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(54) Title: ENZYMATIC TREATMENT OF PAPER PULP

(57) Abstract: The use of a GH8 xylanase in the treating of pulp for preparing paper materials, such as printing and writing paper, tissue and towel, newsprint, carton board, containerboard and packaging papers.

ENZYMATIC TREATMENT OF PAPER PULP

Reference to a Sequence Listing

This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to treatment of paper pulp with a GH8 xylanase.

BACKGROUND

It is well-known to use enzymes in the manufacture of paper materials. Examples of enzymes used for this purpose are proteases, lipases, xylanases, amylases, cellulases, as well as various oxidizing enzymes such as laccases and peroxidases.

The effects of these enzymes are wide-spread, *e.g.*, control of various deposits such as pitch, strength-improvement, deinking, drainage improvement, tissue softening, bleaching etc.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method for treating a paper pulp, comprising contacting the paper pulp with a GH8 xylanase (X-stage).

In another aspect the invention relates to a paper pulp prepared by the method of the present invention.

In another aspect the invention relates to a pulp composition, comprising a paper pulp and a GH8 xylanase.

An additional aspect of the invention relates to use of a GH8 xylanase for treating a paper pulp.

DETAILED DESCRIPTION

The present inventors surprisingly found that lignocellulosic materials (e.g. paper pulp and the resulting paper material) can be modified efficiently with a more controlled (less severe) hydrolysis of xylan by contacting the lignocellulosic material with a GH8 xylanase.

Paper and Pulp

The term “paper material” refers to products, which can be made out of cellulosic pulp, such as printing and writing paper, tissue and towel, newsprint, carton board, containerboard and packaging papers.

The term “pulp” or “paper pulp” means any pulp which can be used for the production of a “paper material”. For example, the pulp can be supplied as a virgin pulp, or can be derived from a recycled source. The pulp may be a wood pulp, a non-wood pulp or a pulp made from

wastepaper. A wood pulp may be made from softwood such as pine, redwood, fir, spruce, cedar and hemlock or from hardwood such as maple, alder, birch, hickory, beech, aspen, acacia and eucalyptus. A non-wood pulp may be made, e.g., from flax, hemp, wheat straw, bagasse, bamboo, cotton or kenaf. A wastepaper pulp may be made by re-pulping wastepaper such as newspaper, mixed office waste, computer print-out, white ledger, magazines, milk cartons, paper cups, packaging materials, etc.

In a particular embodiment, the pulp to be treated comprises both hardwood pulp and softwood pulp.

The wood pulp to be treated may be mechanical pulp (such as ground wood pulp, GP), chemical pulp (such as kraft pulp or sulfite pulp), semi-chemical pulp (SCP), thermo-mechanical pulp (TMP), chemi-thermo-mechanical pulp (CTMP), or bleached chemi-thermo-mechanical pulp (BCTMP).

Mechanical pulp is manufactured by the grinding and refining methods, wherein the raw material is subjected to periodical pressure impulses. TMP is thermo-mechanical pulp, GW is groundwood pulp, PGW is pressurized groundwood pulp, RMP is refiner mechanical pulp, PRMP is pressurized refiner mechanical pulp and CTMP is chemi-thermo-mechanical pulp.

Chemical pulp is manufactured by either an alkaline cooking (e.g. Kraft or soda cooking) or by an acidic-neutral cooking (e.g. sulfite cooking) whereby most of the lignin is removed. In kraft pulping or sulfate cooking sodium sulphide or sodium hydroxide are used as principal cooking chemicals. The sulfite cooking process can cover almost the whole pH range, for instance the pH can be below 1 for sulfur dioxide solutions in water, to above 13 for sodium sulfite solutions with the addition of free sodium hydroxide, but it is more often used in the acidic-neutral range.

The kraft pulp to be treated may be a bleached kraft pulp, which may consist of softwood bleached kraft (SWBK, also called NBKP (Nadel Holz Bleached Kraft Pulp)), hardwood bleached kraft (HWBK, also called LBKP (Laub Holz Bleached Kraft Pulp)) or a mixture of these.

