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NOTICE OF ENTITLEMENT (MICROORGANISM DEPOSITS)

We, GIST-BROCADES B.V., Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft, The Netherlands state the following in connection with Australian Patent No. 28824/95:

1. The person nominated for the grant of the patent is the depositor of the deposits listed hereafter:

2. The deposits referred to above were deposited with Centraalbureau voor Schimmelcultures on 14 April 1994, and accorded the numbers CBS 211.94, CBS 212.94, CBS 213.94, CBS 214.94, CBS 215.94 and CBS 216.94.

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(57) Claim

1. A xylanase wherein it has a significant delignifying activity at a temperature of at least 80°C and at pH 9 and comprises an amino acid sequence that shares at least 70% identity with the amino acid sequence of SEQ ID NO. 13.

12. An isolated microorganism characterized as an anaerobic and thermophilic bacterium and having deposition number CBS 211.94, 212.94, 213.94, 214.94, 215.94 and 216.94.

16. A process for delignifying wood pulp, the process comprising contacting the wood pulp with a xylanase according to any of claims 1 to 7 at a temperature of at least 80°C, under conditions that allow xylanase activity.

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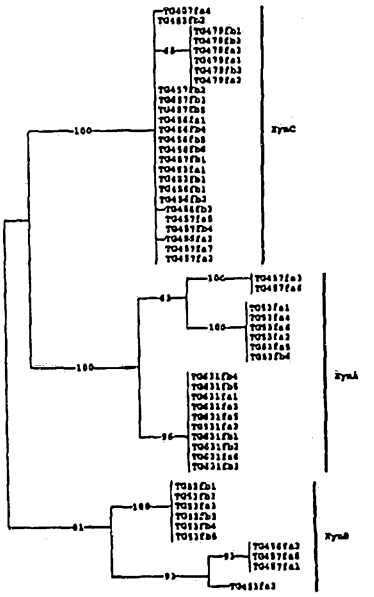
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(57) Abstract

The present invention discloses enzymes having xylanase activity. The xylanases are characterized in that they are active at a temperature of 80 °C or higher. The enzymes are obtainable from anaerobic thermophilic bacteria. The enzymes are suited for use in paper and pulp production processes. The invention also describes cloning and expression of genes having xylanase activity obtained from the deposited strains.

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Thermostable xylanases

Technical field

The present invention relates to novel microorganisms and to novel enzymes. More specifically, the enzymes are thermostable xylanases. These xylanases are obtainable from anaerobic thermophilic bacteria. The xylanases are applicable under conditions used in the paper and pulp industry i.e. a pH of above 9 and a temperature of above 70°C, specifically the enzymes are active at T = 80°C.

Background of the invention

Xylan is a component of plant hemicellulose. Xylan consists of a backbone of 1,4-glycosidically linked β-D-xylose. Usually xylans have side chains or groups comprising xylose and other pentoses, hexoses, uronic acids and acetyl groups.

In the paper production process the bleaching of pulp is an important step. Schematically, the process used for pulp treatment in the paper and pulp industry is performed as follows;

Pulp is treated at pH 10-12 at 80°C to remove most of the lignin in the so-called oxygen delignification step. The remaining pulp contains 2-5% of lignin. This lignin gives the pulp its brown color. Subsequently, the pulp is bleached in a multistage bleaching process. In this bleaching process chemicals such as chlorine, chlorine dioxide, hydrogen peroxide and/or ozone are used, to obtain a bright pulp for high quality paper.

Chlorine and chlorine-containing chemicals are often used in the bleaching process. However, since the use of these chemicals leads to the formation of dioxin and other chlorinated organic compounds, they form a threat to the environment. Therefore, there is a growing tendency to omit the use of chemicals giving rise to this kind of waste products.

This has prompted a tendency to develop chlorine-free processes; total chlorine-free (TCF) and elementary chlorine-free (ECF). In these processes hydrogen peroxide or ozone is used for bleaching. However, the

use of these oxidative chemicals may have a negative effect on the quality of the paper, especially the strength of the paper.

It has been found that certain enzymes make the pulp more accessible to bleaching agents, thereby reducing the amount of bleach. Xylanases, in particular, have been found to be very useful in the paper and pulp processing. Xylanases have been reported to increase the extractability of lignins from the pulp. In current processes, xylanases are mostly used after the oxygen delignification step, because they are not active and do not survive under conditions used during oxygen delignification.

Xylanases cleave the hemicellulose chains which are responsible for the close adherence of lignin to the cellulose network. After xylanase treatment the lignin is more easily removed in the subsequent steps.

Therefore the use of xylanases leads to a reduction of the consumption of active chlorine in prebleaching of 25-30%. This reduction of chlorine does not afflict the quality parameters of the resulting paper (Viikari et al. 1986. Proc. of the third Int. Conf. Biotechnology in Pulp and Paper Ind., Stockholm, p.67-69 and Bajpai and Bajpai. 1992. Process Biochemistry. 27 : 319-325).

The xylanase treatment also reduces the need for other chemicals, such as hydrogen peroxide, in the bleaching process.

The use of xylanases from fungal sources in bleaching of kraft pulp has been reported. These are acidic xylanases and the pH and temperature optima of the enzymes are : pH = 3-5 and T = 30-50°C. These values are not ideal for the use in the bleaching process, where the prevailing conditions are pH $\geq$ 9 and temperature $\geq$ 70°C.

Xylanases from bacterial origin, with higher pH and/or temperature optima, have been reported for use in the bleaching process. Some of these originate from the following species (pH and temperature optima of the reported xylanase activity between brackets):

Bacillus pumilus (pH = 7-9, T = 40°C, Nissen et al. 1992. Progress in Biotechnology 7 : 325-337), Bacillus stearothermophilus (pH = 7, T = 65°C, International patent application WO 91/10724), Dictyoglomus thermophilum (pH = 6-8, T = 70°C, European patent application EP 0 511 933), Rhodothermus (pH = 5.5-6.5, T = 80-100°C, European patent application EP 0 538 177), Thermotoga (pH = 5-6, T = 90°C, International patent application WO 93/19171) and Thermoanaerobacter ethanolicus (T = 68°C, Deblois and Wiegel. 1992. Progress in Biotechnology 7 : 487-490).

Although the use of some of these xylanases for bleaching has been claimed, no xylanases have been reported to date having the desired characteristics of significant delignification activity at temperature ≥ 80°C.

Summary of the invention

The present invention discloses xylanases obtainable from anaerobic thermophilic microorganisms which have been isolated in pure culture from hot springs in New Zealand. The microorganisms have been deposited at the Centraal Bureau voor Schimmelcultures (CBS), Baarn, the Netherlands, on April 14, 1994.

The present invention also discloses enzymes having substantial xylanase activity at a temperature of at least 80°C. Furthermore, the present invention discloses that said xylanases possess significant delignification activity at a temperature of at least 80°C. Said enzymes are obtainable from the deposited strains.

The present invention further describes a process for the preparation of the xylanases which are active at a temperature of 80°C. The process comprises cultivation of the deposited microorganisms of the present invention in a suitable medium, followed by recovery of the enzymes having the indicated activity.

The present invention also discloses the cloning of the genes encoding the subject xylanases. Expression of these genes in a host cell is also disclosed. The enzymes are essentially pure when obtained in this manner. Thus the present invention also discloses another method for obtaining the xylanases, i.e. expression of recombinant DNA.

The present invention further describes the application of the xylanases under conditions equivalent to industrial application conditions, using high temperature and high pH.

Brief description of the figures

Figure 1: Phylogenetic analysis of the internal consensus sequences of the six extremophilic strains. Sequences are named as followed; organism/forward

primer/recombinant number. The branch number displayed are the values obtained from a boot-strap analysis of the sequences.

Figure 2: Sequence alignment of family G internal consensus sequences.

Figure 3: The xylanase domain of xynD from TG456 inserted as a SphI-BamHI fragment in pJLA602.

Figure 4: Paper properties of paper prepared from hardwood pulp TCF bleached as described in Example 15. Ref = no enzyme, 715 = TG456 xynD, 716 = Tg53 xynD. Tensile index (A), porosity (B), tear (C) and burst (D) in relation to Schoppen-Riegler values are given.

Figure 5: Paper properties of paper prepared from hardwood pulp ECF bleached as described in Example 15. Ref = no enzyme, 715 = TG456 xynD, 716 = Tg53 xynD. Tensile index (A), porosity (B), tear (C) and burst (D) in relation to Schoppen-Riegler values are given.

Figure 6: Paper properties of paper prepared from softwood pulp TCF bleached as described in Example 15. Ref = no enzyme, 715 = TG456 xynD, 716 = Tg53 xynD. Tensile index (A), porosity (B), tear (C) and burst (D) in relation to Schoppen-Riegler values are given.

Figure 7: Paper properties of paper prepared from softwood pulp ECF bleached as described in Example 15. Ref = no enzyme, 715 = TG456 xynD, 716 = Tg53 xynD. Tensile index (A), porosity (B), tear (C) and burst (D) in relation to Schoppen-Riegler values are given.

Figure 8: Refining curves as determined in Example 15 for TCF (A) and ECF (B) bleached hardwood pulp and for TCF (C) and ECF (D) bleached softwood pulp. Ref = no enzyme, 715 = TG456 xynD, 716 = Tg53 xynD.

Figure 9: A comparison of the pH optima (A) and temperature optima (B) of the TG456 xynD xylanase and Pulpzyme HB (Novo Nordisk).

Figure 10: A comparison of the thermostabilities of the TG456 xynD xylanase and Pulpzyme HB (Novo Nordisk) at 80°C, pH 7.0 (A) and at 65°C, pH 9.0 (B).

Detailed description of the invention

The present invention discloses microorganisms which have been isolated from hot springs in New Zealand. These microorganisms have been characterized as anaerobic thermophilic bacteria and have been classified according to Rainey (1992. PhD thesis, University of Waikato, Hamilton, New Zealand).

The microorganisms have subsequently been screened using a xylan-agar diffusion assay. Strains which showed a clearing zone in this test were selected as potential xylanase producing strains. The strains were grown under anaerobic conditions at pH 7.0 and T = 75 or 80°C depending on the organism. After concentration by ultrafiltration, the culture broth was analyzed for xylanase activity in an assay at pH = 6.5 and 9 and T = 80°C (Example 1).

Six different strains were found to produce xylanase activity under the indicated conditions. These microorganisms have been deposited at the CBS on April 14, 1994 under deposition numbers: CBS 211.94, CBS 212.94, CBS 213.94, CBS 214.94, CBS 215.94 and CBS 216.94.

The present invention also discloses enzymes having xylanase activity, specifically enzymes having at least a considerable xylanase activity at a temperature of at least 80°C and a pH of 6 or higher. Said enzymes are obtainable from the deposited strains. Said enzymes are also obtainable from mutants and variants of the deposited strains.

With the expression "considerable activity" is meant that the enzymes of the present invention have at least 80°C, at least 40% of the activity they possess at 70°C, preferably this is at least 60%, more preferably about at least 80%, even more preferably about 200%.

The present invention further describes a process for the preparation of the xylanases which are active at a temperature of about 80°C or having a considerable activity under these conditions. The process comprises cultivation of the deposited microorganisms of the present invention in a suitable medium, followed by recovery of the enzymes having the indicated

activity. Isolation and purification of the xylanase can be performed using standard procedures known in the art.

A partial purification of the enzymes is exemplified in Example 2. In this example the cells are first removed by hollow fibre filtration. Subsequently, the protein is further purified by ammonium sulphate addition to a final concentration of 1M. The solution is then brought on a phenyl sepharose column and the protein eluted with 1M NaCl. Finally, the protein is concentrated by ultrafiltration and salt is removed by diafiltration.

To establish the xylan degrading potential of the xylanases at an alkaline pH, a comparison of the activities of the xylanases isolated from the indicated strains at a pH of 7 and 9 has been made using different substrates and a temperature of 70°C (Example 3). The xylanases have been shown to possess considerable activity at alkaline pH. Depending on the substrate and the assay, at pH 9 the xylanases have about 20% or more of the activity they possess at pH 7. One xylanase was shown to retain 80% of its pH 7 activity at pH 9.

The thermostability of the xylanases isolated from the indicated strains varies considerably. Some of the xylanases are very thermostable: they have a half-life of more than 2 hours at pH = 9 and at 80°C (see Example 4). The half-life at pH = 9.0 and 80°C of the xylanases of the invention is at least 10 minutes, preferably the half-life under these conditions is more than 20 minutes, more preferably it is more than 30 minutes, still more preferably it is more than 60 minutes and most preferably the half-life is more than 120 minutes.

The present invention also discloses the cloning and expression of a DNA sequence characterized in that it encodes a xylanase obtainable from an anaerobic thermophilic strain. The invention discloses vectors, which include expression vectors, characterized in that they contain the DNA sequence encoding the xylanases of the present invention. A microbial host cell characterized in that it is transformed with one of these vectors is also disclosed.

Sequence analysis of the DNA sequences encoding the xylanases of the invention revealed that the obtained sequences fall into two groups of enzymes families, i.e. the F-type and G-type xylanases as previously defined by Gilkes et al. (1991, Microbiol. Rev. 55: 303-315). The xynA, xynB and xynC sequences obtained from the microorganisms of the invention belong to the F-type xylanases; the xynD sequences obtained from these

microorganisms belong to the G-type xylanases (see examples 6,7,8 and 9). G-type xylanases from thermophilic microorganisms have not been described previously to the best of our knowledge. The thermophilic microorganisms which are the source of the xylanases of the invention are herein defined as microorganisms with an optimum growth temperature which is higher than 65°C. Preferably the optimum growth temperature is higher than 68°C, more preferably the optimum growth temperature is higher than 70°C, and most preferably the optimum growth temperature is in excess of 75°C.

Expression of the both F-type and G-type xylanase DNA sequences of the invention in E.coli is shown to give an active proteins (see Example 10 and 11). Said proteins are active at a temperature of 80°C.

The cloning and expression demonstrated in the examples of the present description makes possible expression of the genes in homologous organisms. Expression can also be performed in other microorganism using suitable expression and secretion regulating sequences. Organisms of choice are yeast, fungi and bacteria. Specific micro-organisms include strains selected from Aspergillus niger, Kluyveromyces lactis and Bacillus licheniformis.

The enzymes of the present invention have been shown to possess a considerable activity on oat spelts xylan and on birchwood xylan.

The enzymes of the present invention have further been tested for their bleaching capacity. This bleaching capacity is determined by the delignifying activity of the enzymes of the present invention. The invention discloses enzymes which have a significant delignifying activity, as measured on several wood pulps. The enzyme preparations, xylanases, are capable of delignifying wood pulp at a temperature of at least 80°C. The expression "wood pulp" is to be interpreted broadly and is intended to comprise all kinds of lignocellulosic materials.

The enzymes have been tested for their bleaching capacity on both hardwood and softwood pulps. Lignin removal has been measured according to two different methods (Example 5). Lignin in the medium was determined by the A300 test. In this test all preparations showed significant delignifying activity at 80°C on both hard- and softwood. At pH 9 and 80°C the activity on softwood was at least 43% of the activity at pH 6, while on hardwood 3 strains showed at pH 9 activities of more than 50% as compared to pH 6, and 3 others 18-30%. Bleaching of softwood pulp was also measured by determining the decrease in the lignin content of the pulp using the kappa

test. The kappa number was decreased 0.5 to 1.4 units after incubation of the pulp at pH 9 and 80°C with the xylanase preparations.

Additionally, the bleaching capacity of the enzymes of the present invention can be measured by e.g. the increase of ISO brightness of paper produced with conventionally treated pulp, additionally incubated with said enzymes, as compared to incubation without enzymes. Said conventional treatment comprises exposition to bleaching agents such as H_2O_2, ozone, Cl_2 or ClO_2, according to the art.

The cloned F-type and G-type xylanases of the invention as expressed in E.coli have also been tested for their performance in ECF bleaching experiments on both softwood and hardwood pulp (see Example 12). Surprisingly, we have observed that the G-type xylanases (encoded by the xynD genes) show a much better performance in bleaching sequences on both softwood and hardwood pulp as compared to the F-type xylanases, up to 6.8 %ISO delta brightness over the no-enzyme control, which compares to at most 1.2 % ISO brightness increase for the best F-type xylanase. Further testing of the thermostable G-type xylanases showed that they also produce excellent results in TCF bleaching experiments on both softwood and hardwood pulp (see Examples 14 and 15). Verification of the properties of the paper produced from the thus bleached pulp showed no significant differences with the no-enzyme control paper.

The cloned G-type xylanases are highly thermostable, even at high pH. A half-life at 80°C and pH 7.0 of more than 30 minutes was measured. At 65°C and pH 9.0 the half-life of the enzyme is not significantly reduced, even after 120 minutes of incubation (see Example 16). The half-life at pH 9.0 and 65°C of the G-type xylanases of the invention is at least 10 minutes, preferably the half-life under these conditions is more than 20 minutes, more preferably it is more than 30 minutes, still more preferably it is more than 60 minutes and most preferably the half-life is more than 120 minutes.

The invention discloses DNA sequences encoding thermostable G-type xylanases the internal consensus fragment (ICF) of which shows more than 80 % identity with the ICF of SEQ ID NO 12. The G-type ICF is herein defined as the fragment which lies in between the sequences corresponding to SEQ ID NO 4 and SEQ ID NO 5 in SEQ ID NO 12 (nucleotide positions 295 to 623). Preferably the identity with the G-type ICF is more than 87 %, more

preferably the identity is more than 95 %, most preferably, the identity is more than 99 %.

In a further embodiment, the invention discloses G-type xylanase sequences which share at least 70 % identity with the amino acid sequence of SEQ ID NO 13. Preferably this amino acid identity is more than 80 %: more preferably, the amino acid identity is more than 90 %; still more preferably the amino acid identity is more than 95 %; most preferably the amino acid identity is more than 99 %.

The enzymes of the present invention can be used immediately after the oxygen delignification step in the pulp preparation process described above. As a consequence of the temperature and pH characteristics of the enzymes, extensive cooling and pH adaptation can be avoided. In another embodiment of the invention, the enzymes are used before or during the oxygen delignification step. Before this step the lignin concentration is much higher and therefore the effect of the application of the xylanase is much larger.

Furthermore, applications of the enzyme preparations of the present invention are described, particularly a process in which wood pulp is treated with said enzyme preparations according to the invention.

Similarly, a fluff pulp can be treated with the enzyme preparations according to the invention.

The invention further relates to paper, board and fluff pulp made from a wood pulp treated with the enzyme preparations according to the invention.

