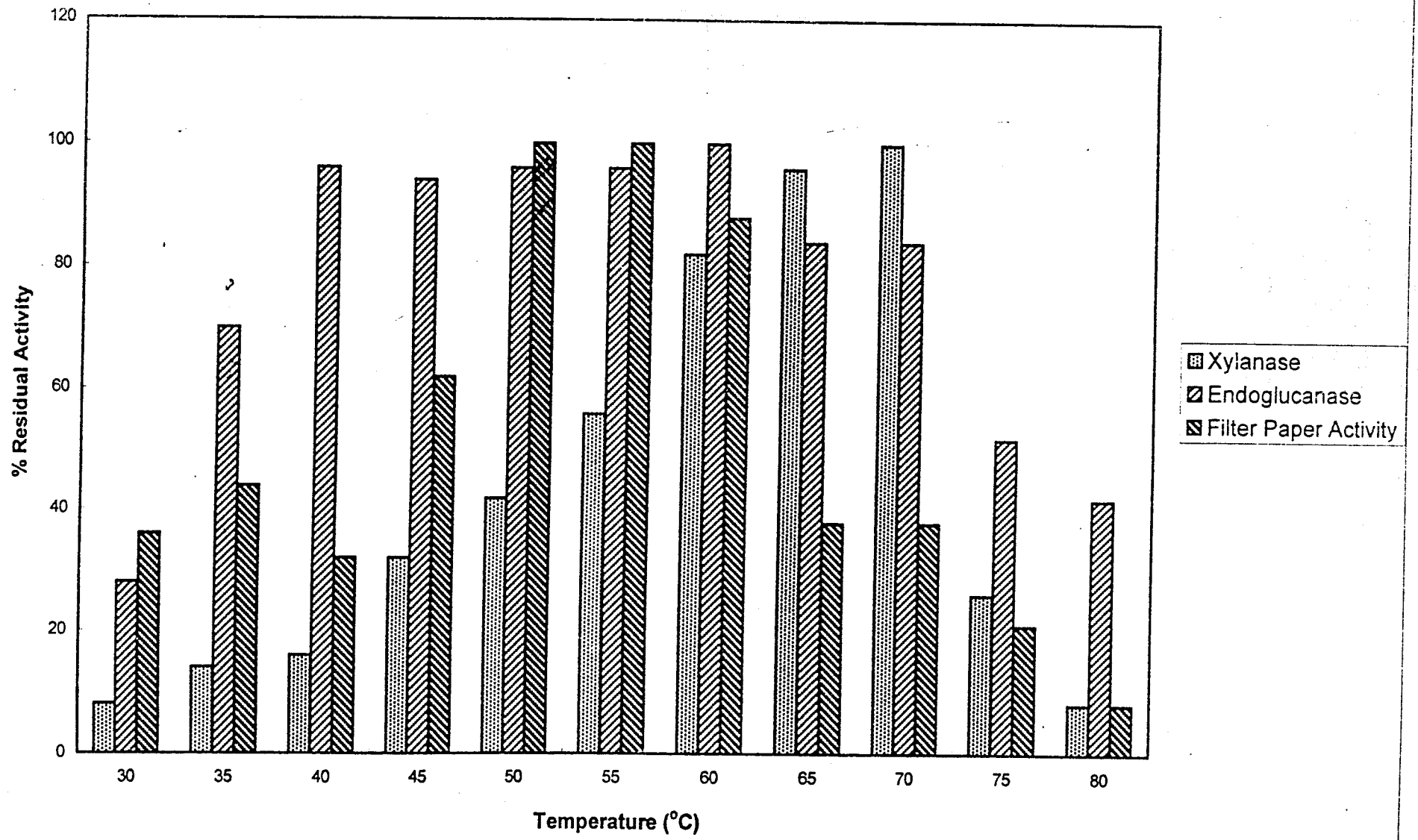
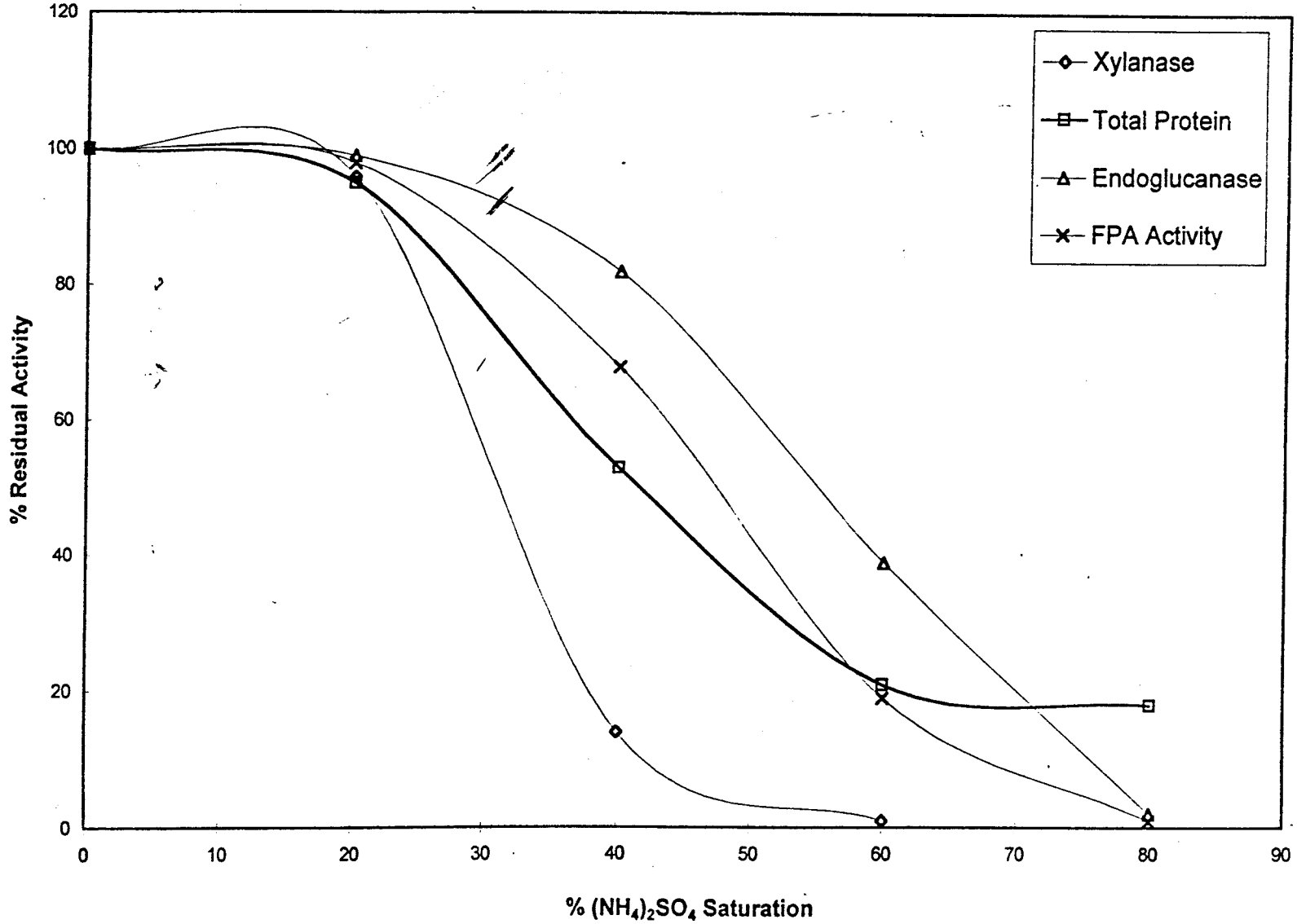


Fig. 9. Enzyme Activities in the Supernatant