The pulp to be used in the process of the invention is a suspension of mechanical or chemical pulp or a combination thereof. For example, the pulp to be used in the process of the invention may comprise 0%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-100% of chemical pulp. In a particular embodiment, a chemical pulp forms part of the pulp being used for manufacturing the paper material. In the present context, the expression "forms part of" means that in the pulp to be used in the process of the invention, the percentage of chemical pulp lies within the range of 1-99%. In particular embodiments, the percentage of chemical pulp lies within the range of 2-98%, 3-97%, 4-96%, 5-95%, 6-94%, 7-93%, 8-92%, 9-91%, 10-90%, 15-85%, 20-80%, 25-75%, 30-70%, 40-60%, or 45-55%.

In a particular embodiment of the use and the process of the invention, the chemical pulp is a kraft pulp, a sulfite pulp, a semi-chemical pulp (SCP), a thermo-mechanical pulp (TMP), a

chemi-thermo-mechanical pulp (CTMP), a bleached chemi-thermo-mechanical pulp (BCTMP). In particular embodiments the kraft pulp is bleached kraft pulp, for example softwood bleached kraft (SWBK, also called NBKP (Nadel Holz Bleached Kraft Pulp)), hardwood bleached kraft (HWBK, also called LBKP (Laub Holz Bleached Kraft Pulp and)) or a mixture thereof.

5 The pulp to be used in the process of the invention may be a never dried pulp or a dried pulp.

GH8 Xylanase

Glycoside hydrolases E.C. 3.2.1. are a widespread group of enzymes that hydrolyse the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-10 carbohydrate moiety. A classification system for glycoside hydrolases, based on sequence similarity, has led to the definition of >100 different families. This classification is available on the CAZy (http://www.cazy.org/GH1.html) web site and also discussed at CAZypedia, an online encyclopedia of carbohydrate active enzymes.

Glycoside hydrolase family 8 in CAZY, GH8, comprises inverting enzymes with several 15 known activities, incl. chitosanase (EC 3.2.1.132); cellulase (EC 3.2.1.4); licheninase (EC 3.2.1.73); endo-1,4-β-xylanase (EC 3.2.1.8); reducing-end-xylose releasing exo-oligoxylanase (EC 3.2.1.156).

The polypeptides in GH8 can be separated into multiple distinct sub-clusters, or clades, where we denoted the clades listed below. Distinct motifs for each clade are described in detail 20 below

(a) DPSY clade

(b) SMDY clade

(c) ALWNW clade

(d) WFAAAL clade.

25 *The DPSY clade*

GH8 xylanases comprise several well-conserved motifs, one example is the motif "[TS]D[PA]SY" or "(Thr/Ser) Asp (Pro/Ala) Ser Tyr" (SEQ ID NO: 15) situated in positions 203-207 of the xylanase amino acid sequence shown in SEQ ID NO: 2 and 3, in positions 193-197 of SEQ ID NO: 6 , and in positions 202-206 of SEQ ID NO: 7 and 8.

30 *The SMDY clade*

The polypeptides of the DPSY clade can be separated into distinct sub-clusters, and one of the sub-clusters we denote "SMDY". A characteristic motif for this subgroup is the motif "MN[FYVILM][GS]MDY" or "Met Asn (Phe/Tyr/Val/Ile/Leu/Met) (Gly/Ser) Met Asp Tyr" (SEQ ID NO: 16).

35 *The ALWNW clade*

The polypeptides of the DPSY clade can be separated into distinct sub-clusters, and one of the sub-clusters we denote "ALWNW". A characteristic motif for this subgroup is the motif

"A[IL]WNW" or "Ala (Ile/Leu) Trp Asn Trp" (SEQ ID NO: 17) corresponding to positions 100 to 104 of the xylanase amino acid sequence shown in SEQ ID NO: 9 and 10, and to positions 101 to 105 in SEQ ID NO: 4 and 5.

The WFAAAL clade

5 The polypeptides of the DPSY clade can be separated into distinct sub-clusters, and one of the sub-clusters we denote "WFAAAL". A characteristic motif for this subgroup is the motif "W[IF]AAAL" or "Trp (Ile/Phe) Ala Ala Ala Leu" (SEQ ID NO: 18) corresponding to positions 134 to 139 of the xylanase amino acid sequence shown in SEQ ID NO: 2 and 3, and to positions. 133 to 138 in SEQ ID NO: 7 and 8.

10 Preferably, the GH8 xylanase comprises an amino acid sequence having a degree of sequence identity to the mature polypeptide of GH8 xylanase of any of SEQ ID NO's: 1-10 of at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 15 98%, or at least 99% or 100% (hereinafter "homologous polypeptides").