Example 1

Isolation of strains producing thermostable xylanase activity

Xylanase-producing microorganisms were obtained from hot springs in various thermal areas of New Zealand. The strains were isolated by in situ enrichment using oat spelts xylan as substrate from hot springs having a temperature of above 70°C, and a pH between 6.0 and 8.5. Cultures of strains were obtained by further anaerobic cultivation in the laboratory on 2/1 medium plus xylan at a temperature (70°, 75° or 85°C) corresponding to the temperature of the site from which the sample was taken.

The composition of the 2/1 medium plus xylan is as follows:

Anaerobic 2/1 medium	
Resazurin (0.1%)	1.0 ml/l
SL10 Trace Elements	1.0 ml/l
$FeCl_3$ soln. (0.28 mg/ml)	1.0 ml/l
Wolin's Vitamins	0.1 ml/l
NaCl	0.9 g/l
$MgCl_2.6H_2O$	0.2 g/l
K_2HPO_4	1.5 g/l
KH_2PO_4	0.75 g/l
NH_4Cl	0.9 g/l
Cysteine	0.75 g/l
Yeast Extract	0.5 g/l
Bacto Tryptone	1.0 g/l
Oat spelts xylan	2 g/l
pH 7.0	
Dispense under N_2 gas	

Wolin's Vitamin Mixture	mg/100ml
Biotin 2.0	
Folic acid	2.0
Pyridoxine HCl	10.0
Riboflavin	5.0
Thiamine	5.0
Nicotinic acid	5.0
Pantothenic acid	5.0
Vitamin B12	0.1

p-Aminobenzoic acid	5.0
Thioctic acid	5.0

SL10 Trace Elements, modified

5M HCL	15ml
	mg/l
$ZnCl_2$	70
$MnCl_2.4H_2O$	100
H_3BO_3	6
$CoCl_2.6H_2O$	130
$CuCl_2.2H_2O$	2
$NiCl_2.2H_2O$	24
$NaMoO_4.2H_2O$	36

Pure cultures of strains were obtained from single colonies in agar roll tubes. Culture concentrates obtained by ultrafiltration were tested for xylanase activity at 70° and 80°C and, at pH 6.5 and 9.0 using a dyed xylan method (see below) and the PAHBAH method (see Example 3).

Six strains which were deposited at the CBS (Centraal Bureau voor Schimmelcultures) are depicted in Table 1.

Table 1: deposition numbers of strains used in this patent application

strain	CBS accession number
TG 53	CBS 211.94
TG 453	CBS 212.94
TG 456	CBS 213.94
TG 457	CBS 214.94
TG 479	CBS 215.94
TG 631	CBS 216.94

Dyed xylan, composed of larchwood xylan coupled to Remazol Brilliant Blue R (Sigma), was prepared following a method described by Biely et al (1985) Anal. Biochem 144, 142-146. Larchwood xylan (6.7 g) was placed into 250 ml water and stirred for several hours at room temperature to dissolve. Remazol Brilliant Blue R (10.0 g) was added to this mixture. After dissolving, 20 ml of a Na-acetate solution (2.7 g Na-acetate in 20 ml distilled

water) was added dropwise. Then a solution of 6 g NaOH in 70 ml water was added and stirring continued for 18 hours. The dyed xylan which had formed was precipitated by the addition of 700 ml 96% ethanol. After allowing to stand for 6 hours the precipitate was separated by filtration (Whatman GF/A). The filter cake was washed with 3 l of a 2:1 mixture of 96% ethanol/0.05 M Na-acetate, followed by 1 l 96% ethanol and 3 l acetone until the filtrate was colourless. The yield was 10.5 g dyed xylan.

For the enzyme assay, 5.1 g dyed xylan was dissolved in 150 ml 0.1 M MOPS buffer, pH 6.8. The mixture was stirred at 70°C for 4 hours to dissolve. After cooling to room temperature insoluble material was removed by centrifugation at 10,000 x g. For activity assays, the stock solution containing 3.34 % w/v dyed xylan was diluted with 0.1 M MOPS buffer, pH 6.8 to give a 0.5 % dyed xylan working solution.

The enzyme assay using dyed xylan was performed by adding 0.9 ml dyed xylan solution (0.5%) to 0.6 ml enzyme preparation in an appropriate buffer with mixing. A portion (0.4 ml) of this mixture was transferred into 0.8 ml 96% ethanol as control (blank). The remaining solution was incubated at 70°C for 90 minutes. The reaction was terminated by transferring 0.4 ml of the test mixture into 0.8 ml 96 % ethanol. This solution was left at room temperature for 30 minutes to allow for the precipitation of uncleaved dyed xylan. The test samples were centrifuged at for 5 minutes at full speed in a Beckman Microfuge E, and the absorbance of the supernatant was measured at 595 nm in a spectrophotometer.

Example 2

Isolation of enzyme preparations

Anaerobic fermentations were carried out in 2 l Schott bottles containing 1800 ml of 2/1 plus xylan medium in stationary incubation at a temperature of either 75 or 80°C (depending on the organism being cultured) for 24 hrs. From 10 l of well-grown culture the cells were removed by hollow fibre filtration in the presence of 0.01% triton X100 (Amicon DC 10 LA and Amicon 0.1 μm H5MP01-43 filter). Ammonium sulphate was added to the cell free culture medium to a final concentration of 1M. The resulting solution was pumped onto a 9 x 15 cm column containing 1 l of phenyl sepharose

(Pharmacia-Fast Flow-low substitution) equilibrated with 1M ammonium sulphate. The flow rate was 150 - 200 ml/minute. The column was washed with 5 l 1M ammonium sulphate. The xylanase was eluted with 5 l 1M NaCl. Fractions were collected in 500 - 1000 ml volumes. The xylanase activity was determined (with the PAHBAH method as described in Example 3) in the fractions and the active fractions were pooled, ultrafiltered to a small volume and diafiltered to reduce the salt concentration using an Amicon Stirred Cell (Model 2000A or 8400) with an YM2 membrane.

Example 3

Characterization of xylanase activities of the partially purified enzyme preparations

Analytical methods

Assays for xylanase activity were performed using modified procedures of the Sumner assay (Sumner et al. 1921. J. Biol. Chem. 47 : 5-9). Alternatively xylanase activity was determined using a modified PAHBAH assay, based on a method from Lever (1973. Biol. Med. 1 : 274-281).

Procedure 1

Sumner assay of xylanase activity on oat spelts xylan

An oat spelts xylan substrate solution is prepared as follows: 4 g oat spelts xylan is suspended in 100 ml demineralized water. The suspension is sonicated for 6 minutes (sonicator: Sonics & Materials, Vibracell type VC 250 B), incubated at 100°C for 10 min., and centrifuged for 10 min. at 10,000 rpm in a Sorvall RC-5B centrifuge. The supernatant is used as a substrate solution and contains about 2% oat spelts xylan.

The assay is carried out as follows: A test tube is filled with 200 µl oat spelts xylan solution, 600 µl aliquots of enzyme preparation (Example 2) diluted in the appropriate buffer. The test tube is incubated under measuring conditions in a waterbath for 15 minutes. After the incubation, 7.2 ml DNS (dinitrosalicylic acid) reagent is added. The mixture is heated in a waterbath at 100°C for 10 minutes, whereafter the test tube is cooled on ice. The absorbance is measured at 575 nm. To eliminate the background absorbance of the enzyme samples a control experiment is executed as follows: a tube

containing substrate without enzyme preparation is incubated under the same conditions as the test samples. After the 15 minutes incubation 7.2 ml DNS and the enzyme sample is added (in this order).

One unit of xylanase (xU) activity is defined as the amount of enzyme producing 1 µmol of xylose-equivalent, measured as reducing sugar.

Actual measuring conditions were pH 7.0, 9.0 and 70°C. At pH 7 a 50 mM phosphate buffer was used, and at pH 9 a 50 mN borate/KOH buffer.

Table 2: relative xylanase activities on oat spelts xylan, measured at 70°C

Strain	Activity (%)	
	pH 7.0	pH 9.0
TG 453	100	57
TG 456	100	39
TG 457	100	31
TG 479	100	19
TG 631	100	31

Procedure 2

Sumner assay of xylanase activity on Birchwood xylan

Essentially the same method as described in procedure 1 is used. Instead of an oat spelts xylan solution a birchwood xylan suspension is used. A birchwood xylan substrate solution is prepared as follows: 4 g birchwood xylan is suspended in 50 ml 0.2 N NaOH and agitated until visibly dissolved. The pH of the solution is adjusted to 7.0 with glacial acetic acid, water is added to 100 ml and the solution is centrifuged at 10,000 rpm in a Sorvall RC-5B centrifuge. The supernatant is used as a substrate solution and contains about 3% birchwood xylan. The test conditions were pH 7 and 9 and 70°C. The results are shown in Table 3.

Table 3: relative xylanase activities on birchwood xylan, measured at 70°C

Strain	Activity (%)	
	pH 7.0	pH 9.0
TG 453	100	40
TG 456	100	20
TG 457	100	21
TG 479	100	12
TG 631	100	21

Procedure 3

PAHBAH assay of xylanase activity on oat spelts xylan

The modification of the PAHBAH method (Lever, 1973) is to the PAHBAH reagent, as follows: 0.05 M trisodium citrate, 0.1 M Na_2SO_3, 0.02 M $CaCl_2$, 0.5 M NaOH and 0.1 M *p*-hydroxybenzoic acid hydrazide (PAHBAH).

For the assay of the enzyme preparations 0.05 ml or 0.1 ml of enzyme preparation is mixed with 0.3 ml substrate buffer (50 mM Bis-Tris-Propane, pH as required, + 0.2% oat spelts xylan in suspension). An appropriate amount of water is added to a final volume of 0.5 ml. Incubation is usually made at 70°C for 30 min. To stop the reaction, 1.0 ml of PAHBAH reagent is added after incubation and the samples are heated at 100°C for 6 minutes. The blanks consist of substrate buffer incubated identically to the sample to which the enzyme is added after the PAHBAH reagent. Before determination of the absorption at 420 nm, all samples are centrifuged 1 min at full speed in a Beckman Microfuge E to remove suspended xylan from the supernatant. The results are shown in Table 4. From this Table it can be concluded that at pH 9.0, 80°C all strains still have a considerable activity as compared with pH 6.0, 70°C.

Table 4: relative xylanase activities on oat spelts xylan, measured with the PAHBAH assay

Strain	Activity (%)	
	pH 6.0, 70°C	pH 9.0, 80°C
TG 53	100	57
TG 453	100	20
TG 456	100	65
TG 457	100	82
TG 479	100	30
TG 631	100	65

Example 4

Thermostability of xylanase activities

The half-life of the xylanase activity of the enzyme preparations was determined as follows. The enzyme preparation was diluted 1/10 in 100 mM TAPS buffer (pH 9.0 at 80°C) and incubated at 80°C. A sample was removed at 0, 10, 20, 40, 60 and 120 minutes and added to 100 mM MOPS buffer (pH 6.0 at 70°C) on ice, to a final dilution of 1/20 to 1/100 as appropriate for the final assay. The samples were kept on ice until they were assayed at 70°C and pH 6.0 using the PAHBAH assay method as described in Example 3, procedure 3. The results are shown in Table 5.

Table 5: thermostability of xylanase activity at pH 9.0 and 80°C, as measured with the PAHBAH assay on oat spelts xylan

Strain	Half-life pH 9.0, 80°C
TG 53	30 minutes
TG 453	60 minutes
TG 456	> 120 minutes
TG 457	> 120 minutes
TG 479	< 10 minutes
TG 631	< 20 minutes

Example 5

Delignification activities

Delignification capacity of the xylanases was determined with two different methods. Using the A300 method the amount of lignin released from the pulp after enzyme treatment is estimated. Using the kappa assay the remaining lignin content of pulp after treatment is measured.

Method 1: A300 test

To measure delignification with the A300 assay enzyme preparations were incubated with soft- or hardwood pulp at a concentration of 2 PAHBAH units per g of wet pulp (about 6 PAHBAH units/g of dry pulp). Incubations were performed for 2 hours at 80°C both at pH 6.0 in MOPS buffer, and at pH 9.0 in TAPS buffer. Pulp concentration in the incubation was 0.1 g wet weight per ml. Two different types of pulp were used: Kraft softwood pulp after oxygen delignification, and Kraft hardwood pulp after oxygen delignification. Properties of these pulps are given in Table 6.

Table 6: properties of the pulp types used in the delignification experiments

	Hardwood	Softwood
	birch	80% spruce, 20% pine
Brightness, % ISO	50.8	35.8
Kappa number	11.0	16.7
Viscosity, dm^3/kg	979	1003
Calcium, ppm	1900	2600
Copper, ppm	0.3	0.6
Iron, ppm	5.1	11
Magnesium, ppm	210	270
Manganese, ppm	25	70

The amount of lignin that was removed from the pulp was determined by measuring the absorbance of the supernatant at 300 nm, after separating the supernatant from the pulp by filtration by suction through a Whatman GF/C filter supported on a sintered glass filter. The results of the A300-test are shown in Table 7. In this assay, Delta A300 values of $\geq$ 0.2 are significantly higher than background levels. Therefore, this example shows that the enzymes possess a significant delignifying activity at 80°C.

Table 7: A300 delignification measured at 80°C, pH 6.0 and 9.0, on soft- and hardwood

Strain	Delta A300					
	Hardwood			Softwood		
	pH 6.0	pH 9.0	%*	pH 6.0	pH 9.0	%*
TG 53	0.7	0.5	71	0.6	0.35	58
TG 453	1.0	0.3	30	0.7	0.3	43
TG 456	1.0	0.3	30	0.6	0.6	100
TG 457	1.0	0.7	70	1.0	0.5	50
TG 479	0.9	0.16	18	0.8	0.6	75
TG 631	1.2	0.7	58	0.8	0.7	88

* Percentage of the activity at pH 9 as compared to pH 6

Method 2: the Kappa test

The kappa tests were performed according to TAPPI protocol T236 (available from TAPPI, Technology Park, Atlanta, USA), with some modifications. The enzyme preparation was added at a dose of 10 xU/g pulp (dry weight), and incubated for 2 hours at pH 9, 80°C. As a control pulp was incubated for the same period under the same conditions without enzyme addition. Oxygen delignified softwood pulp was used, for properties see Table 6.

The difference between the kappanumber with enzyme addition and the kappanumber without enzyme addition is called the kappa reduction, and is a value for delignification. The kappa reductions are shown in Table 8. In this assay, values of $\geq$ 0.5 are significantly higher than background levels. Therefore, as in the previous example, this example shows that the enzymes possess a significant delignifying activity at 80°C.

Table 8: delignification as measured by kappa reduction, determined on softwood pulp, pH 9 and 80°C

Strain	Kappa reduction
TG 453	1.0
TG 456	1.3
TG 457	1.4
TG 479	1.0
TG 631	0.5

Example 6

Cloning and sequence determination of internal consensus fragments of genes encoding thermostable F-type xylanases

6.1. PCR amplification of internal fragments of xylanase genes

Three PCR primers were used to amplify internal consensus fragments of xylanase genes: two different forward primers (xynFA, {5' CAC ACK CTK GTK TGG CA 3', SEQ ID NO 1} and xynFB {5' CAT ACK TTK GTT TGG CA 3', SEQ ID NO 2) and one reverse primer (xynR {TMG TTK ACM ACR TCC CA, SEQ ID NO 3}). The xynFA and xynFB primers bound at the same location, but differed slightly in sequence due to slight differences in the sequence of xylanase genes at the forward consensus region. PCR conditions were as follows: (94°C 1 minute, 50°C 1 minute, 72°C 1 minute) x 30. All family F internal consensus fragments were approximately 350bp.

6.2. Sequence determination of the PCR products

All internal xylanase PCR-products (ca. 350bp) were end-repaired (back-filled) as described below prior to cloning into the SmaI (phosphatased) site of the M13mp10 sequencing vector.

Step 1 - Ammonium acetate precipitation:
(a) make up 50µl PCR mixture to 100µl with TE buffer (10mM Tris-Cl, 1mM EDTA, pH 8.0). (b) add 100µl 4M $CH_3COO\text{-}NH_4^+$ and 250µl 100% CH_3CH_2OH. (c) incubate on ice for 15 minutes (or overnight at -20°C). (d) centrifuge at 16 000rpm for 15 minutes and discard supernatant. (e) wash pellet in 500µl cold 70% CH_3CH_2OH. Re-centrifuge (16 000rpm 5') and discard supernatant. (f) dry pellet under vacuum for 5 minutes and resuspend in 20µl TE buffer.

Step 2 - End-repair of PCR fragments:
(a) to 20µl of precipitated DNA add: 3µl 10x Ligase buffer (Boehringer Mannheim), 1µl 12.5mM dNTP's (Pharmacia DNA polymerisation mixture), 0.5µl (5U) E. coli DNA polymerase large (Klenow) fragment (BRL technologies Ltd), 0.25µl (2.5U) T4 DNA polymerase (Boehringer Mannheim), 0.25µl (2.5U) T4 polynucleotide kinase (Boehringer Mannheim) and H_2O up to 30µl. (b) incubate at 37°C for 30 minutes and heat-kill enzymes by incubating at 70°C for 10 minutes.

Step 3 - Gel-purification of the end-repaired xylanase fragment:
(a) run DNA through 2% LMP agarose in 1x Tris-acetate buffer (pH 7.8). (b) excise the 350bp xylanase band from the agarose. (c) purify the DNA from the agarose slice using the GeneClean©) procedure (Bio101 Inc.).

Step 4 - Ligation into M13mp10 (SmaI-phosphatased):
(a) mix 1µl M13mp10 vector DNA (appropriately diluted to ca. 10ng/µl), 20-50ng insert DNA (xylanase consensus primer fragment), 1µl 10x ligase buffer (Boehringer Mannheim), 1µl T4 DNA ligase (Boehringer Mannheim), and H2O up to 10µl.
(b) incubate overnight at room temperature.

Step 5 - Transformation of ligation mixture into Escherichia coli strain JM101:
(a) transform JM101 with the entire 10µl ligation mixture using the DMSO-mediated transformation technique of Chung et al. (1989. Proc. Natl. Acad. Sci. 86: 2172-2175.). (b) Plate the M13/JM101, and isolate recombinant M13 plasmids using standard procedures (Sambrook et al. 1989. Cold Spring Harbour Laboratory Press).