at different % saturation of $(NH_4)_2SO_4$



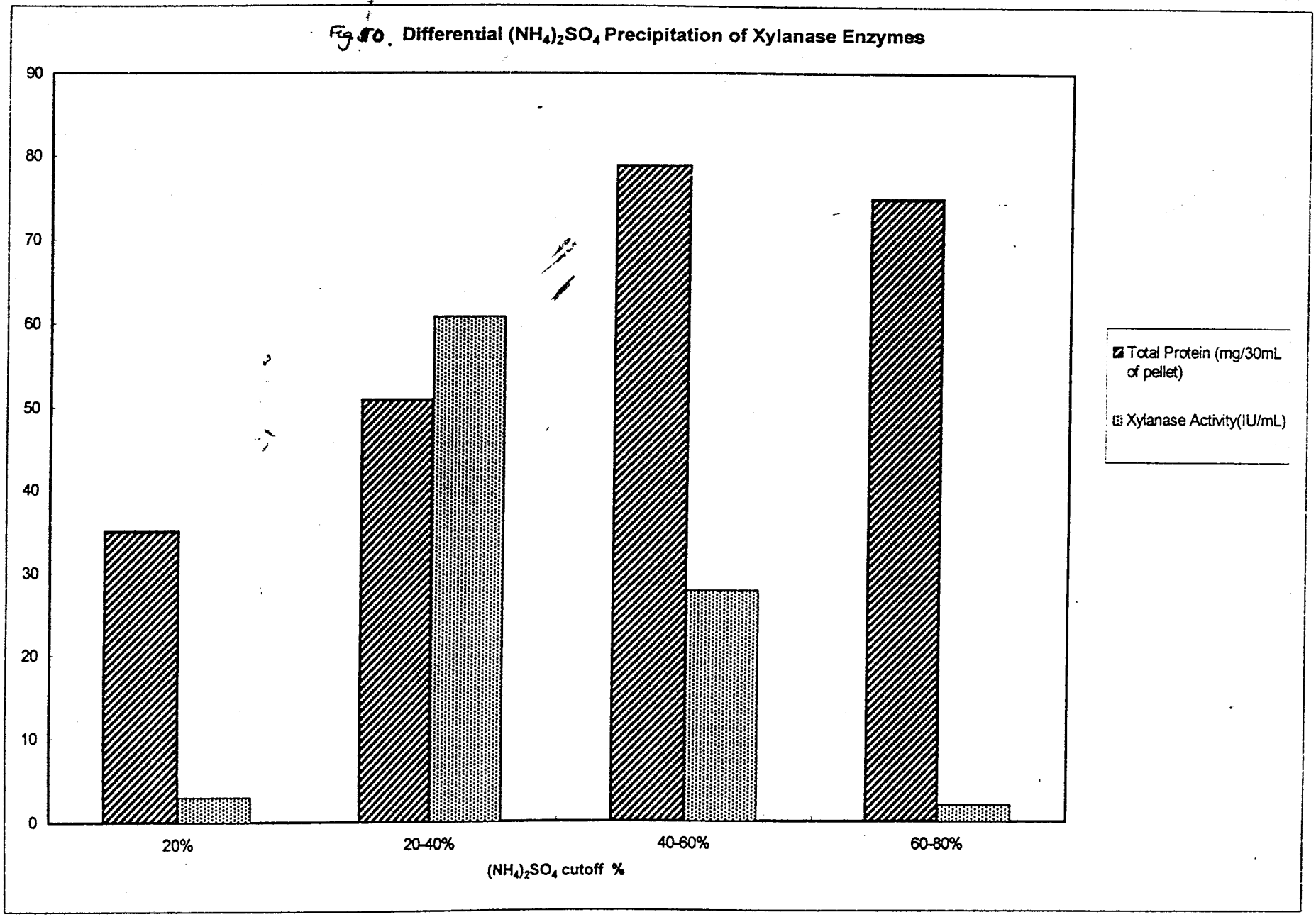


Fig 10. Differential $(NH_4)_2SO_4$ Precipitation of Xylanase Enzymes
90
80
70
60
50
40
30
20
10
0
20%
20-40%
40-60%
60-80%
$(NH_4)_2SO_4$ cutoff %
Total Protein (mg/30mL of pellet)
Xylanase Activity(IU/mL)

Fig. 4. Thermal stability of Xylanases at different temperatures

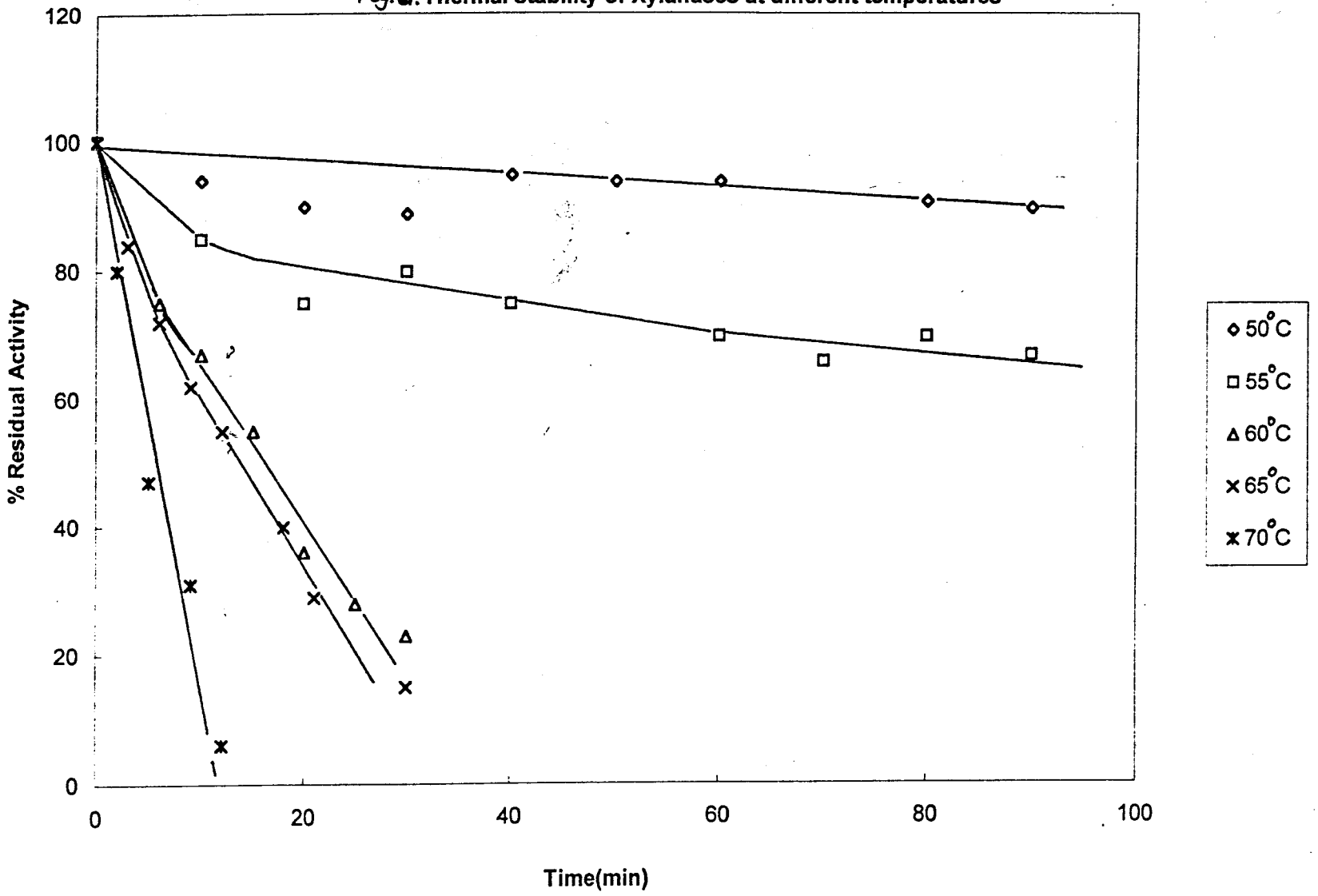
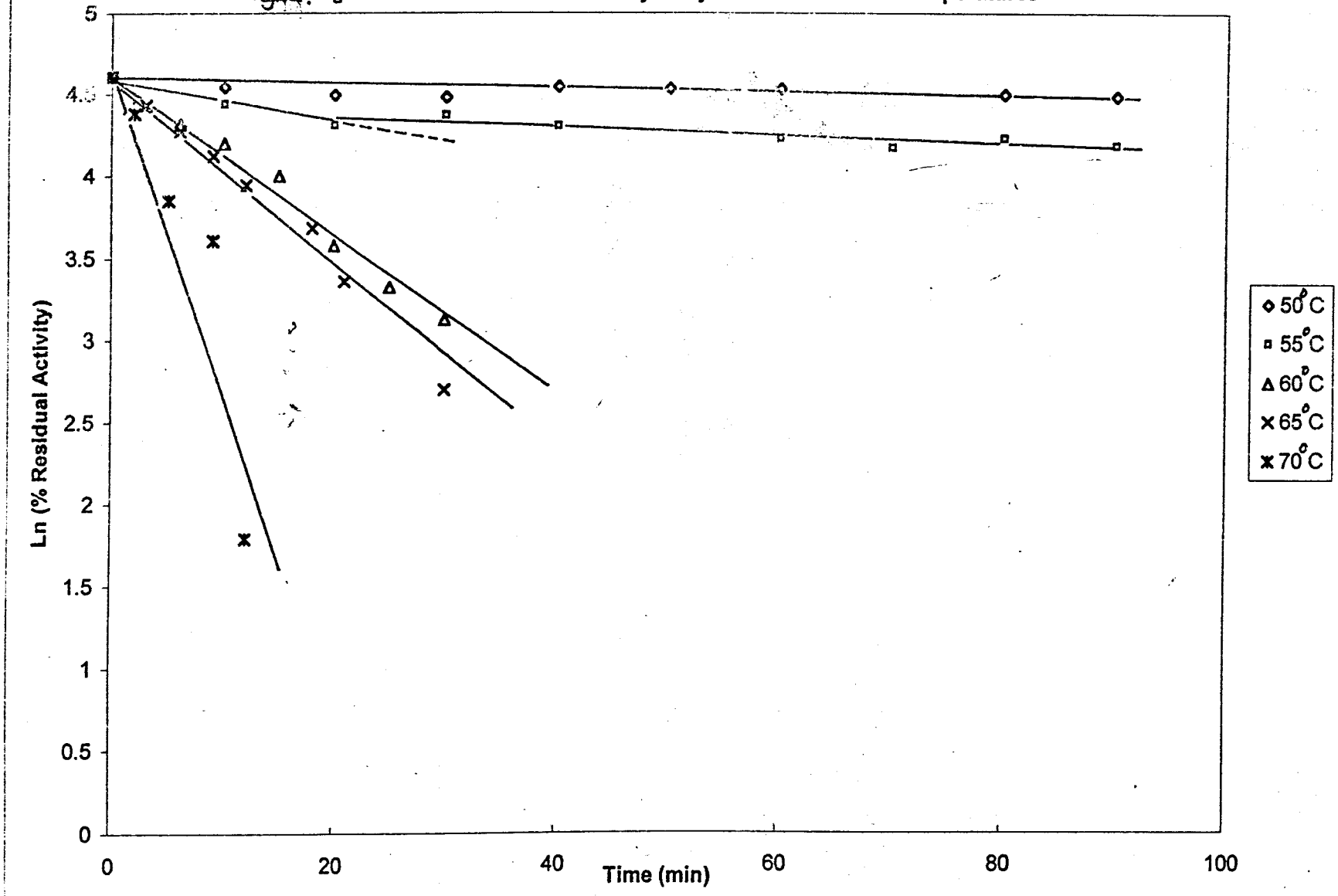


Fig 4.2 Logarithm of the Thermal Stability of Xylanase at Different