Even more preferable, is where the mature polypeptide comprises or consists of amino acids 29 to 433 of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10.

For purposes of the present invention, the sequence identity between two amino acid 20 sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of 25 BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

In a preferred aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by nine amino acids, eight amino acids, seven amino acids, 30 six amino acids, five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of any of SEQ ID NO's: 1-10. GH8 xylanase comprises the mature polypeptide of any of SEQ ID NO's: 1-10 or an allelic variant thereof; or a fragment thereof having xylanase activity. In a preferred aspect, the GH8 xylanase comprises the amino 35 acid sequence of any of SEQ ID NO's: 1-10 or the mature polypeptide thereof. In another preferred aspect, the GH8 xylanase comprises the mature polypeptide of any of SEQ ID NO's: 1-10. In another preferred aspect, the GH8 xylanase consists of the amino acid sequence of any

of SEQ ID NO's: 1-10. In another preferred aspect, the GH8 xylanase consists of the mature polypeptide of any of SEQ ID NO's: 1-10.

In a preferred embodiment, the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

According to the present invention, the GH8 xylanase is a polypeptide having GH8 xylanase activity.

In a preferred embodiment, the GH8 xylanase comprises artificial variants comprising a substitution, deletion, and/or insertion of one or more (or several) amino acids of the mature polypeptide of any of SEQ ID NO's: 1-10, or a homologous sequence thereof. Preferably, amino acid changes are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-*N*-methyl lysine, 2-aminoisobutyric acid, isovaline, and alpha-methyl serine) may be substituted for amino acid residues of a wild-type polypeptide. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for amino acid residues. "Unnatural amino acids" have been

modified after protein synthesis, and/or have a chemical structure in their side chain(s) different from that of the standard amino acids. Unnatural amino acids can be chemically synthesized, and preferably, are commercially available, and include pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the GH8 xylanases are altered. For example, amino acid changes may improve the thermal stability of the GH8 xylanase, alter the substrate specificity, change the pH optimum, and the like.

Essential amino acids in the parent GH8 xylanase can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (*i.e.*, xylanase activity) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identities of essential amino acids can also be inferred from analysis of identities with GH8 xylanases that are related to a GH8 xylanase according to the invention.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, Lowman *et al.*, 1991, *Biochem.* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized GH8 xylanases expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). Mutagenized DNA molecules that encode active GH8 xylanases can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide GH8 xylanase of interest, and can be applied to GH8 xylanases of unknown structure.

In an embodiment, the total number of amino acid substitutions, deletions and/or insertions of the mature polypeptide of any of SEQ ID NO's: 1-10 is 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1.

Bleaching

5 Pulp bleaching is defined as a process aimed at removal of colour in cellulosic pulps derived from residual lignin or other colored impurities. Native wood is only slightly colored, whereas residual lignin of a chemical pulp after cooking is highly colored. Traditional concepts for bleaching of pulp include chlorine and oxygen based oxidants which selectively remove chromophore structures present in the pulp. Examples of bleaching are bleaching stages with 10 chlorine (C-stage), chlorine dioxide (D-stage), hydrogen peroxide (P-stage), ozone (Z-stage) and oxygen (O-stage) as well as alkaline extraction stages (E-stage) that can be reinforced by small amount of oxygen and/or hydrogen peroxide. The progress in bleaching is followed by measuring the brightness, which is defined as the reflectance of visible blue light from a pad of pulp sheets using a defined spectral band of light having an effective wavelength of 457 nm. 15 Official ISO standard methods are ISO 2469 or ISO 2470. Bleaching to full brightness (> 88% ISO) requires multi-stage application of bleaching chemicals. The first stages in a bleaching sequence are often conceived as delignification, where the majority of residual lignin is removed. The last stages are often referred to brightening stages, in which the chromophores in the pulps are eliminated to attain a high brightness level.

20 According to the invention, the ISO brightness of the pulp is determined according to ISO 2470 using pulp handsheets prepared according to ISO 3688.

Process Conditions

The process of the invention is particularly applicable to the modification of lignocellulosic materials, for example, in improving the bleaching of a pulp and/or quality of the final pulp in a 25 process for making paper material.