Recombinant M13 phage containing internal xylanase consensus fragments were sequenced from ssDNA (Sambrook et al. 1989) on an Applied Biosystems 373A automated DNA sequencer using dye-primer chemistry (sequencing primer used was the universal M13 forward {dye-labelled} primer, ABI Ltd). All DNA sequence data were analysed and manipulated using the G C G sequence analysis software (installed on Irix) run on a Silicon Graphics Personal Iris Workstation.

Family F xylanase internal fragment sequence results:

Based on the PCR fragments which were amplified from the six strains depicted in Table 1 using the xylanase consensus primers, it was predicted that each organism contained between 1 to 3 family F xylanase genes. The results have been analysed via the well known boot-strap analysis (Wisconsin Molecular Biology Package, Devereux, 1984, Nucleic Acids Res. 12, 387-394). In Figure 1 a dendogram of the various xylanases and strains is shown. From the nucleotide sequences of the family F consensus fragments, it now appears that each organism will contain three different family F genes. Each of the family F xylanase genes of each organism belongs to a separate xylanase cluster (based on nucleotide and primary amino acid sequence homologies), now designated as cluster A, cluster B and cluster C. The full-length xylanase amplified from TG 456 belongs to cluster A, and has subsequently been named TG 456 xynA. In addition, an internal consensus fragments from a TG 456 cluster B xylanase (TG 456 xynB) and a cluster C xylanase (TG 456 xynC) have been identified.

Example 7

Cloning and sequence determination of internal consensus fragments of genes encoding thermostable G-type xylanases

7.1 PCR amplification of internal fragments of G-type xylanase genes

The Family G internal consensus fragments (ICFs) were isolated with the Polymerase Chain Reaction (PCR), using the forward and reverse family G consensus primers (GF and GR). The PCR profile was as follows:

Approximately 10ng of the end-repaired and gel-purified 300 ICFs were ligated into the SmaI site of M13mp10 using BM T4 DNA ligase, in 10uL reactions.

7.3 Sequence determination of the PCR products

Six independent phages originating from strains TG457 and TG53 were sequenced. It appears from the alignments (Figure 2) that only a single G-type xylanase gene is present in each of these strains. In addition M13mp10 recombinants containing family G ICFs from TG453, TG456, TG479 and TG631 were sequenced. These organisms contained all a family G xylanase gene encoding an essentially identical xylanase, although variations in the DNA sequence are up to 13 %.

Example 8

Sequence of full-length F-type xylanases

On the basis of the internal PCR fragments 3 different type of F-xylanase genes have been identified: xynA, xynB and xynC.
Using Genome Walking PCR (GWPRCR) full length xylanase genes have been isolated from most of the strains.

Full length sequences of the TG456 xynA gene have been determined. The complete sequence of the xynA gene is given in SEQ ID NO 6. The encoded amino acid sequence of the TG456 xylanase A is provided in SEQ ID NO 7.

For xynB and xynC it was discovered that the genes encode multi-domain enzymes, with one xylanase domain. These xylanase domains have been subcloned using PCR primers designed on sequences at the border of the xylanase domain.
The partial sequence information for the xynB and xynC genes originating from TG456 are given in SEQ ID NO 8 and SEQ ID NO 10, respectively. The encoded amino acid sequences of the TG456 xylanases B and C are given in SEQ ID NO 9 and SEQ ID NO 11, respectively.

n.b. °C refers to degrees celsius.
x refers to the number of cycles (i.e. x1 equals ONE cycle)

(94°C, 4 minutes) x 1
(94°C, 1 minute; 45°C, 1 minute; 72°C, 1 minute) x 35 (94°C, 1 minute; 45°C, 1 minute; 72°C, 6 minute) x 1

The PCRs were performed in 50uL reactions using the following ingredients: 100ng GF primer; 100ng GR primer; 0.25mM dNTPs; 1 Unit Taq Polymerase; 0.25 mM MgCl2; 10mM Tris-HCl, pH 8.8; 50mM KCl; 0.001% Gelatin; 1-10 ng template DNA.

Two PCR primers were used to amplify consensus fragments of xylanase genes:
GF: TATNTGRSTNTMTATGGWTGG (forward internal consensus primer) SEQ ID NO 4.
GR: CCGCTNCTTTGGTANCCTTC (reverse internal consensus primer) SEQ ID NO 5.

With all six strains a PCR fragment was found upon amplification with the consensus primers.

Two species of PCR-products (DNA fragments) were amplified: a 300bp fragment of the expected size, plus an unexpected 600bp PCR-product - this 600bp fragment was a head-to-tail dimer of the 300bp PCR-product; presumably this 600bp species was a result of self-priming during the PCR reactions, as a consequence of homology between the GF and GR primers.

7.2 Sequence determination of the PCR products

The 300bp fragments amplified from each organism were end-repaired (see example 6).
The end-repaired fragments were then purified from a 1% low melting temperature agarose gel (run in Tris-acetate running buffer) using the Geneclean (Bio 101, La Jolla, Calif.) procedure.

Example 9

Complete sequence of G-type xylanase genes

Using Genome Walking PCR (GWPRCR) the full length xynD genes have been isolated from most of the strains.

The complete sequence for the xynD gene of TG456 is given in SEQ ID NO 12 and the encoded TG456 xylanase D amino acid sequence is provided in SEQ ID NO 13.

Example 10

Construction of expression vectors and hosts for xylanase production from cloned genes

In the consensus PCR primers appropriate restriction sites have been designed, which allow the subcloning of xylanase genes in suitable expression vectors.

The xynA and xynC genes are inserted NcoI-BamHI into the pJLA602 expression vector [Schauder, B., Blucker, H., Frank, R., and McCarthy, J. E. G. (1987). Inducible Expression Vectors Incorporating the Escherichia coli atpE Transcriptional Initiation Region. Gene 52: 279-283.]) for in-frame fusion with the lambda L and R promoters [Gibbs, M.G, Saul D. J, Luthi, E., and Bergquist, P.L. (1992). The beta-mannanase from "Caldocellum saccharolyticum" is Part of a Multidomain Enzyme. Appl. Environ. Microbiol. 58 (12): 3864-3867.]

XynB and *xynD* genes were inserted into the unique SphI-BamHI sites of pJLA602.

Constructs were transferred into the hosts *E.coli* JM101 and *E.coli* DH5α using standard transformation techniques.

Figure 3 shows the sequence of the xylanase domain of xynD from TG456 inserted in pJLA602 as a SphI-BamHI fragment.

Example 11

Production and recovery of cloned xylanases

In order to obtain protein samples from the cloned F-type xylanases the *E.coli* clones were fermented in a 10.0 litre capacity LH fermenter (series 210). The culture was maintained at 30°C until induction (42°C) with continuous aeration (4.8 litres/min), agitation (500 rpm) and pH control (pH 7.1). The media used and other additions are given below:

Luria Broth (for inoculum cultures):

Tryptone	10 g
Yeast Extract	5 g
NaCl	10 g
Water	to 1000 ml

BGM2 bulk growth medium for fermenter runs (quantities are for final medium, i.e. 2 batches of 8.5 litres):

NH_4Cl	3.22 g
KH_2PO_4	1.05 g
$MgSO_4.7H_2O$	0.135 g
K_2SO_4	0.043 g
$FeSO_4.7H_2O$	0.003 g
SL-10 Trace Elements	1 ml
Tryptone	10 g
Yeast Extract	4.7 g
Glycerol	34 ml
Water	to 1000 ml

After the fermenter vessel, with approximately 6 litres of water in it, was sterilized by autoclaving at 121°C for 30 miniutes, it was cooled to 30°C. The media concentrate (8.5 fold concentrate) was pumped through a sterile 0.2 μm cartridge filter (Sartorius). Prior to inoculation the following additions were made:

Antifoam (Bevaloid 5901)	10 ml (autoclaved)
$CaCl_2$ (17 mg/ml stock)	1 ml (autoclaved)
Ampicillin	850 mg (filter sterilized)
Thiamine (JM101 cultures only)	8.7 mg (filter sterilized)

The liquid volume was then adjusted to 8.5 litres. The pH of the media was adjusted to 7.1. Filter-sterilized air was sparged through the fermenter at a flow rate of 4.8 litres per minute. The fermenter temperature was held at 30°C by hot and cold water circulation through finger probes. pH control was by addition of autoclaved NaOH (5M) or H_4SO_4 (2M). The fermenter stirrer speed was 500 rpm.

Inoculation was initiated with an aliquot (approximately 10.0 ml) of a fresh culture of the *E.coli* clone grown in Luria Broth with 100 µg/ml ampicillin at an OD_{650} of 0.2 to 0.4. The cells were grown to an OD_{650} of between 11 and 13 at 30°C, then they fed-batched with a medium concentrate, allowed to recover and grown to an OD_{650} of between 14 and 16.They were subsequently induced by raising the temperature to 42°C.

The cells were harvested 4 hours after maximum OD_{650} was reached. The cells were recovered by hollow fibre filtration (0.1 µm, Amicon) and resuspended in 50 mM Tris-HCl (pH 8.0), 7.5 mM EDTA, 7.5 mM ß-mercaptoethanol, 0.02% PMSF, 1.0 µM pepstatin A, 0.02 % DNase and 0.02% lysozyme and incubated at 4°C for 1 hour (total volume of approximately 1 litre). The cells were sonicated on ice in 160 ml batches for 4 - 6 minutes in one minute blasts until lysed (monitored by microscopy). An aliquot of 56 mM PMSF (in isopropanol) was added (each addition equivalent to 160 µM) for each two minutes of sonication to a final concentration of no more than 1 mM PMSF. After lysis was complete, cell debris was removed by centrifugation. The supernatant was heat treated at 70°C until obvious precipitation of protein occurred (usually about 20-30 minutes) and the denatured protein was removed by centrifugation. Prior to phenyl-sepharose chromatography, ammonium sulphate was added to the heat-treated supernatant to a final concentration of 1.0 M (this is termed the 1° heat treatment extract). The cell pellet derived from the sonicated cells was re-extracted by suspension in 1.0 litre of 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 7 mM ß-mercaptoethanol, followed by a second heat treatment at 70°C for 15 minutes and the precipitated protein was again removed by centrifugation. This was found to extract an additional 20 - 40% of the xylanase and was termed 2° heat treatment extract. Ammonium sulphate was added to this 2° heat treatment extract to 1.0 M and the 1° and 2° heat treatment extracts were pooled prior to phenyl-sepharose separation. Xylanase activity was step-eluted from the 1100 ml bed volume phenyl-sepharose column (after

extensive washing with 1.0 M ammonium sulphate and return to baseline) using 1.0 M NaCl and the eluted protein was concentrated and desalted by ultrafiltration with a YM3 membrane (Amicon). The final concentration of the preparations are 25 mM Tris-HCl (pH 8.0), 5 mM EDTA, 7 mM ß-mercaptoethanol, approximately 250 mM NaCl, 20% glycerol and 0.05% sodium azide.

For production of the G-type xylanases the method was nearly identical to that described above except that HEPES buffer was used in place of TrisHCl for both the extraction (pH 8.0) and the desalting (pH 7.3) steps. The final preparation had a buffer composition of 50 mM HEPES, pH 7.3, 1 mM EDTA, 7 mM ß-mercaptoethanol, 0.05% sodium Azide, 20.0% glycerol and ± 250 mM NaCl.

In total 6 F-type preparations (5 xynA and 1 xynB) and 2 G-type preparation of sufficient purity were obtained. For both the F-type and the G-type preparation the protein concentration was determined by the standard BCA method (Pierce, Rockford, Illinois, USA). The purity of the samples was roughly estimated by running an SDS-PAGE gel (Phast-system, Pharmacia, 20 % gel) and comparing the thickness of the band at about 40 kDa for the F-type, and about 25 kDa for the G-type with the thickness of the bands of the impurities. The purity of the samples varied between 20 and 70% (Table 9).

Table 9: Purity of F-type and G-type xylanase preparations

Enzyme	Purity (%)
TG53xynA	30
TG53xynD (G-type)	20
TG456xynA	70
TG456xynD (G-type)	30
TG457xynA	70
TG479xynA	70
TG631xynA	70
TG631xynB	20

Example 12

ECF bleaching results with cloned F- and G-type xylanases

ECF-bleaching was performed using oxygen delignified Kraft pulp from a Swedish pulp mill. The softwood (SW) pulp had a kappa number of 16.0 and the hardwood (HW) pulp a kappa number of 10.6. A XDED-sequence was used under conditions as presented in Table 10.

Table 10: Bleaching conditions

STAGE	X	D_0	E	D_1
Cons	10 %	10 %	10 %	10 %
Time	120 min	60 min	60 min	120 min.
Temp	65°C	60°C	60°C	70°C
pH	9	≈ 2.5	≈ 10	≈ 3.5
Chemical Dosage	15 µg xyl. protein/ g pulp	chlorine multiple: 0.15	NaOH: 7.2 kg/t (SW) 5.9 kg/t (HW)	aCl_2: 10 kg/t (S/HW)

X = enzyme, D_0,D_1 = chlorine dioxide, E = extraction with NaOH.

The results of these experiments are presented in Table 11. It can be seen that the G-type xylanases perform significantly better as compared with the F-type xylanases, when compared on the basis of enzyme protein.

Table 11: ECF bleaching with cloned F- and G-type xylanases.

Enzyme	Delta brightness (%ISO)	
	SW	HW
TG53xynA	1.0[2]	1.2[5]
TG53xynD (G-type)	5.9[3]/6.8[4]	3.0[6]
TG456xynA	1.2[1]/0.7[2]	1.0[5]
TG456xynD (G-type)	4.3[3]/5.9[4]	4.0[5]/2.1[6]
TG457xynA	0[1]	0[6]
TG479xynA	0.3[2]	0.3[5]
TG631xynA	0[1]	0[6]
TG631xynB	0[2]	-

[1-6] refer to six separate experiments. The reference ISO brightness values of these experiments were as follows: expt 1: 73.5; expt 2: 78.3; expt 3: 52.0; expt 4: 52.3; expt 5: 79.0; expt 6: 80.5.

Example 13

ECF dose-response curves with cloned G-type xylanases

Using the same XDED bleaching sequence as described in Example 12 the dosage of the two G-type xylanases was varied. The results are shown in Table 12. Dosages of 1 to 3 μg/g pulp already give an increase in ISO brightness of at least two points.

Table 12: Dose-response curves of two G-type xylanases

Enzyme	dosage (µg xyl · / g pulp)	Brightness HW (XDED)		Brightness SW (XDED)			
		%ISO	Δ %ISO	%ISO	Δ %ISO	%ISO	Δ %ISO
TG456 xynD	0	78.5	-	55.85	-	62.92	-
	1	80.73	2.23	56.80	0.95	65.73	2.81
	3	81.03	2.53	58.06	2.21	68.12	5.20
	6	80.99	2.49	59.20	3.35	68.32	5.40
	15					70.45	7.53
TG53 xynD	0	78.5	-	55.85	-	66.75	-
	1	79.73	1.23	57.28	1.43	69.04	2.29
	3	80.13	1.63	57.87	2.02	69.35	2.60
	6	81.32	2.82	58.23	2.38	69.92	3.17
	15					72.39	5.64

Example 14

TCF bleaching results with cloned G-type xylanases

The two G-type xylanases were tested in a TCF bleaching sequence using the XQPP sequence as described in Example 15 (below). Oxygen delignified hardwood kraftpulp was used (30 g o.d. per sample). The dosage of the G-type xylanases was varied as indicated in Table 13. ISO brightness values obtained for each sample after X, P1 and P2 stages are given in Table 13. The values represent the average of duplicate samples.

Table 13: TCF bleaching - dose response experiment with G-type xylanases.

Sample	dosage (µg protein/ g. pulp)	X brightness	P1 brightness	P2 brightness
Control	0	50.3	79.5	81.0
TG456 xynD	15	53.8	81.8	84.3
	6	52.9	81.0	83.8
TG53 xynD	15	55.2	82.2	85.1
	6	53.6	82.7	85.1

The experiment was repeated using dosages of 3 and 6 µg protein per g pulp. Only the brightness values obtained after the P2 stage were determined. Results are presented in Table 14.

Table 14. TCF bleaching - dose response experiment with G-type xylanases

Sample	dosage (µg protein/g pulp)	P2 brightness
Control	0	84.5
TG456 xynD	6	85.5
	3	86.5
TG53 xynD	6	86.1
	3	86.8

Example 15

ECF and TCF results, including paper properties

Xylanase samples were tested for bleaching effect in ECF & TCF bleaching of a Kraft H/W and a Kraft S/W pulp supplied from a Swedish pulp mill. The cloned TG456 xynD and TG53 xynD (in this Example referred to as 715 and 716, respectively) are compared with "no-enzyme" reference samples. The refining tests in a Lampen ball mill showed that 715 gave the

overall best performance with considerable improvements in Tear Index and no significant loss in Tensile and Burst Indices.

Screening Protocol

Hardwood		Softwood	
1. TCF Ref	QPP	1. TCF Ref	QPP
2. Enz 715	XQPP	2. Enz 715	XQPP
3. Enz 716	XQPP	3. Enz 716	XQPP
4. ECF Ref	No X DEDED	4. ECF Ref	No X DEDED
5. Enz 715	XDEDED	5. Enz 715	XDEDED
6. Enz 716	XDEDED	6. Enz 716	XDEDED
7. Chem Ref	DEDED	7. Chem Ref	DEDED

Unbleached pulp properties

Pulp	Kappa No	% ISO Bright-ness	Viscosity dm^3/kg
Kraft H/W	10.8	53	1104
Kraft S/W	16.1	36.4	1003

Bleaching parameters to be tested

TCF

% ISO Brightness	Chemical consumption	Kappa No	Viscosity	Paper Strength
After X	XQP	XQPP	XQPP	XQPP
XQP	XQPP			
XQPP				

ECF

% ISO Brightness	Chemical consumption	Kappa No	Viscosity	Paper Strength
After X	XDE	XDE	XDEDED	XDEDED
XDE	XDED			
XDED	XDEDED			
XDEDED				

Bleaching conditions

A) TCF H/W & S/W (Same bleaching conditions for both pulps)

Stage	X	Q	P	P
Consistency (%)	10	9	10	10
Time (mins)	120	5*4 secs in mixer	180	180
Temp. (°C)	65	65	85	85
pH	9	4-5	10.5-11	10.5-11
Enz Charge	3	-	-	-
EDTA Charge (kg/t)	-	3	-	-
NaOH Charge (kg/t)	-	-	20	10
H_2O_2 Charge (kg/t)	-	-	20	10

B) ECF H/W

Stage	X	D	E	D	E	D
Consistency (%)	10	10	10	10	10	10
Time (mins)	120	60	60	120	60	180
Temp (°C)	65	60	60	70	60	70
pH	9	2-3	~11.5	2.5-3	~11.5	4-5
Enz Charge (μg/g)	3	-	-	-	-	-
aCl Charge (kg/t)	-	0.18*Kappa =19.4 for X ref & all X's 21.6 for Chem Ref	-	18	-	8
NaOH Charge (kg/t)	-	-	11.6 for X ref & all X's 13 for Chem Ref	-	10	-

C) ECF S/W

Stage	X	D	E	D	E	D
Consistency (%)	10	10	10	10	10	10
Time (mins)	120	60	60	120	60	180
Temp (°C)	65	60	60	70	60	70
pH	9	2-3	~11.5	2.5-3	~11.5	4-5
Enz Charge (µg/g)	3	-	-	-	-	-
aCl Charge (kg/t)	-	0.18*Kappa =28.8 for X ref & all X's 32 for Chem Ref	-	18	-	8
NaOH Charge (kg/t)	-	-	17.3 for X ref & all X's 19.2 for Chem Ref	-	10	-

The results obtained in the above experiments are present in Tables 15 (Hardwood) and 16 (Softwood).