Temperatures



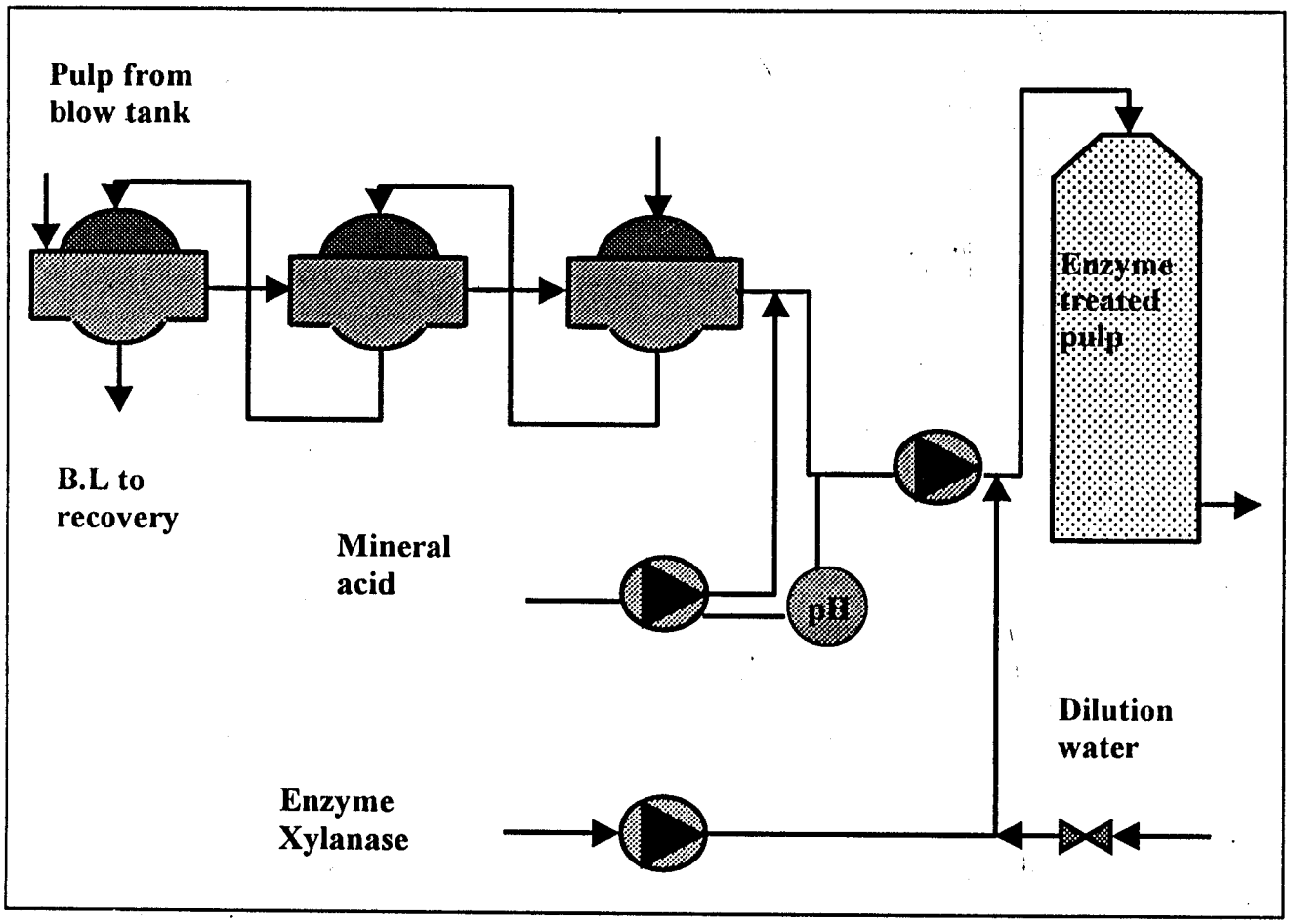


FIG.13 PROPOSED PLAN FOR ENZYMATIC PREBLEACHING OF PULP IN A MILL