In the case of paper and pulp processing, the process according to the invention can be carried out at any pulp and paper production stage. The enzyme can be added to any holding tank, e.g. to a pulp storing container (storage chest), storage tower, mixing chest or metering chest. The enzyme treatment can be performed before the bleaching of pulp, in connection with 30 the pulp bleaching process or after the bleaching. When carried out in connection with pulp bleaching the enzyme preparation may be added together with bleaching chemicals such as chlorine or chlorine dioxide. Applying oxygen gas, hydrogen peroxide or ozone or combinations thereof may also carry out the bleaching of pulp. The enzyme preparation may also be added together with these substances. Preferably the enzyme preparation is added before or after such 35 bleaching steps. The enzyme can also be added to the circulated process water (white water) originating from bleaching and process water (brown water) originating from the mechanical or

chemimechanical pulping process. In a particular embodiment of a kraft pulping process, the enzyme is added during the brown-stock washing.

In the present context, the term “process water” comprises *inter alia* 1) water added as a raw material to the paper manufacturing process; 2) intermediate water products resulting from any step of the process for manufacturing the paper material; as well as 3) waste water as an output or by-product of the process. In a particular embodiment, the process water is, has been, is being, or is intended for being circulated (re-circulated), *i.e.*, re-used in another step of the process. The term “water” in turn means any aqueous medium, solution, suspension, e.g. ordinary tap water, and tap water in admixture with various additives and adjuvants commonly used in paper manufacturing processes. In a particular embodiment the process water has a low content of solid (dry) matter, e.g. below 20%, 18%, 16%, 14%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 20% or below 1% dry matter.

The process of the invention may be carried out at conventional conditions in the paper and pulp processing. The process conditions will be a function of the enzyme(s) applied, the reaction time and the conditions given.

The enzyme of the invention should be added in an effective amount. By the term “effective amount” is meant the amount sufficient to achieve the desired and expected effect in pulp modification, such as increasing the amount of aldehyde groups in the pulp or removing colored compounds and contaminants leading to a desired bleaching and/or deinking effect, and/or strengthening effect etc.

In a particular embodiment, the dosage of the GH8 xylanase and additional enzymes, if any, is from about 0.1 mg enzyme protein to about 100,000 mg enzyme protein (of each enzyme) per ton of paper pulp.

In further particular embodiments, the amount of the GH8 xylanase and additional enzymes, if any, is in the range of 0.00001-20; or 0.0001-20 mg of enzyme (calculated as pure enzyme protein) per gram (dry weight) of pulp material, such as 0.0001-10 mg/g, 0.0001-1 mg/g, 0.001-1 mg/g, 0.001-0.1, or 0.01-0.1 mg of enzyme per gram of pulp material. Again, these amounts refer to the amount of each enzyme.

The enzymatic treatment can be done at conventional pulp consistency, e.g. 0.5-15% dry substance. In particular embodiments, the consistency is within the range of 0.5-45%; 0.5-40%; 0.5-35%; 0.5-30%; 0.5-25%; 0.5-20%; 0.5-15%; 0.5-10%; 0.5-8%; 0.5-6%; or 0.5-5% dry substance.

The enzymatic treatment may be carried out at a temperature of from about 10°C to about 100°C. Further examples of temperature ranges (all “from about” and “to about”) are the following: 20-120°C, 30-120°C, 35-120°C, 37-120°C, 40-120°C, 50-120°C, 60-120°C, 70-120°C, 10-100°C, 10-90°C, 10-80°C, 10-70°C, 10-60°C, and 30-60°C, as well as any combination of the upper and lower values here indicated. A typical temperature is from about 20 to 90°C, or 20

to 95°C, preferably from about 40 to 70°C. Usually, the enzymatic treatment is carried out at atmospheric pressure. But when the temperature exceeds 100°C, the treatment is carried out at a pressure of 1-2 bar (up to 1 bar above atmospheric pressure).

The enzymatic treatment is carried out at a pH of from about 2 to about 12, preferably at a pH from about 4 to about 9, more preferably at a pH from about 5 to about 8, and most preferably at a pH from about 5.5 to about 7.