Table 15: A summary of the Hardwood bleaching results

Hardwood					
	P2 % ISO	P2 Kappa	P2 Visc	Total P	
TCF Ref	78.4	6.7	866	25.2	
TCF 715	81	6.2	850	26.1	+ +--
TCF 716	81.7	6.2	740	27.7	+ +--
	D2 % ISO	XDE Kappa	D2 Visc	Total aCl	
ECF Ref	88.6	4.6	1050	46.1	
ECF 715	89.1	4.5	1035	44.8	+ +-+
ECF 716	88.6	4.6	1040	45	00-+
Chem Ref	88.6	4.9	1020	47	

Table 16: A summary of the Softwood bleaching results.

Softwood					
	P2 % ISO	P2 Kappa	P2 Visc	Total P	
TCF Ref	69.1	7.3	814	18.5	
TCF 715	72.1	6.7	805	16	+ +-+
TCF 716	71	6.7	833	16.7	+ + +
	D2 % ISO	XDE Kappa	D2 Visc	Total aCl	
ECF Ref	85.5	4.6	941	54.5	
ECF 715	86.5	4.4	935	54.4	+ +-0
ECF 716	86.7	4.3	935	54.5	+ +-0
Chem Ref	87	3.7	962	57.3	

Verification of paper properties

After all the sequences were fully bleached, 30 g samples were taken and refined in a Lampen ball mill for 10, 20 and 30 thousand revolutions. Schopper-Rieglers were measured, after which approximately 2 g sheets were made for Tensile Index, Porosity, Tear Index and Burst Index. The sheets were left to condition for 24 hours at 23°C and 50% relative humidity. The results obtained are presented in Figures 4 to 8.

Example 16

pH optimum, temperature optimum and thermostability of TG456 xynD

Activities were measured as described in Example 2, procedure 1, with oat spelt xylane as substrate. All assays were performed in 50 mM phosphate buffer, at the pH and temperature as indicated. The results are presented in Figures 9 and 10.

The TG456 xylanase D is much more thermostable than the reference enzyme Pulpzyme HB (Novo Nordisk), and it has a slightly higher pH optimum.

Throughout the description and claims of the specification the word "comprise" and variations of the word, such as "comprising" and "comprises" is not intended to exclude other additives, components, integers or steps.

AUSTRALIAN PATENT OFFICE

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Gist-brocades B.V.
(B) STREET: Wateringseweg 1
(C) CITY: Delft
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 2611 XT

(ii) TITLE OF INVENTION: Thermostable xylanases

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: xynFA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CACACKCTKG TKTGGCA 17

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: xynFB

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CATACKTTKG TTTGGCA 17

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: xynR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TMGTTKACMA CRTCCCA 17

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: GF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TATNTGRSTN TMTATGGWTG G 21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: GR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCGCTNCTTT GGTANCCTTC 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1065 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Extremophile
(C) INDIVIDUAL ISOLATE: TG456

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..1058
(D) OTHER INFORMATION: /product= "xylanase A"
/gene= "xynA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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CC ATG GAC CTT TAT TCA ATC TCA GAT GAA AAT TGG GGG CAG CCT GTG          47
   Met Asp Leu Tyr Ser Ile Ser Asp Glu Asn Trp Gly Gln Pro Val
    1               5                  10                  15

CCT GAT TAT AAA CTG CCA TCA CTT TGT GAA AAG TAC AAA AAC TAT TTC          95
Pro Asp Tyr Lys Leu Pro Ser Leu Cys Glu Lys Tyr Lys Asn Tyr Phe
                20                  25                  30

AAG ATT GGA GTT GCT GTG CCC TAC AGG GCT CTG ACA AAT CCA GTT GAT         143
Lys Ile Gly Val Ala Val Pro Tyr Arg Ala Leu Thr Asn Pro Val Asp
            35                  40                  45

GTG GAG ATG ATA AAA AGG CAT TTC AAC AGC ATA ACA CCG GAG AAC GAG         191
Val Glu Met Ile Lys Arg His Phe Asn Ser Ile Thr Pro Glu Asn Glu
        50                  55                  60

ATG AAA CCA GAG AGC CTT CAG CCT TAT GAA GGT GGT TTT AGC TTT AGC         239
Met Lys Pro Glu Ser Leu Gln Pro Tyr Glu Gly Gly Phe Ser Phe Ser
    65                  70                  75

ATT GCA GAT GAG TAT ATA GAT TTT TGC AAA AAG AAC AAT ATC TCA CTG         287
Ile Ala Asp Glu Tyr Ile Asp Phe Cys Lys Lys Asn Asn Ile Ser Leu
80                  85                  90                  95

CGA GGG CAC ACK CTT GTT TGG CAT CAG CAA ACC CCG AGC TGG TTC TTT         335
Arg Gly His Thr Leu Val Trp His Gln Gln Thr Pro Ser Trp Phe Phe
                100                 105                 110

ACA AAT CCT GAG ACG GGC GAA AAA CTT ACT AAC AGT GAG AAG GAC AAG         383
Thr Asn Pro Glu Thr Gly Glu Lys Leu Thr Asn Ser Glu Lys Asp Lys
            115                 120                 125

RAA ATA CTA TTG GAT AGG CTA AAG AAG CAC ATC CAG ACA GTT GTT GGC         431
Xaa Ile Leu Leu Asp Arg Leu Lys Lys His Ile Gln Thr Val Val Gly
        130                 135                 140
```

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AGG TAT AAG GGG AAA GTA TAT GCA TGG GAC GTT GTG AAT GAG GCG ATT      479
Arg Tyr Lys Gly Lys Val Tyr Ala Trp Asp Val Val Asn Glu Ala Ile
    145                 150                 155

GAT GAG AAT CAG CCG GAT GGG TAT AGA AGA AGT GAC TGG TAC AAT ATC      527
Asp Glu Asn Gln Pro Asp Gly Tyr Arg Arg Ser Asp Trp Tyr Asn Ile
160                 165                 170                 175

TTR GGA CCG GAG TAC ATT GAA AAG GCA TTT ATC TGG GCG CAT GAA GCA      575
Xaa Gly Pro Glu Tyr Ile Glu Lys Ala Phe Ile Trp Ala His Glu Ala
                180                 185                 190

GAC CCG AAA GCA AAG CTT TTC TAC AAT GAC TAC AGT ACA GAA GAM CCA      623
Asp Pro Lys Ala Lys Leu Phe Tyr Asn Asp Tyr Ser Thr Glu Xaa Pro
            195                 200                 205

TAT AAA AGA GGG AAT TTA TAT ACA CTA ATT AAA AAY TTA AAA GCM AAA      671
Tyr Lys Arg Gly Asn Leu Tyr Thr Leu Ile Lys Asn Leu Lys Ala Lys
        210                 215                 220

GGT GTG CCA GTT CAT GGT GTT GGG CTT CAG TGT CAT ATT TCA CTT GAC      719
Gly Val Pro Val His Gly Val Gly Leu Gln Cys His Ile Ser Leu Asp
    225                 230                 235

TGG CCG GAT GTG AGT GAA ATC GAG GAG ACT GTC AAA TTA TTT AGC AGG      767
Trp Pro Asp Val Ser Glu Ile Glu Glu Thr Val Lys Leu Phe Ser Arg
240                 245                 250                 255

ATT CCA GGA CTT GAA ATA CAC TTC ACA GAA ATT GAT ATA AGT ATT GCT      815
Ile Pro Gly Leu Glu Ile His Phe Thr Glu Ile Asp Ile Ser Ile Ala
                260                 265                 270

AAA AAC ATG ACC GAT GAT GAT GCA TAT AAC CGC TAT CTT TTG ATT CAG      863
Lys Asn Met Thr Asp Asp Asp Ala Tyr Asn Arg Tyr Leu Leu Ile Gln
            275                 280                 285

CAG GCA CAA AAA TTA AAA GCA ATT TTT GAT GTT TTG AAA AAG TAC AGA      911
Gln Ala Gln Lys Leu Lys Ala Ile Phe Asp Val Leu Lys Lys Tyr Arg
        290                 295                 300

AAT GTA GTT ACA AGT GTT ACA TTC TGG GGA CTG AAG GAT GAT TAC TCA      959
Asn Val Val Thr Ser Val Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser
    305                 310                 315

TGG CTA CGG GGA GAT ATG CCA CTT TTA TTC GAT AAA GAC TAC CAG CCA     1007
Trp Leu Arg Gly Asp Met Pro Leu Leu Phe Asp Lys Asp Tyr Gln Pro
320                 325                 330                 335

AAG TTT GCG TTC TGG AGC TTA ATT GAC CCA TCA GTT GTC CCA AAA GAG     1055
Lys Phe Ala Phe Trp Ser Leu Ile Asp Pro Ser Val Val Pro Lys Glu
                340                 345                 350
```

TAATGGATCC 1065

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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Met Asp Leu Tyr Ser Ile Ser Asp Glu Asn Trp Gly Gln Pro Val Pro
 1               5                  10                  15

Asp Tyr Lys Leu Pro Ser Leu Cys Glu Lys Tyr Lys Asn Tyr Phe Lys
            20                  25                  30

Ile Gly Val Ala Val Pro Tyr Arg Ala Leu Thr Asn Pro Val Asp Val
        35                  40                  45

Glu Met Ile Lys Arg His Phe Asn Ser Ile Thr Pro Glu Asn Glu Met
    50                  55                  60

Lys Pro Glu Ser Leu Gln Pro Tyr Glu Gly Gly Phe Ser Phe Ser Ile
65                  70                  75                  80

Ala Asp Glu Tyr Ile Asp Phe Cys Lys Lys Asn Asn Ile Ser Leu Arg
                85                  90                  95

Gly His Thr Leu Val Trp His Gln Gln Thr Pro Ser Trp Phe Phe Thr
            100                 105                 110

Asn Pro Glu Thr Gly Glu Lys Leu Thr Asn Ser Glu Lys Asp Lys Xaa
        115                 120                 125

Ile Leu Leu Asp Arg Leu Lys Lys His Ile Gln Thr Val Val Gly Arg
    130                 135                 140

Tyr Lys Gly Lys Val Tyr Ala Trp Asp Val Val Asn Glu Ala Ile Asp
145                 150                 155                 160

Glu Asn Gln Pro Asp Gly Tyr Arg Arg Ser Asp Trp Tyr Asn Ile Xaa
                165                 170                 175

Gly Pro Glu Tyr Ile Glu Lys Ala Phe Ile Trp Ala His Glu Ala Asp
            180                 185                 190
```

```
Pro Lys Ala Lys Leu Phe Tyr Asn Asp Tyr Ser Thr Glu Xaa Pro Tyr
        195                 200                 205

Lys Arg Gly Asn Leu Tyr Thr Leu Ile Lys Asn Leu Lys Ala Lys Gly
    210                 215                 220

Val Pro Val His Gly Val Gly Leu Gln Cys His Ile Ser Leu Asp Trp
225                 230                 235                 240

Pro Asp Val Ser Glu Ile Glu Glu Thr Val Lys Leu Phe Ser Arg Ile
                245                 250                 255

Pro Gly Leu Glu Ile His Phe Thr Glu Ile Asp Ile Ser Ile Ala Lys
            260                 265                 270

Asn Met Thr Asp Asp Asp Ala Tyr Asn Arg Tyr Leu Leu Ile Gln Gln
        275                 280                 285

Ala Gln Lys Leu Lys Ala Ile Phe Asp Val Leu Lys Lys Tyr Arg Asn
    290                 295                 300

Val Val Thr Ser Val Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser Trp
305                 310                 315                 320

Leu Arg Gly Asp Met Pro Leu Leu Phe Asp Lys Asp Tyr Gln Pro Lys
                325                 330                 335

Phe Ala Phe Trp Ser Leu Ile Asp Pro Ser Val Val Pro Lys Glu
            340                 345                 350
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1633 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Extremophile
(C) INDIVIDUAL ISOLATE: TG456

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1632
(D) OTHER INFORMATION: /partial
/product= "xylanase B"
/gene= "xynB"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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TGT ACC ATA AAA TGG CAG CAA ACA GTT CCA TCT GGG GTT TGG ACA GAA      48
Cys Thr Ile Lys Trp Gln Gln Thr Val Pro Ser Gly Val Trp Thr Glu
 1               5                  10                  15

GTT TCT GGT TCA TAT ACA GTA CCA CAG ACA GCA ACC CAG CTC ATA TTC      96
Val Ser Gly Ser Tyr Thr Val Pro Gln Thr Ala Thr Gln Leu Ile Phe
            20                  25                  30

TAT GTG GAA TCG CCA AAT GCA ACA CTT GAC TTT TAC CTT GAC GAC TTT     144
Tyr Val Glu Ser Pro Asn Ala Thr Leu Asp Phe Tyr Leu Asp Asp Phe
        35                  40                  45

ACT GTA ATA GAC AAA AAC CCG GTT ACA ATA CCT GCT GCG GCA AAA GAG     192
Thr Val Ile Asp Lys Asn Pro Val Thr Ile Pro Ala Ala Ala Lys Glu
    50                  55                  60

CCA GAG TTG GAG ATT CCA TCA CTT TGC CAG CAA TAC AGC CAG TAC TTT     240
Pro Glu Leu Glu Ile Pro Ser Leu Cys Gln Gln Tyr Ser Gln Tyr Phe
65                  70                  75                  80

TCA ATT GGT GTT GCA ATA CCA TAT AGA GTG CTC CAA AAC CCG GTA GAA     288
Ser Ile Gly Val Ala Ile Pro Tyr Arg Val Leu Gln Asn Pro Val Glu
                85                  90                  95

AGA GCA ATG GTT TTA AAG CAT TTC AAC AGT ATT ACT GCT GAA AAT GAG     336
Arg Ala Met Val Leu Lys His Phe Asn Ser Ile Thr Ala Glu Asn Glu
            100                 105                 110

ATG AAG CCC GAT GCT ATA CAA AGA ACA GAA GGG CAG TTC AAT TTC GAT     384
Met Lys Pro Asp Ala Ile Gln Arg Thr Glu Gly Gln Phe Asn Phe Asp
        115                 120                 125

GTT GCA GAC CAG TAT GTT GAC TTT GCA CAG AGC AAT AAT ATT GGA ATA     432
Val Ala Asp Gln Tyr Val Asp Phe Ala Gln Ser Asn Asn Ile Gly Ile
    130                 135                 140

AGA GGT CAT ACA CTG GTT TGG CAT CAA CAA ACT CCA GAT TGG TTT TTC     480
Arg Gly His Thr Leu Val Trp His Gln Gln Thr Pro Asp Trp Phe Phe
145                 150                 155                 160

CAG CAT TCT GAC GGT TCG CCA CTT GAT CCA AAC AAT TCT GAA GAC AAG     528
Gln His Ser Asp Gly Ser Pro Leu Asp Pro Asn Asn Ser Glu Asp Lys
```

```
                    165                 170                 175

    CAG CTT TTG AGA AAT AGG TTA AAA ACA CAC ATT CAG ACA CTT GTT GGA      576
    Gln Leu Leu Arg Asn Arg Leu Lys Thr His Ile Gln Thr Leu Val Gly
5               180                 185                 190

    AGA TAT GCA GAG AAA GTT TAT GCA TGG GAT GTT GTA AAT GAA GCA ATT      624
    Arg Tyr Ala Glu Lys Val Tyr Ala Trp Asp Val Val Asn Glu Ala Ile
            195                 200                 205
10
    GAT GAA AAT CAA CCG GAT GGA TAT AGA AGA AGT GAA TGG TAC AGA ATT      672
    Asp Glu Asn Gln Pro Asp Gly Tyr Arg Arg Ser Glu Trp Tyr Arg Ile
        210                 215                 220

15  TTA GGA CCA ACT CCA GAA ACA GGC GGA ATA CCA GAG TAT ATA ATC CTT      720
    Leu Gly Pro Thr Pro Glu Thr Gly Gly Ile Pro Glu Tyr Ile Ile Leu
    225                 230                 235                 240

    GCA TTC CAG TAT GCA CGG GAA GCT GAC CCG AAC GCA AAA CTT TTC TAC      768
20  Ala Phe Gln Tyr Ala Arg Glu Ala Asp Pro Asn Ala Lys Leu Phe Tyr
                    245                 250                 255

    AAC GAT TAC AGC ACT GAA AAT CCA AAG AAG AGA CAG TTT ATT TAC AAC      816
    Asn Asp Tyr Ser Thr Glu Asn Pro Lys Lys Arg Gln Phe Ile Tyr Asn
25              260                 265                 270

    ATG GTC AAA GCT TTG CAT GAT AGA GGT CTC ATT GAT GGT GTT GGT CTG      864
    Met Val Lys Ala Leu His Asp Arg Gly Leu Ile Asp Gly Val Gly Leu
            275                 280                 285
30
    CAG GGA CAT ATT AAT GTG GAT TCG CCT GCA GTC AAA GAA ATA GAA GAT      912
    Gln Gly His Ile Asn Val Asp Ser Pro Ala Val Lys Glu Ile Glu Asp
        290                 295                 300

35  ACA ATC AAT TTA TTC AGC ACA ATA CCG GGT CTT CAA ATT CAA ATA ACA      960
    Thr Ile Asn Leu Phe Ser Thr Ile Pro Gly Leu Gln Ile Gln Ile Thr
    305                 310                 315                 320