A suitable duration of the enzymatic treatment may be in the range from a few seconds to several hours, e.g. from about 30 seconds to about 48 hours, or from about 1 minute to about 24 hours, or from about 1 minute to about 18 hours, or from about 1 minute to about 12 hours, or from about 1 minute to 5 hours, or from about 1 minute to about 2 hours, or from about 1 minute to about 1 hour, or from about 1 minute to about 30 minutes. A typical reaction time is from about 10 minutes to 3 hours, 10 minutes to 10 hours, preferably 15 minutes to 1 hour, or 15 minutes to 2 hours.

Various additives over and above the GH8 xylanase and additional enzymes, if any, can be used in the process or use of the invention. Surfactants and/or dispersants are often present in, and/or added to a pulp. Thus the process and use of the present invention may be carried out in the presence of an anionic, non-ionic, cationic and/or zwitterionic surfactant and/or dispersant conventionally used in a pulp. Examples of anionic surfactants are carboxylates, sulfates, sulphonates or phosphates of alkyl, substituted alkyl or aryl. Examples of non-ionic surfactants are polyoxyethylene compounds, such as alcohol ethoxylates, propoxylates or mixed ethoxy-/propoxylates, poly-glycerols and other polyols, as well as certain block-copolymers. Examples of cationic surfactants are water-soluble cationic polymers, such as quartenary ammonium sulfates and certain amines, e.g. epichlorohydrin/dimethylamine polymers (EPI-DMA) and cross-linked solutions thereof, polydiallyl dimethyl ammonium chloride (DADMAC), DADMAC/Acrylamide co-polymers, and ionene polymers, such as those disclosed in US patents nos. 5,681,862; and 5,575,993. Examples of zwitterionic or amphoteric surfactants are betains, glycinates, amino propionates, imino propionates and various imidazolin-derivatives. Also the polymers disclosed in US patent no. 5,256,252 may be used.

Also according to the invention, surfactants such as the above, including any combination thereof may be used in a paper making process together with a GH8 xylanase, as defined herein, and included in a composition together with such enzyme. The amount of each surfactant in such composition may amount to from about 1 to about 1000 ppm of the composition. In particular embodiments the amount of each surfactant is from about 10 to about 1000 ppm, or from about 10 to about 500 ppm, or from about 50 to about 500 ppm.

In another particular embodiment, each of the above ranges refers to the total amount of surfactants.

In further particular embodiments of the above method, and of the process of the invention, the GH8 xylanase is used in an amount of 0.005-50 ppm (mg/L), or 0.01-40, 0.02-30, 0.03-25, 0.04-20, 0.05-15, 0.05-10, 0.05-5, 0.05-1, 0.05-0.8, 0.05-0.6, or 0.1-0.5 ppm. The amount of enzyme refers to mg of a well-defined enzyme preparation.

5 In the process of the invention, the GH8 xylanase may be applied alone or together with an additional enzyme. The term “an additional enzyme” means at least one additional enzyme, e.g. one, two, three, four, five, six, seven, eight, nine, ten or even more additional enzymes.

The term “applied together with” (or “used together with”) means that the additional enzyme may be applied in the same, or in another step of the process of the invention. The other 10 process step may be upstream or downstream in the paper manufacturing process, as compared to the step in which the pulp is treated with a GH8 xylanase.

In particular embodiments the additional enzyme (see also below) is an enzyme which has protease, lipase, xylanase, cutinase, oxidoreductase, cellulase, endoglucanase, amylase, mannanase, steryl esterase, the lytic polysaccharide monooxygenase and/or cholesterol 15 esterase activity. Examples of oxidoreductase enzymes are enzymes with laccase, and/or peroxidase activity. In a preferred embodiment, the additional enzyme is cellulase.

The term “a step” of a process means at least one step, and it could be one, two, three, four, five or even more process steps. In other words, the GH8 xylanase may be applied in at least one process step, and the additional enzyme(s) may also be applied in at least one process 20 step, which may be the same or a different process step as compared to the step where the GH8 xylanase is used.

The term “enzyme preparation” means a product containing at least one GH8 xylanase. The enzyme preparation may also comprise enzymes having other enzyme activities. In addition to the enzymatic activity, such a preparation preferably contains at least one adjuvant. Examples 25 of adjuvants, which are used in enzyme preparations for the paper and pulp industry are buffers, polymers, surfactants and stabilizing agents.

Additional enzymes

Any enzyme having protease, lipase, xylanase, cutinase, oxidoreductase, cellulase, endoglucanase, amylase, mannanase, steryl esterase, polysaccharide monooxygenase 30 (LPMO) and/or cholesterol esterase activity can be used as additional enzymes in the use and process of the invention. Below some non-limiting examples are listed of such additional enzymes. The enzymes written in capitals are commercial enzymes available from Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark. The activity of any of those additional enzymes can be analyzed using any method known in the art for the enzyme in question, 35 including the methods mentioned in the references cited.

Examples of cutinases are those derived from *Humicola insolens* (US 5,827,719); from a strain of *Fusarium*, e.g. *F. roseum culmorum*, or particularly *F. solani pisi* (WO 90/09446; WO

94/14964, WO 94/03578). The cutinase may also be derived from a strain of *Rhizoctonia*, e.g. *R. solani*, or a strain of *Alternaria*, e.g. *A. brassicicola* (WO 94/03578), or variants thereof such as those described in WO 00/34450, or WO 01/92502.

Examples of proteases are the ALCALASE, ESPERASE, SAVINASE, NEUTRASE and DURAZYM proteases. Other proteases are derived from *Nocardiopsis*, *Aspergillus*, *Rhizopus*, *Bacillus alcalophilus*, *B. cereus*, *B. natto*, *B. vulgatus*, *B. mycoide*, and subtilisins from *Bacillus*, especially proteases from the species *Nocardiopsis sp*. and *Nocardiopsis dassonvillei* such as those disclosed in WO 88/03947, and mutants thereof, e.g. those disclosed in WO 91/00345 and EP 415296.

Examples of amylases are the BAN, AQUAZYM, TERMAMYL, and AQUAZYM Ultra amylases. An example of a lipase is the RESINASE A2X lipase. An example of a xylanase is the PULPZYME HC hemicellulase. Examples of endoglucanases are the NOVOZYM 613, 342, and 476 enzyme products.

Examples of mannanases are the *Trichoderma reesei* endo-beta-mannanases described in Ståhlbrand et al, J. Biotechnol. 29 (1993), 229-242.

Examples of steryl esterases. peroxidases, laccases, and cholesterol esterases are disclosed in the references mentioned in the background art section hereof. Further examples of oxidoreductases are the peroxidases and laccases disclosed in EP 730641; WO 01/98469; EP 719337; EP 765394; EP 767836; EP 763115; and EP 788547. In the present context, whenever an oxidoreductase enzyme is mentioned that requires or benefits from the presence of acceptors (e.g. oxygen or hydrogenperoxide), enhancers, mediators and/or activators, such compounds should be considered to be included. Examples of enhancers and mediators are disclosed in EP 705327; WO 98/56899; EP 677102; EP 781328; and EP 707637. If desired a distinction could be made by defining an oxidoreductase enzyme system (e.g. a laccase, or a peroxidase enzyme system) as the combination of the enzyme in question and its acceptor, and optionally also an enhancer and/or mediator for the enzyme in question.

Examples of the lytic polysaccharide monooxygenase (LPMO) are those derived from *Thielavia terrestres* (mature polypeptide of SEQ ID NO: 8 of WO 2010/080532A); from *Lentinus similis* (mature polypeptide of SEQ ID NO: 6 of WO2014/066141A); from *Thermoascus aurantiacus* (mature polypeptide of SEQ ID NO: 2 of WO2005/074656A).

Compositions, methods and uses

In a first aspect, the invention provides a method for treating a paper pulp, comprising contacting the paper pulp with a GH8 xylanase.

In an embodiment, the GH8 xylanase is an endo-beta-1,4-xylanase.

In an embodiment, the GH8 xylanase is a member of DPSY clade; preferably GH8 xylanase is a member of at least one of the following clades as defined herein: SMDY clade, ALWNW clade, and WFAAAL clade.

In an embodiment, the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

The amino acid sequence of the GH8 xylanase may have at least 60% sequence identity, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably at least 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95%, 96%, 97%, 98%, 99%, and most preferably 100% sequence identity to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.

In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; or up to 5, *e.g.*, 1, 2, 3, 4, or 5.

In an embodiment, the GH8 xylanase is a GH8 xylanase fragment that has GH8 xylanase activity.

In an embodiment, the GH8 xylanase is derived from *Bacillus*, such as *Bacillus* sp. KK-1, or *Bacillus licheniformis*; Metagenome from environmental sample; *Dyella*, such as *Dyella* sp-62206.