    GAG CTT GAT ATC AGC GTA TAT ACA AGC AGC ACT CAG CAA TAT GAC ACA     1008
40  Glu Leu Asp Ile Ser Val Tyr Thr Ser Ser Thr Gln Gln Tyr Asp Thr
                    325                 330                 335

    TTA CCA CAG GAT ATT ATG ATT AAA CAG GCT TTA AAA TTC AAA GAG CTG     1056
    Leu Pro Gln Asp Ile Met Ile Lys Gln Ala Leu Lys Phe Lys Glu Leu
45              340                 345                 350

    TTT GAA ATG TTA AAG CGC CAC AGC GAC AGA ATC ACA AAT GTT ACA CTT     1104
    Phe Glu Met Leu Lys Arg His Ser Asp Arg Ile Thr Asn Val Thr Leu
            355                 360                 365
50
    TGG GGT CTC AAA GAT GAT TAT CCA TGG CTG TCA AAA GAT AGA AGT AAC     1152
    Trp Gly Leu Lys Asp Asp Tyr Pro Trp Leu Ser Lys Asp Arg Ser Asn
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```
        370                 375                 380

    TGG CCA CTG CTA TTT GAT AGT AAC TAC CAG GCA AAA TAC AAT TAC TGG      1200
    Trp Pro Leu Leu Phe Asp Ser Asn Tyr Gln Ala Lys Tyr Asn Tyr Trp
385                 390                 395                 400

    GCT ATT GTA GAA CCT TCG GTG TTG CCT GTT GCT ATA AAT AAG GGA TAT      1248
    Ala Ile Val Glu Pro Ser Val Leu Pro Val Ala Ile Asn Lys Gly Tyr
                405                 410                 415

    GCG AAC AAT GCA CAG CCA AGA ATT GAT GGG ATT ATG GAT AAA GAA TAC      1296
    Ala Asn Asn Ala Gln Pro Arg Ile Asp Gly Ile Met Asp Lys Glu Tyr
            420                 425                 430

    AAA GGA ACC ATT CCA CTT TCG GTT TTG AAT GAT GCA GGG CAG GAT ATT      1344
    Lys Gly Thr Ile Pro Leu Ser Val Leu Asn Asp Ala Gly Gln Asp Ile
        435                 440                 445

    GCT CAG GTA AGG GCA CTG TGG AGT GGC AAT GAG CTT TGT CTT TAT GTC      1392
    Ala Gln Val Arg Ala Leu Trp Ser Gly Asn Glu Leu Cys Leu Tyr Val
    450                 455                 460

    ACT GTA AAT GAT TCA AGT GTG GAT GCT AAC AAT GAT AGG GTT GTA ATT      1440
    Thr Val Asn Asp Ser Ser Val Asp Ala Asn Asn Asp Arg Val Val Ile
465                 470                 475                 480

    TTC ATT GAT CAG GAC AAT GGA AAG TTG CCA GAG TTA AAA GAT GAT GAC      1488
    Phe Ile Asp Gln Asp Asn Gly Lys Leu Pro Glu Leu Lys Asp Asp Asp
                485                 490                 495

    TTC TGG GTT TCA ATT TCG AGA AAT GGC ACA AAG AAT CAA TCC AAA ACT      1536
    Phe Trp Val Ser Ile Ser Arg Asn Gly Thr Lys Asn Gln Ser Lys Thr
            500                 505                 510

    GGC TAT GTA AAA GAT TAT GTA GTG TTA CAG CAA TTA AAT GGA TAT ACA      1584
    Gly Tyr Val Lys Asp Tyr Val Val Leu Gln Gln Leu Asn Gly Tyr Thr
        515                 520                 525

    ATG GAG GTT AAG CTG CTT TTA AAC AAC AGT TTA GCA ATT AAC ACA AAT      1632
    Met Glu Val Lys Leu Leu Leu Asn Asn Ser Leu Ala Ile Asn Thr Asn
    530                 535                 540

    A                                                                   1633
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 544 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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Cys Thr Ile Lys Trp Gln Gln Thr Val Pro Ser Gly Val Trp Thr Glu
 1               5                  10                  15

Val Ser Gly Ser Tyr Thr Val Pro Gln Thr Ala Thr Gln Leu Ile Phe
            20                  25                  30

Tyr Val Glu Ser Pro Asn Ala Thr Leu Asp Phe Tyr Leu Asp Asp Phe
        35                  40                  45

Thr Val Ile Asp Lys Asn Pro Val Thr Ile Pro Ala Ala Ala Lys Glu
    50                  55                  60

Pro Glu Leu Glu Ile Pro Ser Leu Cys Gln Gln Tyr Ser Gln Tyr Phe
65                  70                  75                  80

Ser Ile Gly Val Ala Ile Pro Tyr Arg Val Leu Gln Asn Pro Val Glu
                85                  90                  95

Arg Ala Met Val Leu Lys His Phe Asn Ser Ile Thr Ala Glu Asn Glu
            100                 105                 110

Met Lys Pro Asp Ala Ile Gln Arg Thr Glu Gly Gln Phe Asn Phe Asp
        115                 120                 125

Val Ala Asp Gln Tyr Val Asp Phe Ala Gln Ser Asn Asn Ile Gly Ile
    130                 135                 140

Arg Gly His Thr Leu Val Trp His Gln Gln Thr Pro Asp Trp Phe Phe
145                 150                 155                 160

Gln His Ser Asp Gly Ser Pro Leu Asp Pro Asn Asn Ser Glu Asp Lys
                165                 170                 175

Gln Leu Leu Arg Asn Arg Leu Lys Thr His Ile Gln Thr Leu Val Gly
            180                 185                 190

Arg Tyr Ala Glu Lys Val Tyr Ala Trp Asp Val Val Asn Glu Ala Ile
        195                 200                 205

Asp Glu Asn Gln Pro Asp Gly Tyr Arg Arg Ser Glu Trp Tyr Arg Ile
    210                 215                 220

Leu Gly Pro Thr Pro Glu Thr Gly Gly Ile Pro Glu Tyr Ile Ile Leu
225                 230                 235                 240

Ala Phe Gln Tyr Ala Arg Glu Ala Asp Pro Asn Ala Lys Leu Phe Tyr
```

245 250 255

Asn Asp Tyr Ser Thr Glu Asn Pro Lys Lys Arg Gln Phe Ile Tyr Asn
260 265 270

Met Val Lys Ala Leu His Asp Arg Gly Leu Ile Asp Gly Val Gly Leu
275 280 285

Gln Gly His Ile Asn Val Asp Ser Pro Ala Val Lys Glu Ile Glu Asp
290 295 300

Thr Ile Asn Leu Phe Ser Thr Ile Pro Gly Leu Gln Ile Gln Ile Thr
305 310 315 320

Glu Leu Asp Ile Ser Val Tyr Thr Ser Ser Thr Gln Gln Tyr Asp Thr
325 330 335

Leu Pro Gln Asp Ile Met Ile Lys Gln Ala Leu Lys Phe Lys Glu Leu
340 345 350

Phe Glu Met Leu Lys Arg His Ser Asp Arg Ile Thr Asn Val Thr Leu
355 360 365

Trp Gly Leu Lys Asp Asp Tyr Pro Trp Leu Ser Lys Asp Arg Ser Asn
370 375 380

Trp Pro Leu Leu Phe Asp Ser Asn Tyr Gln Ala Lys Tyr Asn Tyr Trp
385 390 395 400

Ala Ile Val Glu Pro Ser Val Leu Pro Val Ala Ile Asn Lys Gly Tyr
405 410 415

Ala Asn Asn Ala Gln Pro Arg Ile Asp Gly Ile Met Asp Lys Glu Tyr
420 425 430

Lys Gly Thr Ile Pro Leu Ser Val Leu Asn Asp Ala Gly Gln Asp Ile
435 440 445

Ala Gln Val Arg Ala Leu Trp Ser Gly Asn Glu Leu Cys Leu Tyr Val
450 455 460

Thr Val Asn Asp Ser Ser Val Asp Ala Asn Asn Asp Arg Val Val Ile
465 470 475 480

Phe Ile Asp Gln Asp Asn Gly Lys Leu Pro Glu Leu Lys Asp Asp Asp
485 490 495

Phe Trp Val Ser Ile Ser Arg Asn Gly Thr Lys Asn Gln Ser Lys Thr
500 505 510

Gly Tyr Val Lys Asp Tyr Val Val Leu Gln Gln Leu Asn Gly Tyr Thr

515 520 525

Met Glu Val Lys Leu Leu Leu Asn Asn Ser Leu Ala Ile Asn Thr Asn
530 535 540

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1125 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Extremophile
(C) INDIVIDUAL ISOLATE: TG456

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1125
(D) OTHER INFORMATION: /partial
/product= "xylanase C"
/gene= "xynC"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CGC GAG TTA TTA CTT TAT GTT GAG GCG CAA AAT GCA AAT TTG GCT TTC 48
Arg Glu Leu Leu Leu Tyr Val Glu Ala Gln Asn Ala Asn Leu Ala Phe
1 5 10 15

TGG GTT GAT GAT TTA AAG ATT TAT GAT TTA TCC AAG CTG GCT GAA CCT 96
Trp Val Asp Asp Leu Lys Ile Tyr Asp Leu Ser Lys Leu Ala Glu Pro
20 25 30

GAA TGG GAG ATA CCA TCT TTG ATA GAA AAG TAT AAA GAT TAT TTC AAA 144
Glu Trp Glu Ile Pro Ser Leu Ile Glu Lys Tyr Lys Asp Tyr Phe Lys
35 40 45

GTA GGG GTA GCT TTG TCT TAC AAA AGC ATT GCT YCT GAT ACA GAG AAG 192
Val Gly Val Ala Leu Ser Tyr Lys Ser Ile Ala Xaa Asp Thr Glu Lys
50 55 60

```
AAG ATG GTT TTG AAG CAT TTC AAT AGT ATT ACT GCA GGG AAT GAA ATG      240
Lys Met Val Leu Lys His Phe Asn Ser Ile Thr Ala Gly Asn Glu Met
 65                  70                  75                  80

AAA CCA TCA GAG TTA CTT ATC AGT GAA AAT AAT TAT AAC TTT AGT AAA      288
Lys Pro Ser Glu Leu Leu Ile Ser Glu Asn Asn Tyr Asn Phe Ser Lys
                85                  90                  95

GCA GAT GAA TTT GTA AAT TTT GCA ACA AGT AAC AAC ATT GCC ATC AGA      336
Ala Asp Glu Phe Val Asn Phe Ala Thr Ser Asn Asn Ile Ala Ile Arg
            100                 105                 110

GGT CAT ACA CTG GTT TGG CAT GAG CAA ACA CCC GAC TGG TTT TTC AAG      384
Gly His Thr Leu Val Trp His Glu Gln Thr Pro Asp Trp Phe Phe Lys
        115                 120                 125

GAT GCA AAT GGA AAT ACC TTG AGC AAG GAT GCA TTG CTA AGC AGA TTA      432
Asp Ala Asn Gly Asn Thr Leu Ser Lys Asp Ala Leu Leu Ser Arg Leu
    130                 135                 140

AAG CAG TAT ATT TAT ACG GTA GTG GGA AGA TAT AAA GGG AAG GTT TAT      480
Lys Gln Tyr Ile Tyr Thr Val Val Gly Arg Tyr Lys Gly Lys Val Tyr
145                 150                 155                 160

GCA TGG GAT GTG GTA AAT RAA GCA ATA GAT GAA AGT CAA GGT AAT GGA      528
Ala Trp Asp Val Val Asn Xaa Ala Ile Asp Glu Ser Gln Gly Asn Gly
                165                 170                 175

TTC AGG AGA TCT AAC TGG TAC AAC ATT TGT GGT CCC GAA TAT ATT GAA      576
Phe Arg Arg Ser Asn Trp Tyr Asn Ile Cys Gly Pro Glu Tyr Ile Glu
            180                 185                 190

AAG GCT TTT ATA TGG GCA CAT GAR GCC GAT CCA GAC GCA AAA TTG TTT      624
Lys Ala Phe Ile Trp Ala His Glu Ala Asp Pro Asp Ala Lys Leu Phe
        195                 200                 205

TAC AAC GAT TAC AAC ACA GAA AAC AGT CAG AAG AGA CAG TTT ATT TMC      672
Tyr Asn Asp Tyr Asn Thr Glu Asn Ser Gln Lys Arg Gln Phe Ile Xaa
    210                 215                 220

AAC ATG ATT AAG AGT CTC AAG GAA AAA GGT GTT CCA ATT CAT GGA ATA      720
Asn Met Ile Lys Ser Leu Lys Glu Lys Gly Val Pro Ile His Gly Ile
225                 230                 235                 240

GGA TTG CGG TGT CAT ATA AAT CTT GAT TGG CCC TCG ATT AGC GAG ATA      768
Gly Leu Arg Cys His Ile Asn Leu Asp Trp Pro Ser Ile Ser Glu Ile
                245                 250                 255

GAG AAC ACC ATA AAA TTG TTC AGC TCT ATA CCT GGA TTG GAG ATA CAC      816
Glu Asn Thr Ile Lys Leu Phe Ser Ser Ile Pro Gly Leu Glu Ile His
            260                 265                 270
```

```
ATT ACG GAG CTT GAT ATG AGT TTT TAT CAG TGG GGT TCG AGT ACC AGT        864
Ile Thr Glu Leu Asp Met Ser Phe Tyr Gln Trp Gly Ser Ser Thr Ser
        275                 280                 285

TAT TCA ACG CCA CCM AGA GAT CTC CTG ATA AAA CAG GCA ATG AGA TAT        912
Tyr Ser Thr Pro Pro Arg Asp Leu Leu Ile Lys Gln Ala Met Arg Tyr
    290                 295                 300

AAG GAG TTA TTC GAT TTA TTT AAA AAG TAC AAT GTA ATA ACT AAT GTA        960
Lys Glu Leu Phe Asp Leu Phe Lys Lys Tyr Asn Val Ile Thr Asn Val
305                 310                 315                 320

ACA TTC TGG GGA CTA AAG GAT GAT TAC TCA TGG CTG AGT CAA AAC TTT       1008
Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser Trp Leu Ser Gln Asn Phe
                325                 330                 335

GGA AAA AGT GAT TAC CCG TTG TTA TTT GAT GGA AAC TAT AAG TCA AAA       1056
Gly Lys Ser Asp Tyr Pro Leu Leu Phe Asp Gly Asn Tyr Lys Ser Lys
            340                 345                 350

TAT GCC TTT TGG AGC CTG ATT GAG CCA ACT GTG GTG CCG GTT ACC GGT       1104
Tyr Ala Phe Trp Ser Leu Ile Glu Pro Thr Val Val Pro Val Thr Gly
        355                 360                 365

CAT AGC TGT TTT TGC GCC ATG                                          1125
His Ser Cys Phe Cys Ala Met
    370                 375
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Arg Glu Leu Leu Leu Tyr Val Glu Ala Gln Asn Ala Asn Leu Ala Phe
  1               5                  10                  15

Trp Val Asp Asp Leu Lys Ile Tyr Asp Leu Ser Lys Leu Ala Glu Pro
            20                  25                  30

Glu Trp Glu Ile Pro Ser Leu Ile Glu Lys Tyr Lys Asp Tyr Phe Lys
        35                  40                  45

Val Gly Val Ala Leu Ser Tyr Lys Ser Ile Ala Xaa Asp Thr Glu Lys
```

50 55 60

Lys Met Val Leu Lys His Phe Asn Ser Ile Thr Ala Gly Asn Glu Met
65 70 75 80

Lys Pro Ser Glu Leu Leu Ile Ser Glu Asn Asn Tyr Asn Phe Ser Lys
85 90 95

Ala Asp Glu Phe Val Asn Phe Ala Thr Ser Asn Asn Ile Ala Ile Arg
100 105 110

Gly His Thr Leu Val Trp His Glu Gln Thr Pro Asp Trp Phe Phe Lys
115 120 125

Asp Ala Asn Gly Asn Thr Leu Ser Lys Asp Ala Leu Leu Ser Arg Leu
130 135 140

Lys Gln Tyr Ile Tyr Thr Val Val Gly Arg Tyr Lys Gly Lys Val Tyr
145 150 155 160

Ala Trp Asp Val Val Asn Xaa Ala Ile Asp Glu Ser Gln Gly Asn Gly
165 170 175

Phe Arg Arg Ser Asn Trp Tyr Asn Ile Cys Gly Pro Glu Tyr Ile Glu
180 185 190

Lys Ala Phe Ile Trp Ala His Glu Ala Asp Pro Asp Ala Lys Leu Phe
195 200 205

Tyr Asn Asp Tyr Asn Thr Glu Asn Ser Gln Lys Arg Gln Phe Ile Xaa
210 215 220

Asn Met Ile Lys Ser Leu Lys Glu Lys Gly Val Pro Ile His Gly Ile
225 230 235 240

Gly Leu Arg Cys His Ile Asn Leu Asp Trp Pro Ser Ile Ser Glu Ile
245 250 255

Glu Asn Thr Ile Lys Leu Phe Ser Ser Ile Pro Gly Leu Glu Ile His
260 265 270

Ile Thr Glu Leu Asp Met Ser Phe Tyr Gln Trp Gly Ser Ser Thr Ser
275 280 285

Tyr Ser Thr Pro Pro Arg Asp Leu Leu Ile Lys Gln Ala Met Arg Tyr
290 295 300

Lys Glu Leu Phe Asp Leu Phe Lys Lys Tyr Asn Val Ile Thr Asn Val
305 310 315 320

Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser Trp Leu Ser Gln Asn Phe
325 330 335

```
Gly Lys Ser Asp Tyr Pro Leu Leu Phe Asp Gly Asn Tyr Lys Ser Lys
            340                 345                 350

Tyr Ala Phe Trp Ser Leu Ile Glu Pro Thr Val Val Pro Val Thr Gly
        355                 360                 365

His Ser Cys Phe Cys Ala Met
    370                 375
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1244 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Extremophile
(C) INDIVIDUAL ISOLATE: TG456

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1107
(D) OTHER INFORMATION: /partial
/product= "xylanase D"
/gene= "xynD"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
AAA GTC TTG CTG GCT GCT CTG ATG TGT GTT GTG TTG GCT AAT CCT TTT        48
Lys Val Leu Leu Ala Ala Leu Met Cys Val Val Leu Ala Asn Pro Phe
  1               5                  10                  15

TAT GCA CAG GCA GCC ATG ACA TTT ACC TCT AAT GCA ACT GGG ACA TAC        96
Tyr Ala Gln Ala Ala Met Thr Phe Thr Ser Asn Ala Thr Gly Thr Tyr
            20                  25                  30

GAT GGT TAC TAC TAC GAG TTG TGG AAG GAC ACA GGG AAT ACT ACC ATG       144
Asp Gly Tyr Tyr Tyr Glu Leu Trp Lys Asp Thr Gly Asn Thr Thr Met
        35                  40                  45

ACA GTT GAC ACA GGA GGA AGA TTT AGC TGT CAG TGG AGT AAC ATT AAC       192
```

```
Thr Val Asp Thr Gly Gly Arg Phe Ser Cys Gln Trp Ser Asn Ile Asn
     50                  55                  60

AAT GCA CTC TTC AGA ACA GGT AAA AAG TTT AGC ACT GCA TGG AAT CAG      240
Asn Ala Leu Phe Arg Thr Gly Lys Lys Phe Ser Thr Ala Trp Asn Gln
 65              70                  75                  80

CTT GGG ACT GTA AAG ATT ACC TAC TCT GCT ACC TAC AAT CCA AAT GGC      288
Leu Gly Thr Val Lys Ile Thr Tyr Ser Ala Thr Tyr Asn Pro Asn Gly
                 85                  90                  95

AAT TCC TAT CTC TGC ATT TAT GGA TGG TCA AGA AAT CCA CTT GTT GAA      336
Asn Ser Tyr Leu Cys Ile Tyr Gly Trp Ser Arg Asn Pro Leu Val Glu
            100                 105                 110

TTT TAT ATC GTT GAA AGC TGG GGC TCA TGG CGT CCG CCC GGG GCA ACG      384
Phe Tyr Ile Val Glu Ser Trp Gly Ser Trp Arg Pro Pro Gly Ala Thr
        115                 120                 125

TCA CTT GGC ACT GTA ACA ATT GAT GGA GCA ACA TAT GAT ATT TAT AAG      432
Ser Leu Gly Thr Val Thr Ile Asp Gly Ala Thr Tyr Asp Ile Tyr Lys
    130                 135                 140

ACA ACT CGT GTT AAT CAG CCA TCT ATC GAA GGA ACA AGA ACA TTT GAT      480
Thr Thr Arg Val Asn Gln Pro Ser Ile Glu Gly Thr Arg Thr Phe Asp
145                 150                 155                 160

CAG TAC TGG AGT GTT AGG ACA TCA AAG AGA ACA AGT GGT ACT GTT ACT      528
Gln Tyr Trp Ser Val Arg Thr Ser Lys Arg Thr Ser Gly Thr Val Thr
                165                 170                 175

GTA ACT GAT CAT TTC AAA GCA TGG GCT GCA AAA GGT TTG AAC CTG GGT      576
Val Thr Asp His Phe Lys Ala Trp Ala Ala Lys Gly Leu Asn Leu Gly
            180                 185                 190

ACA ATT GAC CAG ATT ACA CTC TGT GTG GAA GGY TAC CAR AGC AGC GGC      624
Thr Ile Asp Gln Ile Thr Leu Cys Val Glu Gly Tyr Gln Ser Ser Gly
        195                 200                 205

TCA GCA AAT ATA ACA CAG AAT ACA TTT ACT ATT GGT GGT TCG AGT AGT      672
Ser Ala Asn Ile Thr Gln Asn Thr Phe Thr Ile Gly Gly Ser Ser Ser
    210                 215                 220

GGC TCA AGT AAT GGT TCA AAT AAC GGT TCA AAT GAT GGT TCC AAT GGA      720
Gly Ser Ser Asn Gly Ser Asn Asn Gly Ser Asn Asp Gly Ser Asn Gly
225                 230                 235                 240

GGA ACA AAT GCA GGA ATT TCA ACY GCA AGC AGG ATA GAA TGT GAA AGT      768
Gly Thr Asn Ala Gly Ile Ser Thr Ala Ser Arg Ile Glu Cys Glu Ser
                245                 250                 255

ATG TCG CTC AGC GGY CCT TAT GTT TCA AGA ATT ACT TAT CCA TTT AAT      816
```

```
Met Ser Leu Ser Gly Pro Tyr Val Ser Arg Ile Thr Tyr Pro Phe Asn
            260                 265                 270

GGT ATA GCA CTT TAT GCG AAC GGA GAT AGA GCA ACG GCA AAT GTA AAC      864
Gly Ile Ala Leu Tyr Ala Asn Gly Asp Arg Ala Thr Ala Asn Val Asn
        275                 280                 285

TTT TCA GCA AGC CGT AAC TAT ACT TTT AAA TTA CGT GGA TGT GGA AAT      912
Phe Ser Ala Ser Arg Asn Tyr Thr Phe Lys Leu Arg Gly Cys Gly Asn
    290                 295                 300

AAC AAT AAT TTG GCA TCA GTT GAT TTA CTG ATA GAT GGA AAG AAA GTA      960
Asn Asn Asn Leu Ala Ser Val Asp Leu Leu Ile Asp Gly Lys Lys Val
305                 310                 315                 320

GGT TCG TTC TAT TAT AAG GGA ACA TAT CCT TGG GAA GCY TCT ATA AAT     1008
Gly Ser Phe Tyr Tyr Lys Gly Thr Tyr Pro Trp Glu Ala Ser Ile Asn
                325                 330                 335

AAT GTG TAT GTA AGT GCA GGT ACC CAC AGA GWG GAG CTT GTA CTT TCT     1056
Asn Val Tyr Val Ser Ala Gly Thr His Arg Xaa Glu Leu Val Leu Ser
            340                 345                 350

GCT GAT AAT GGT ACA TGG GAT GTC TAT GCG GAT TAT TTG TTA ATA CAA     1104
Ala Asp Asn Gly Thr Trp Asp Val Tyr Ala Asp Tyr Leu Leu Ile Gln
        355                 360                 365

TGAAATTCGG AAATGTTTTT AAAAATACTG CTTCGRAGAA GCAGGTATTT TTTTATGTTC    1164

ACTATTATAA AGCGATTGAA GTCAGTCTTA GTTCATCCTA GTTGTTCTTC ARACCGRTCA    1224

TAGCTGTTTC CKGCGCCATG                                               1244
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 368 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Lys Val Leu Leu Ala Ala Leu Met Cys Val Val Leu Ala Asn Pro Phe
  1               5                  10                  15

Tyr Ala Gln Ala Ala Met Thr Phe Thr Ser Asn Ala Thr Gly Thr Tyr
            20                  25                  30
```

```
Asp Gly Tyr Tyr Tyr Glu Leu Trp Lys Asp Thr Gly Asn Thr Thr Met
          35                  40                  45

Thr Val Asp Thr Gly Gly Arg Phe Ser Cys Gln Trp Ser Asn Ile Asn
      50                  55                  60

Asn Ala Leu Phe Arg Thr Gly Lys Lys Phe Ser Thr Ala Trp Asn Gln
 65                  70                  75                  80

Leu Gly Thr Val Lys Ile Thr Tyr Ser Ala Thr Tyr Asn Pro Asn Gly
                 85                  90                  95

Asn Ser Tyr Leu Cys Ile Tyr Gly Trp Ser Arg Asn Pro Leu Val Glu
            100                 105                 110

Phe Tyr Ile Val Glu Ser Trp Gly Ser Trp Arg Pro Pro Gly Ala Thr
        115                 120                 125

Ser Leu Gly Thr Val Thr Ile Asp Gly Ala Thr Tyr Asp Ile Tyr Lys
    130                 135                 140

Thr Thr Arg Val Asn Gln Pro Ser Ile Glu Gly Thr Arg Thr Phe Asp
145                 150                 155                 160

Gln Tyr Trp Ser Val Arg Thr Ser Lys Arg Thr Ser Gly Thr Val Thr
                165                 170                 175

Val Thr Asp His Phe Lys Ala Trp Ala Ala Lys Gly Leu Asn Leu Gly
            180                 185                 190

Thr Ile Asp Gln Ile Thr Leu Cys Val Glu Gly Tyr Gln Ser Ser Gly
        195                 200                 205

Ser Ala Asn Ile Thr Gln Asn Thr Phe Thr Ile Gly Gly Ser Ser Ser
    210                 215                 220

Gly Ser Ser Asn Gly Ser Asn Asn Gly Ser Asn Asp Gly Ser Asn Gly
225                 230                 235                 240

Gly Thr Asn Ala Gly Ile Ser Thr Ala Ser Arg Ile Glu Cys Glu Ser
                245                 250                 255

Met Ser Leu Ser Gly Pro Tyr Val Ser Arg Ile Thr Tyr Pro Phe Asn
            260                 265                 270

Gly Ile Ala Leu Tyr Ala Asn Gly Asp Arg Ala Thr Ala Asn Val Asn
        275                 280                 285

Phe Ser Ala Ser Arg Asn Tyr Thr Phe Lys Leu Arg Gly Cys Gly Asn
    290                 295                 300
```

```
Asn Asn Asn Leu Ala Ser Val Asp Leu Leu Ile Asp Gly Lys Lys Val
305                 310                 315                 320

Gly Ser Phe Tyr Tyr Lys Gly Thr Tyr Pro Trp Glu Ala Ser Ile Asn
                325                 330                 335

Asn Val Tyr Val Ser Ala Gly Thr His Arg Xaa Glu Leu Val Leu Ser
            340                 345                 350

Ala Asp Asn Gly Thr Trp Asp Val Tyr Ala Asp Tyr Leu Leu Ile Gln
        355                 360                 365
```

1. A xylanase wherein it has a significant delignifying activity at a temperature of at least 80°C and at pH 9 and comprises an amino acid sequence that shares at least 70% identity with the amino acid sequence of SEQ ID NO. 13.

2. A xylanase according to claim 1 having a half-life at 80°C and pH 9.0 of more than 10 minutes.

3. A xylanase according to claim 1 or 2 wherein:

a. the xylanase is a G-type xylanase, and

b. the xylanase is derived from a thermophilic organism with an optimum growth temperature in excess of 65°C.

4. A xylanase according to claim 3 wherein the thermophilic organism is anaerobic.

5. A xylanase according to any preceding claim wherein the xylanase has a half-life at 80°C and at pH 7.0 of more than 10 minutes.

6. A xylanase according to any preceding claim wherein the xylanase has an half-life at 65°C and at pH 9.0 of more than 10 minutes.

7. A xylanase according to any preceding claim wherein the xylanase is derived from a strain deposited under one of the deposition numbers: CBS 211.94, 212.94, 213.94, 214.94, 215.94 and 216.94.

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8. An isolated and purified DNA sequence wherein it encodes a xylanase according to any preceding claim.

9. A DNA sequence according to claim 8 comprising a sequence having at least 80% identity with the sequence SEQ ID NO. 12.

10. A vector wherein it contains the DNA sequence of claim 8 or 9.

11. A microbial host cell wherein it has been transformed with the vector of claim 10 or contains, as heterologous DNA, a sequence of claim 8 or 9.

12. An isolated microorganism characterized as an anaerobic and thermophilic bacterium and having deposition number CBS 211.94, 212.94, 213.94, 214.94, 215.94 and 216.94.

13. A process for the preparation of a xylanase, the process comprising cultivating a microorganism of claim 12 or a host cell according to claim 11 in a suitable medium, followed by recovery of the enzyme.

14. A process for the degradation of xylan, the process comprising contacting a xylanase according to any of claims 1 to 7 with a composition containing the xylan.

15. A process according to claim 14 wherein the xylanase is used at a temperature of 80°C or higher.

16. A process for delignifying wood pulp, the process comprising contacting the wood pulp with a xylanase according to any of claims 1 to 7 at a temperature of at least 80°C, under conditions that allow xylanase activity.

AUSTRALIAN

17. A process according to claim 16 wherein characterized in that the xylanase is used before, during or after an oxygen delignification step.

18. A product obtained after the treatment of wood pulp or the degradation of xylan using a process according to any one of the claims 14 to 18.

19. A product according to claim 18 which is treated wood pulp or paper, board or fluff pulp made using said wood pulp.

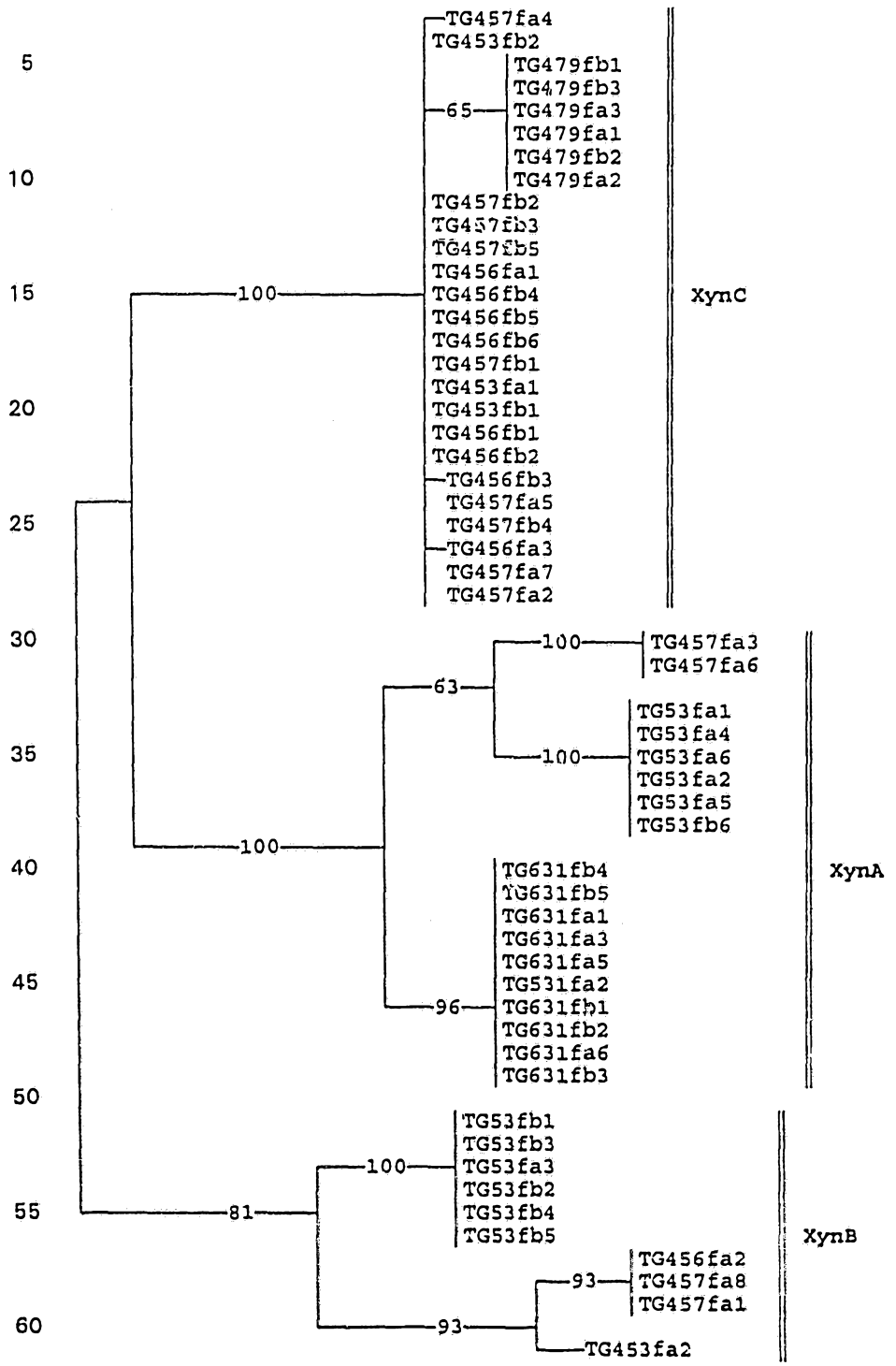
20. A composition suitable for the degradation of xylan or the treatment of wood pulp comprising a xylanase according to any of claims 1 to 7.

21. A xylanase according to claim 1 substantially as hereinbefore described with reference to any one of the Examples.

DATED: 15 May, 1998
PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
GIST-BROCADES B.V.

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Figure 1.


TG457fa4
TG453fb2
TG479fb1
TG479fb3
65
TG479fa3
TG479fa1
TG479fb2
TG479fa2
TG457fb2
TG457fb3
TG457fb5
TG456fa1
100
TG456fb4
TG456fb5
TG456fb6
TG457fb1
TG453fa1
TG453fb1
TG456fb1
TG456fb2
TG456fb3
TG457fa5
TG457fb4
TG456fa3
TG457fa7
TG457fa2
XynC
100
TG457fa3
TG457fa6
63
TG53fa1
TG53fa4
100
TG53fa6
TG53fa2
TG53fa5
TG53fb6
100
TG631fb4
TG631fb5
TG631fa1
TG631fa3
TG631fa5
TG531fa2
96
TG631fb1
TG631fb2
TG631fa6
TG631fb3
XynA
TG53fb1
TG53fb3
100
TG53fa3
TG53fb2
TG53fb4
81
TG53fb5
TG456fa2
93
TG457fa8
TG457fa1
93
TG453fa2
XynB

```
TG457   .CGCTGCTTTGGTATCCTTCCACACAGAGTGTAATCTGGTCAATTGTACC
TG457   CCGCTGCTTTGGTATCCTTCCACACAGAGTGTAATCTGGTCAATTGTACC
TG457   CCGCTGCTTTGGTAGCCTTCCACACAGAGTGTAATCTGGTCAATTGTACC
TG53    CCGCTACTTTGGTAACCTTCAACGCAAAGAGTAATCTGATCGATTGTACC
TG53    .CGCTGCTTTGGTATCCTTCAACGCAAAGAGTAATCTGATCGATTGTACC
TG53    ................CTTCAACGCAAAGAGTAATCTGATCGATTGTACC

        51                                                100
TG457   CAGGTtCAAACCTTTTGCAGCCCATGCTTTGAAATGATCAGTTACAGTAA
TG457   CAGGTtCAAACCTTTTGCAGCCCATGCTTTGAAATGATCAGTTACAGTAA
TG457   CAGGTTCAAACCTTTTGCAGCCCATGCTTTGAAATGATCAGTTACAGTAA
TG53    AAGATTCAAACCTTTTGCAGCCCATGCCTTGAAATGATCTGTAACTGTTA
TG53    AAGATTCAAACCTTTTGCAGCCCATGCCTTGAAATGATCTGTAACTGTTA
TG53    AAGATTCAAACCTTTTGCAGCCCATGCCTTGAAATGATCTGTAACTGTTA