In an embodiment, the pulp is wood pulp. In an embodiment, the pulp is non-wood cellulosic pulp.

In an embodiment, the pulp is a chemical pulp, such as kraft pulp.

In an embodiment, the method of the invention further comprises a step of bleaching, and/or alkaline extraction (E stage).

In an embodiment, the method of the invention comprises a sequence of treatments selected from the group consisting of:

a) X-B;

b) B-X;

c) XB;

wherein,

X is a GH8 xylanase treatment stage,

B is a bleaching stage, preferably a chlorine dioxide bleaching stage, and

XB is a GH8 xylanase treatment together with a bleaching stage.

In an embodiment, the method of the invention further comprises contacting the pulp with one or more additional enzyme(s) having protease, lipase, xylanase, cutinase, oxidoreductase, cellulase, endoglucanase, amylase, mannanase, steryl esterase, polysaccharide monooxygenase (LPMO) and/or cholesterol esterase activity, preferably a cellulase.

In an embodiment, an average degree of polymerization of the xylosaccharides in a filtrate from the pulp obtained from the method of the present invention is 3.5-8, preferably 4-7.

In an embodiment, the method of the invention produces a more controlled (less severe) hydrolysis of xylan and thereby having a more xylan retaining modification of pulp xylan as compared to the other xylanase families typically used in lignocellulosic processing (particularly GH10 and GH11 families) while still improving pulp properties, such as brightness and strength. This has a significant importance as xylan loss needs to be minimized in paper pulp production.

In an embodiment, the method of the present invention results in an improved benefit/harm balance with regard to a positive effect on strength and/or brightness development and/or dewatering of the pulp while minimizing a negative effect on pulp yield, and/or COD of the resulting filtrate/effluent, compared with a method with the GH8 xylanase treatment replaced by a GH10 xylanase treatment or GH11 xylanase treatment.

In an embodiment, the method of the invention further comprises a final step of preparing a paper material from the pulp.

In a further aspect, the present invention relates to a paper pulp prepared by the method of the present invention.

In a further aspect, the invention provides a pulp composition, comprising a paper pulp and a GH8 xylanase.

In a preferred embodiment, the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of a GH8 xylanase selected from the group of GH8 xylanases shown in any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the mature polypeptide of a GH8 xylanase shown in any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanse activity.

In an embodiment, the pulp is wood pulp.

In an embodiment, the pulp is a chemical pulp, such as kraft pulp.

In a further aspect, the invention provides a paper material, which is made from the pulp composition of the invention, or which is made from a paper pulp subjected to the method of the invention.

5 The invention also provides for use of the methods and compositions above for treating a paper pulp.

In an embodiment, the GH8 xylanase boosts bleaching and/or increases brightness and/or increases strength and/or improves dewatering of a paper pulp.

Various references are cited herein, the disclosures of which are incorporated by reference 10 in their entireties.

One specific embodiment of the present invention is described in the set of items herein below.

Items:

1. A method for treating a paper pulp, comprising contacting the paper pulp with a GH8 15 xylanase (X-stage).

2. The method of item 1, wherein the GH8 xylanase is an endo-beta-1,4-xylanase.

3. The method of item 1 or 2, wherein the GH8 xylanase is a member of DPSY clade; preferably GH8 xylanase is a member of at least one of the following clades as defined herein: SMDY clade, ALWNW clade, and WFAAAL clade.

20 4. The method of any of items 1 to 3, wherein the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at 25 least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

30 (c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

5. The method of any of items 1 to 4, wherein the GH8 xylanase is derived from *Bacillus*, such as *Bacillus* sp. KK-1, or *Bacillus* licheniformis; Metagenome from environmental sample; *Dyella*, such as *Dyella* sp-62206.

6. The method of any of items 1 to 5, wherein the pulp is wood pulp.

35 7. The method of any of items 1 to 6, wherein the pulp is a chemical pulp, such as kraft pulp.

8. The method of any of items 1 to 7, which further comprises a step of bleaching and/or alkaline extraction.

9. The method of any of items 1 to 8, comprises a sequence of treatments selected from the group consisting of:

5 a) X-B;

b) B-X;

c) XB;

wherein,

X is a GH8 xylanase treatment stage,

10 B is a bleaching stage, preferably a chlorine dioxide bleaching stage, and

XB is a GH8 xylanase treatment together with a bleaching stage.