        101                                               150
TG457   CAGTACCACtTGTTCTCTTTGATGTCCTAACACTCCAGTACTGATCAAAT
TG457   CAGTACCACtTGTTCTCTTTGATGTCCTAACACTCCAGTACTGATCAAAT
TG457   CAGTACCACTTGTTCTCTTTGATGTCCTAACACTCCAGTACTGATCAAAT
TG53    CTGTACCGCTGGTTCTCTTCGAGGTTCTAACGCTCCAGTATTGATCAAAT
TG53    CTGTACCGCTGGTTCTCTTCGAGGTTCTAACGCTCCAGTATTGATCAAAT
TG53    CTGTACCGCTGGTTCCCTTCGAGGTTCTAACGCTCCAGTATTGATCAAAT

        151                                               200
TG457   GTTCTTGTTCCTTCGATAGATGGCTGATTAACACGAGTTGTCTTATAAAT
TG457   GTTCTTGTTCCTTCGATAGATGGCTGATTAACACGAGTTGTCTTATAAAT
TG457   GTTCTTGTTCCTTCGATAGATGGCTGATTAACACGAGTTGTCTTATAAAT
TG53    GTTGTTGTCCCTTCAATAGATGGTTGATTTACACGAGTTGTCTTGTAAAT
TG53    GTTGTTGTCCCTTCAATAGATGGTTGATTTACACGAGTTGTCTTGTAAAT
TG53    GTTGTTGTCCCTTCAATAGATGGTTGATTTACACGAGTTGTCTTGTAAAT

        201                                               250
TG457   ATCATATGTTCCTCCATCAATTGTTACAGTGCCAAGTGACGTTGCCCCGG
TG457   ATCATATGTTCCTCCATCAATTGTTACAGTGCCAAGTGACGTTGCCCCGG
TG457   ATCATATGTTGCTCCATCAATTGTTACAGTGCCAAGTGACGTTGCCCCGG
TG53    ATCATATGTTCCTCCATCGATTGTTACAGTACCAAGTGATGTTGCTCC.G
TG53    ATCATATGTTCCTCCATCGATTGTTACAGTACCAAGTGATGTTGCTCCGG
TG53    ATCATATGTTCCTCCATCGATTGTTACAGTACCAAGTGATGTTGCTCCGG

        251                                               300
TG457   GCGGACGCCATGAGCCCCAGCTTTCAACGATATaAAATTCAACAAGTGGA
TG457   GCGGACGCCATGAGCCCCAGCTTTCAACGATATaAAATTCAACAAGTGGA
TG457   GCGGACGCCATGAGCCCCAGCTTTCAACGATATAAAaTTCAACAAGTGGA
TG53    GCGGACGCCATGAACCCCAGCTTTCAACAATATAGAATTCAACAAGTGGA
TG53    GCGGACGCCATGAACCCCAGCTTTCAACAATATAGAATTCAACAAGTGGA
TG53    gCGGACGCCATGAACCCCAGCTTTCAACAATATAGAATTCAACAAGTGGA

        301                         329
TG457   T.........................
TG457   TTTCTTGACCaTC.............
TG457   TTTCTTGACCATCCATATACAcCCAA...
TG53    TTTCTTGACCATCCATAGAArCCCATATA
TG53    TTTCTTGACCATCCAT.............
TG53    TTTCTTGACCATCCATATACAGCCACATA
```

Figure 2.

Figure 3.

```
          10         20         30         40         50         60
ACGAAACGAAGCATTGGCGCCTCGAGTAATTTACCAACACTACTACGTTTTAACTGAAAC

          70         80         90SpHI    100        110        120
AAACAAACTGGAGACTGCCATGGCATATGGCATGCCATTTACCTCTAATGCAACTGGGAC
                                    + P  F  T  S  N  A  T  G  T

        130        140        150        160        170        180
ATACGATGGTTACTACTACGAGTTGTGGAAGGACACAGGGAATACTACCATGACAGTTGA
  Y  D  G  Y  Y  Y  E  L  W  K  D  T  G  N  T  T  +  T  V  D

        190        200        210        220        230        240
CACAGGAGGAAGATTTAGCTGTCAGTGGAGTAACATTAACAATGCACTCTTCAGAACAGG
  T  G  G  R  F  S  C  Q  W  S  N  I  N  N  A  L  F  R  T  G

        250        260        270        280        290        300
TAAAAAGTTTAGCACTGCATGGAATCAGCTTGGGACTGTAAAGATTACCTACTCTGCTAC
  K  K  F  S  T  A  W  N  Q  L  G  T  V  K  I  T  Y  S  A  T

        310        320        330        340        350        360
CTACAATCCAAATGGCAATTCCTATCTCTGCATTTATGGATGGTCAAGAAATCCACTTGT
  Y  N  P  N  G  N  S  Y  L  C  I  Y  G  W  S  R  N  P  L  V

        370        380        390        400        410        420
TGAATTTTATATCGTTGAAAGCTGGGGCTCATGGCGTCCGCCCGGGGCAACGTCACTTGG
  E  F  Y  I  V  E  S  W  G  S  W  R  P  P  G  A  T  S  L  G

        430        440        450        460        470        480
CACTGTAACAATTGATGGAGCAACATATGATATTTATAAGACAACTCGTGTTAATCAGCC
  T  V  T  I  D  G  A  T  Y  D  I  Y  K  T  T  R  V  N  Q  P

        490        500        510        520        530        540
ATCTATCGAAGGAACAAGAACATTTGATCAGTACTGGAGTGTTAGGACATCAAAGAGAAC
  S  I  E  G  T  R  T  F  D  Q  Y  W  S  V  R  T  S  K  R  T

        550        560        570        580        590        600
AAGTGGTACTGTTACTGTAACTGATCATTTCAAAGCATGGGCTGCAAAAGGTTTGAACCT
  S  G  T  V  T  V  T  D  H  F  K  A  W  A  A  K  G  L  N  L

        610        620        630        640        650        660
GGGTACAATTGACCAGATTACACTCTGTGTGGAAGGYTACCARAGCAGCGGCTCAGCAAA
  G  T  I  D  Q  I  T  L  C  V  E  G  Y  Q  S  S  G  S  A  N

        670        680        690        700BamHI   710        720
TATAACACAGAATACATTTACTATTGGTGGTTCGAGTAGTGGATCCTAAGTAAGTAGAAT
  I  T  Q  N  T  F  T  I  G  G  S  S  S  G  S  *

        730
TCTGAGTAGGTAA
```

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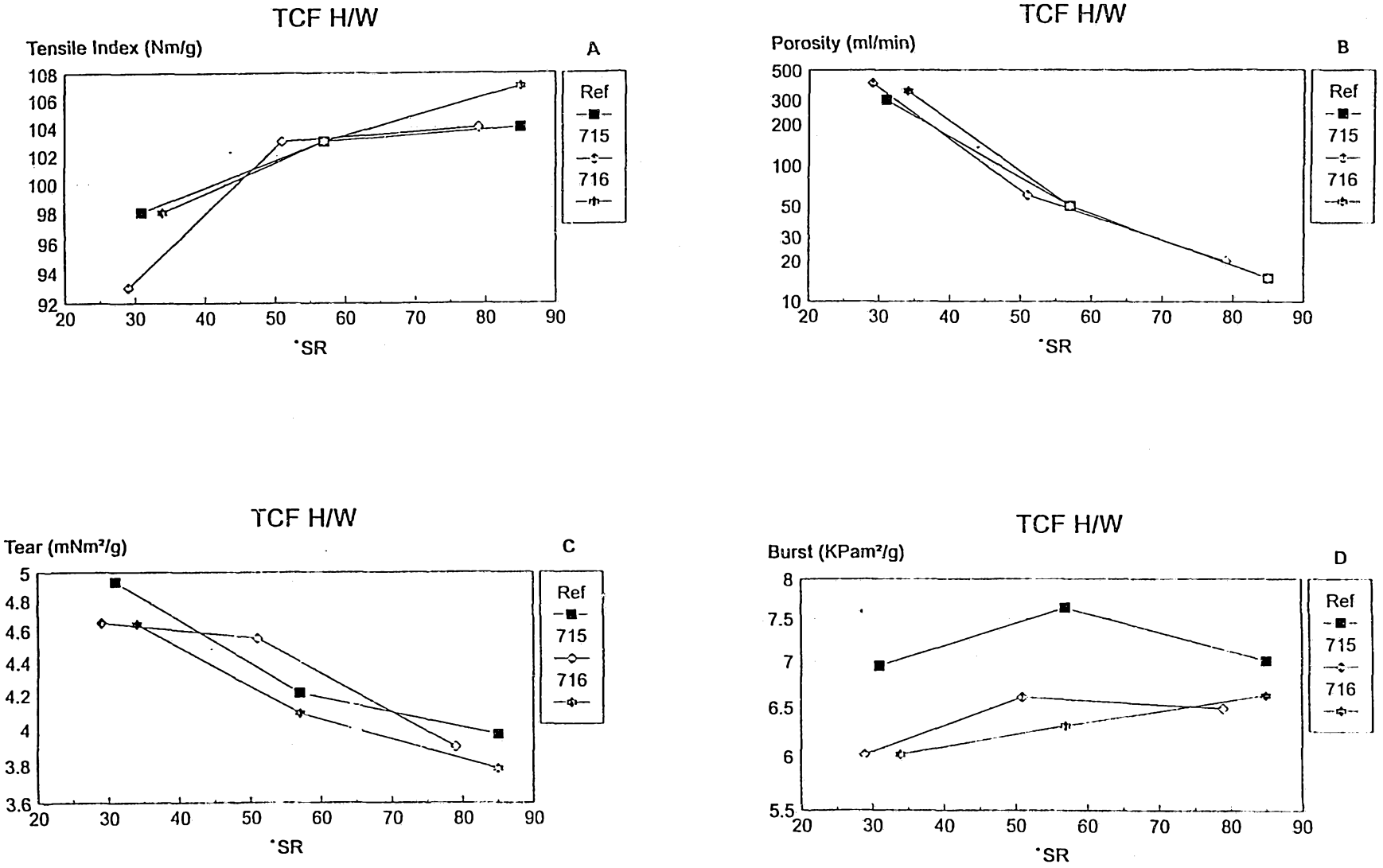
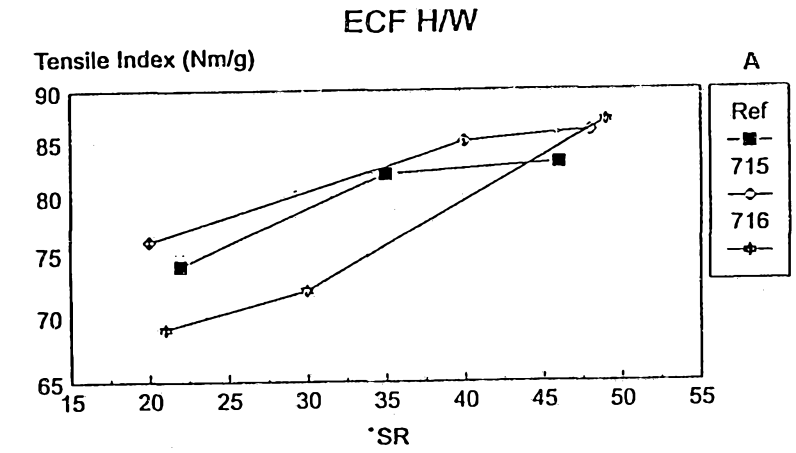
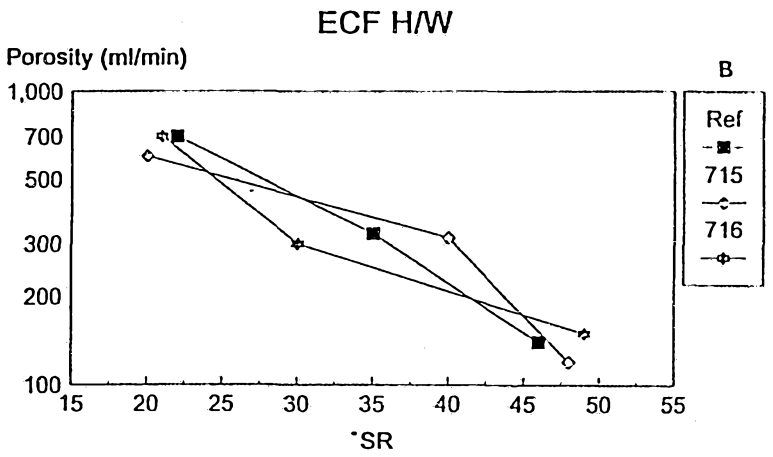
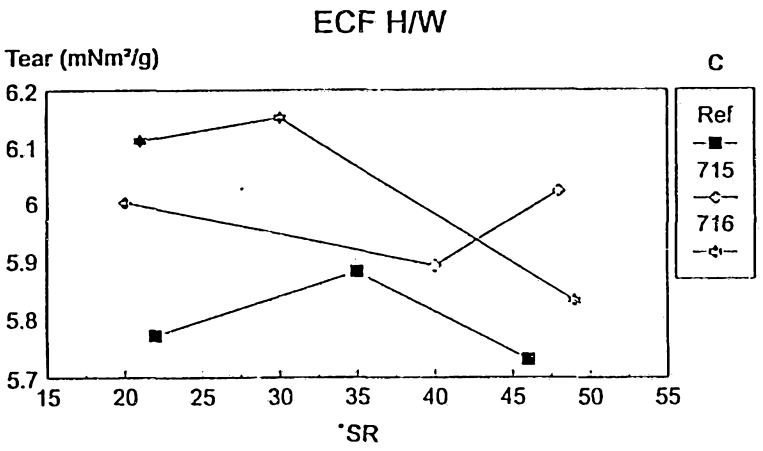


Figure 4


ECF H/W
Tensile Index (Nm/g)
A
90
85
80
75
70
65
15
20
25
30
35
40
45
50
55
°SR
Ref
715
716


ECF H/W
Porosity (ml/min)
B
1,000
700
500
300
200
100
15
20
25
30
35
40
45
50
55
°SR
Ref
715
716


ECF H/W
Tear (mNm²/g)
C
6.2
6.1
6
5.9
5.8
5.7
15
20
25
30
35
40
45
50
55
°SR
Ref
715
716

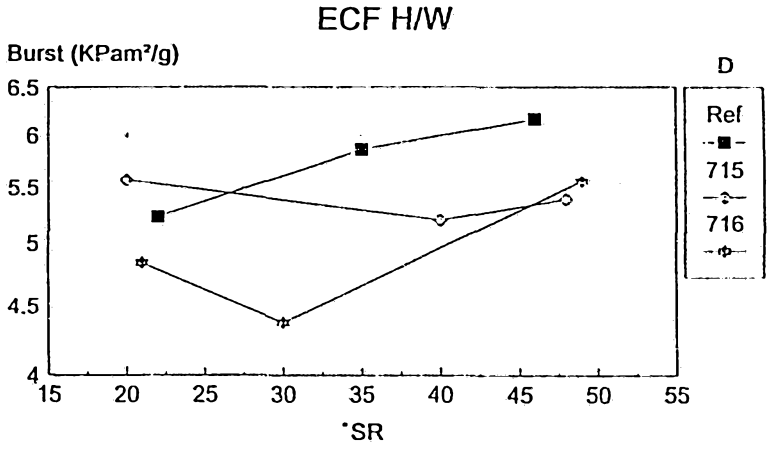
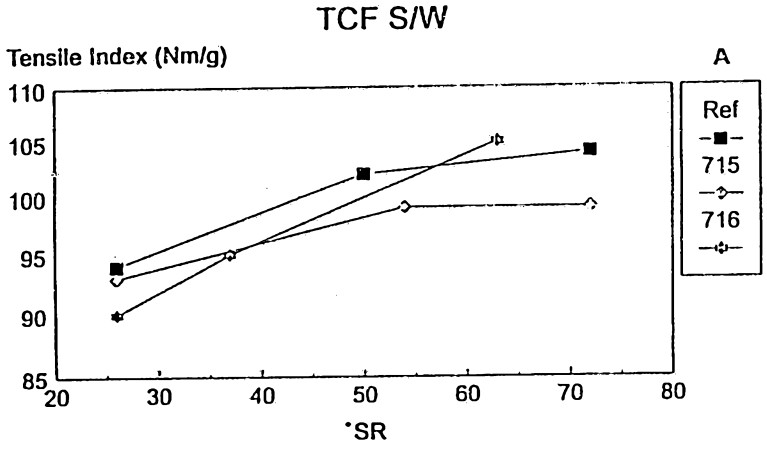
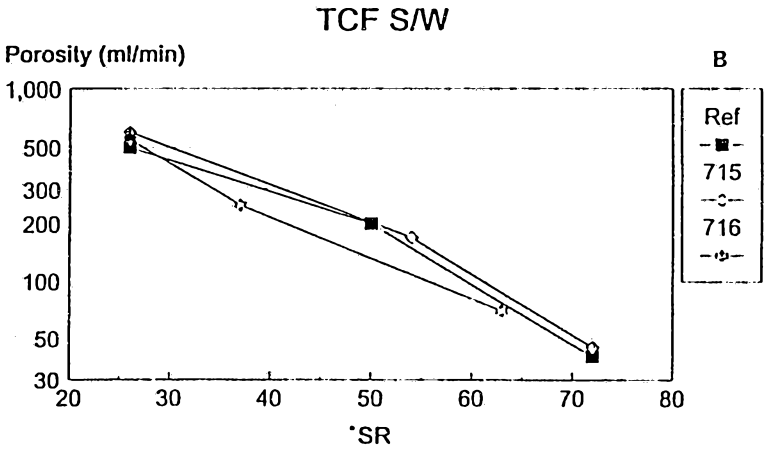
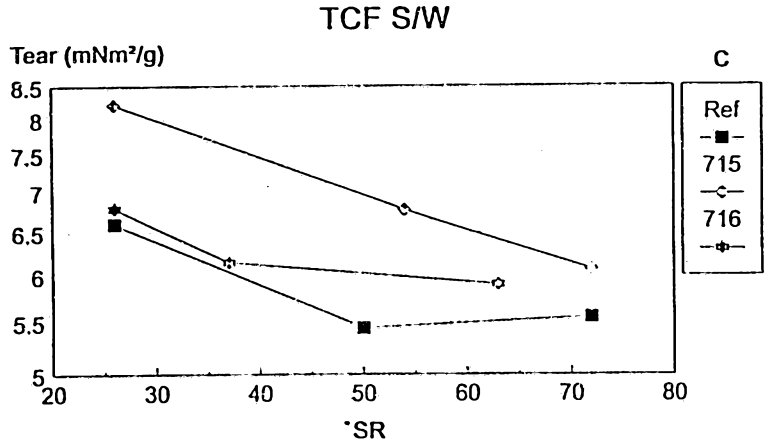

ECF H/W
Burst (KPam²/g)
D
6.5
6
5.5
5
4.5
4
15
20
25
30
35
40
45
50
55
°SR
Ref
715
716

Figure 5







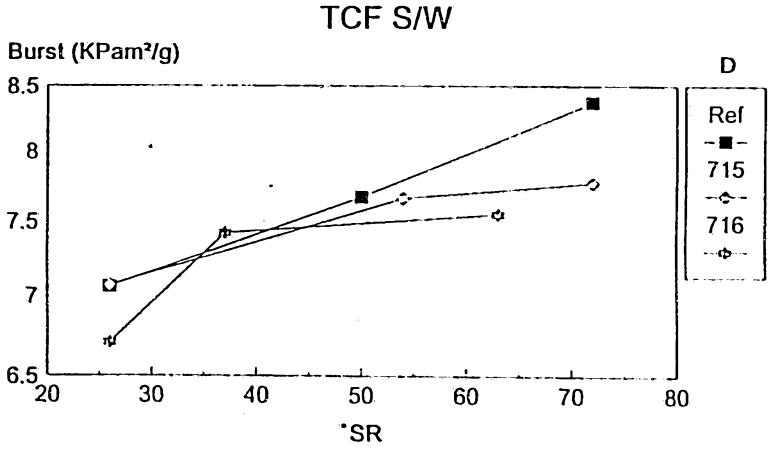


Figure 6

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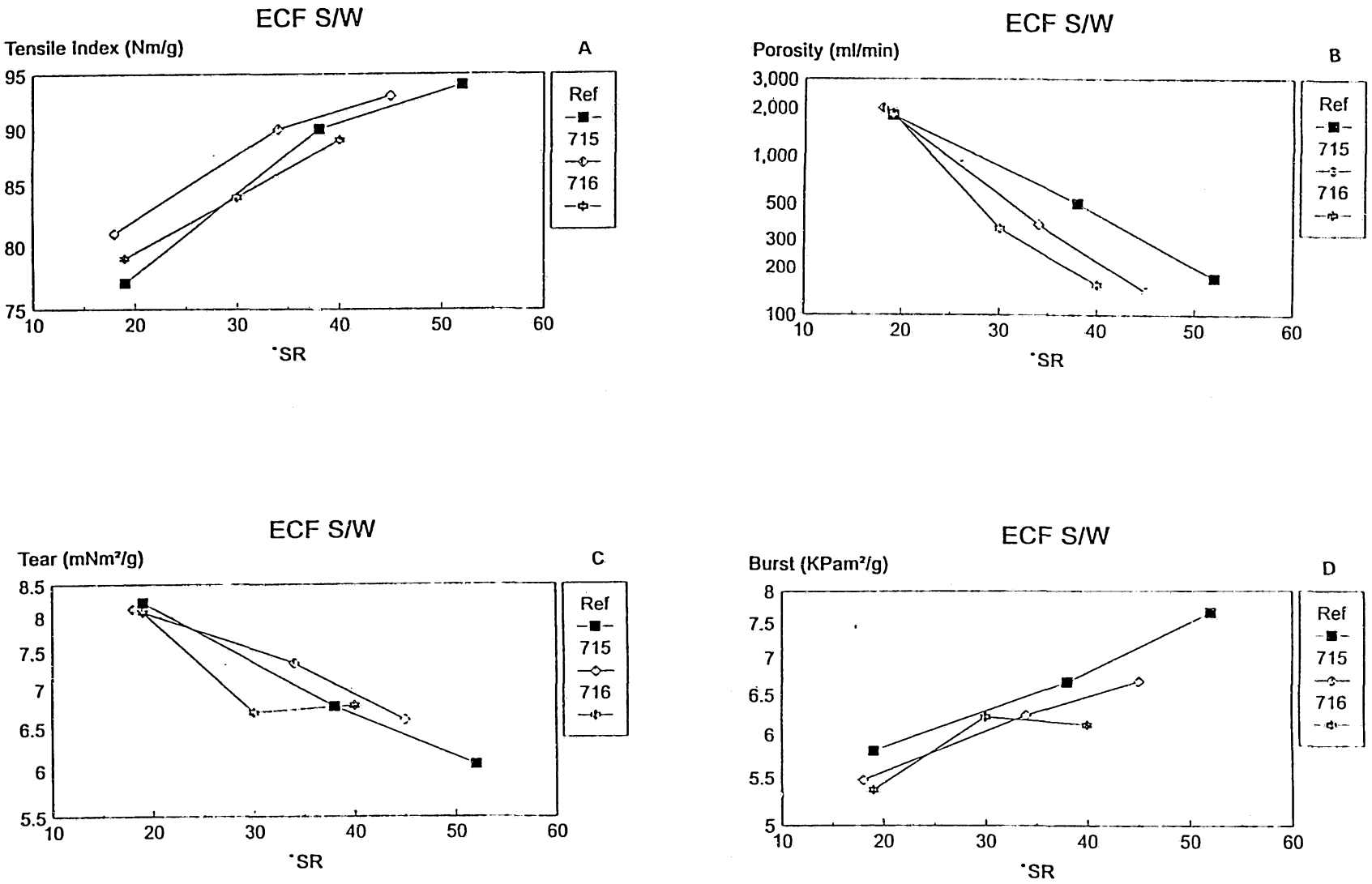


Figure 7

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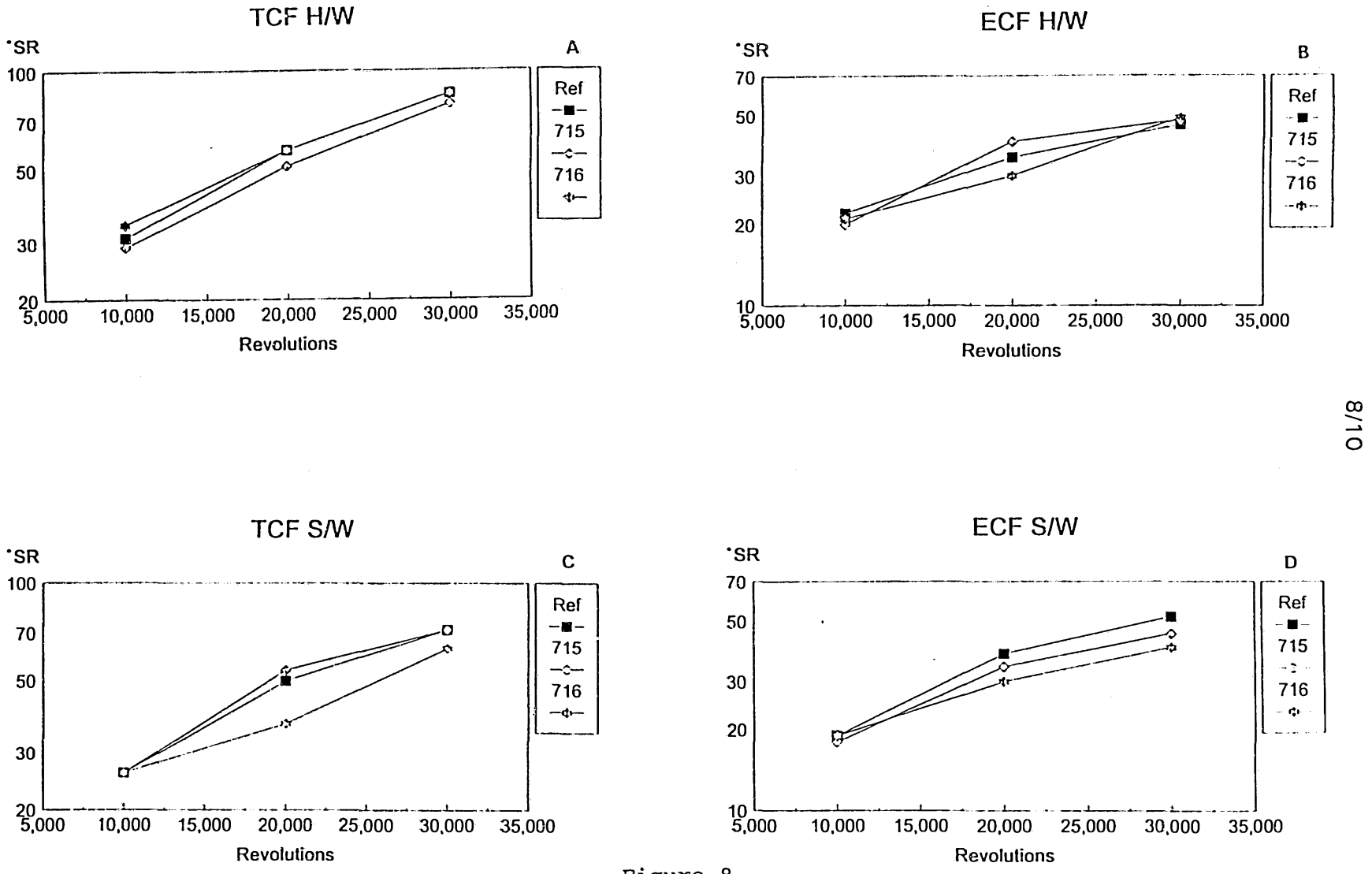
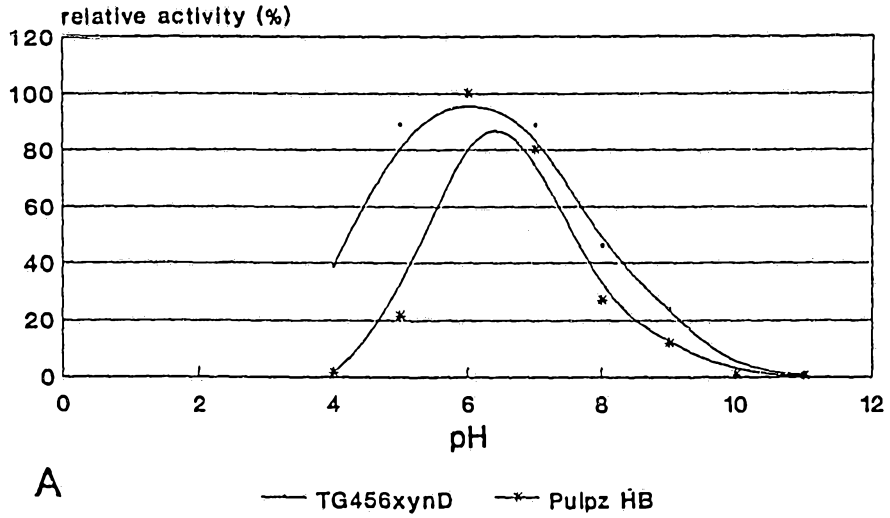


Figure 8

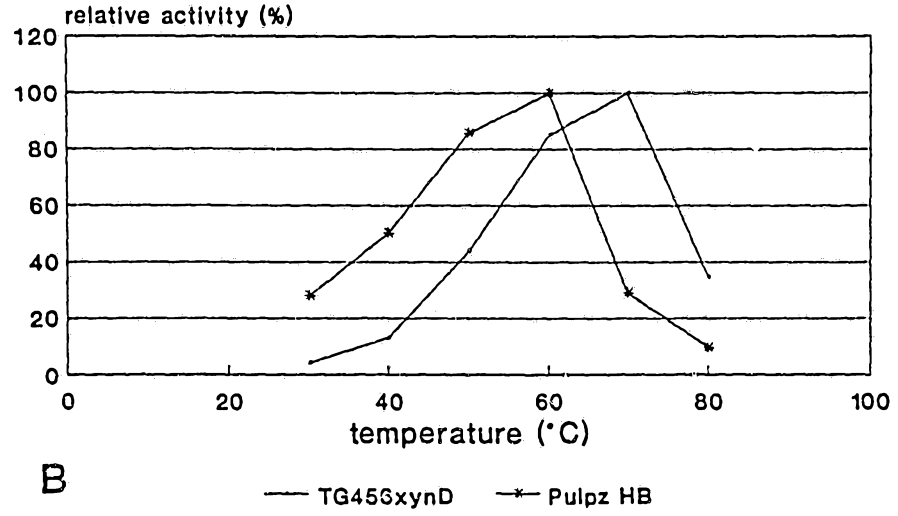
SUBSTITUTE SHEET (RULE 26)

pH Optima of different enzymes.
temperature= 60°C



A

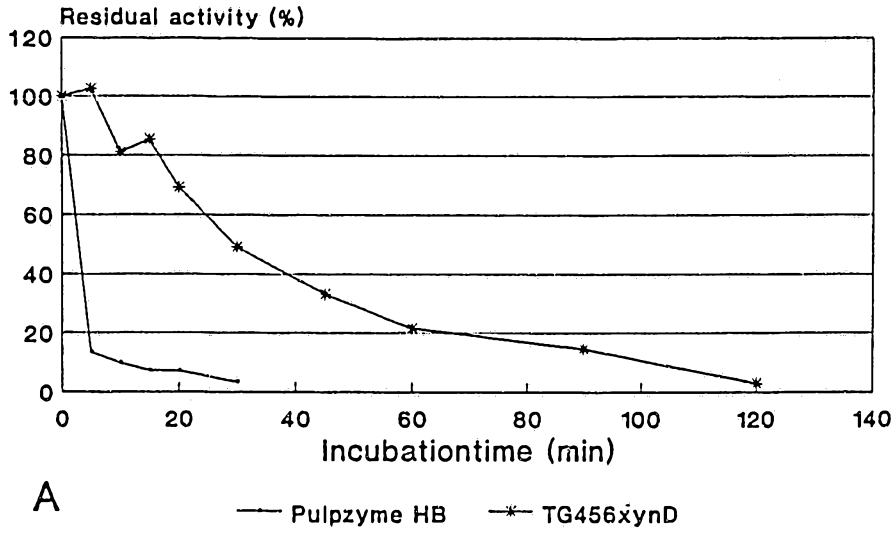
Temp optima of different enzyme
pH = 7 (TG)/6 (Pulp HB)



B

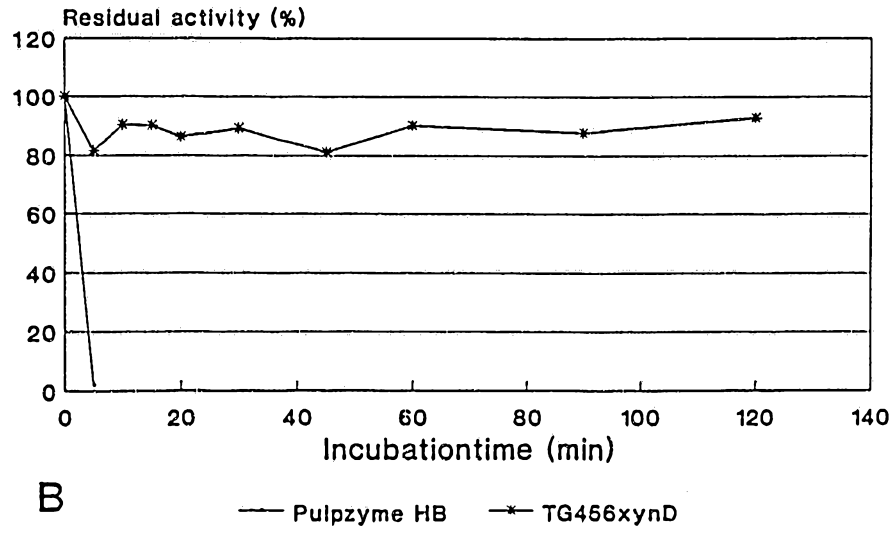
Figure 9

Thermostability of TG456xynD and Pulpzyme HB at 80°C (pH 7).



A

Thermostability of TG456xynD and Pulpzyme HB at 65°C (pH 9).



B

Figure 10

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 95/02299

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/56 C12N9/24 C12N1/21 D21C3/00 C12S3/08
//(C12N9/24,C12R1:00),(C12N1/21,C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C21C C12S

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where pracical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 511 933 (NOVO NORDISK A/S) 4 November 1994 cited in the application see page 8; figures 4,6,8; examples 3-8 ---	1-6, 14-20
X	EP,A,0 538 177 (NOVO NORDISK A/S) 21 April 1993 cited in the application see page 6; figure 1 ---	1-3,6,7, 14-20
X	WO,A,93 19171 (NOVO NORDISK A/S) 30 September 1993 cited in the application see page 11 --- -/--	1-4,6,7, 13-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 1995	23.11.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Espen, J

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INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 95/02299

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLARK D. S. & D. A. ESTELL (EDS.) 'Enzyme Engineering XI' 1992 , N. Y. ACADEMY OF SCIENCES , N. Y. vol. 672, Ruttersmith L. D. et al.: Cellulolytic and hemicellulolytic enzymes functional above 100 degree C see page 137 - page 141 ---	1-4,6,7, 15-20
X	BIOSCI. BIOTECH. BIOCHEM., vol. 57, no. 202, February 1993 pages 273-277, KAZUO SAKKA ET AL. 'Nucleotide sequence of the Clostridium stercorarium xynA gene encoding xylanase A: Identification of catalytic and cellulose binding domains' see the whole document ---	1-4,6,7, 9-11, 13-20
X	AGRICULTURAL AND BIOLOGICAL CHEMISTRY, vol. 54, no. 2, 1990 TOKYO JP, pages 337-342, KAZUO SAKKA ET AL. 'Cloning and expression in Escherichia coli of Clostridium stercorarium Strain F-9 genes related to xylan hydrolysis' see page 340, right column - page 341; figure 3 ---	1-4,6,7, 9,10, 13-20
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P,X	WO,A,95 12668 (CORNELL RESEARCH FOUNDATION, INC.) 11 May 1995 see the whole document -----	1-3,6,7, 9,10, 13-20

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/EP 95/02299

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		EP-A-	0580710	02-02-94
		JP-T-	6506593	28-07-94
		NZ-A-	242410	28-03-95
EP-A-0538177	21-04-93	WO-A-	9308275	29-04-93
		EP-A-	0610371	17-08-94
		FI-A-	941760	15-04-94
		JP-T-	7500730	26-01-95
		NO-A-	941379	15-04-94
		NZ-A-	244764	26-07-94
		US-A-	5395765	07-03-95
WO-A-9319171	30-09-93	EP-A-	0631621	04-01-95
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WO-A-9512668	11-05-95	NONE		