10. The method of any of items 1 to 9, which further comprises contacting the pulp with one or more additional enzyme(s) having protease, lipase, xylanase, cutinase, oxidoreductase, cellulase, endoglucanase, amylase, mannanase, steryl esterase, polysaccharide
15 monooxygenase (LPMO) and/or cholesterol esterase activity, preferably a cellulase.

11. The method of any of items 1-10, wherein an average degree of polymerization of the xylosaccharides in a filtrate from the pulp obtained from the method is 3.5-8, preferably 4-7.

12. The method of any of items 1 to 11, wherein the method results in an improved benefit/harm balance with regard to a positive effect on strength and/or brightness development
20 and/or dewatering of the pulp while minimizing a negative effect on pulp yield, and/or COD of the resulting filtrate/effluent, compared with a method with the GH8 xylanase treatment replaced by a GH10 xylanase treatment or GH11 xylanase treatment.

13. A paper pulp prepared by the method of any one of items 1 to 12.

14. A pulp composition, comprising a paper pulp and a GH8 xylanase.

25 15. The pulp composition of item 14, wherein the GH8 xylanase is an endo-beta-1,4-xylanase.

16. The pulp composition of item 14 or 15, wherein the GH8 xylanase is a member of DPSY clade; preferably GH8 xylanase is a member of at least one of the following clades as defined herein: the SMDY clade, ALWNW clade, and WFAAAL clade.

30 17. The pulp composition of any of items 14 to 16, wherein the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at
35 least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

18. The pulp composition of any of items 14 to 17, wherein the paper pulp is wood pulp.

5 19. The pulp composition of any of items 14 to 18, wherein the paper pulp is a chemical pulp, such as kraft pulp.

20. The pulp composition of any of items 14 to 19, wherein an average degree of polymerization of the xylosaccharides in a filtrate from the pulp is 3.5-8, preferably 4-7.

21. Use of a GH8 xylanase for treating a paper pulp.

10 22. The use of item 21, wherein GH8 xylanase improves the brightness and/or strength and/or dewatering of the paper pulp.

The present invention is further described by the following examples which should not be construed as limiting the scope of the invention.

15 **EXAMPLES**

Preparation of pulp handsheets

For the measurement of the "ISO brightness" (diffuse blue reflectance factor; ISO 2470-1), pulp handsheets were prepared according to ISO 3688 and using a Color Touch PC spectrophotometer from Technidyne.

20 For physical testing, e.g. tensile and burst strength, pulp handsheets were prepared according to TAPPI standard T205. The handsheets were conditioned for at least 24 hours in a controlled humidity (50.0% ± 2.0% RH) and temperature (23.0 ± 1.0°C) atmosphere before being measured in the same room. The physical testing of the handsheets was conducted as described in TAPPI standard T220. The tensile test procedure was based on Tappi standard T using the
25 Instron 5564 tester. The burst test procedure was based on Tappi standard T 403 using a L&W Burst-O-Matic tester.

Aldehyde group measurement in pulp

The amount of aldehyde groups in pulps was measured spectrophotometrically (UV-Vis spectrophotometer HP 8453A, model G1103A) according to the procedure described by
30 Obolenskaya et al., "Determination of aldehyde groups in oxidized pulps", Laboratory Manipulations in Wood and Cellulose Chemistry, Ecologia, Moscow, 211-212, 1991, which is based on the reaction of 2,3,5-triphenyltetrazolium chloride (TTC) with the aldehyde groups leading to the formation of formazan (red colorant).

Chemical Oxygen Demand (COD) determination

35 The COD determination in the pulp filtrates was performed using a COD Cell Test from Merck. The reaction cells with the diluted filtrate were put in a thermo reactor TR 620 at 148 ºC for 2 hours and then allowed to cool down before measurement in the photometer Spectroquant®

NOVA60 within 60 min after the reaction. The Absorbance of the pulp filtrates was measured with a UV-Vis spectrophotometer HP 8453A, model G1103A.

Measurement of reducing sugars in filtrates

For the measurement of the reducing sugars (CHO groups) in the pulp filtrates, a microtiter plate method based on p-Hydroxybenzoic acid hydrazide (PHBAH) was utilized as reducing sugars react with hydrazides of benzoic acids in an alkaline medium to yield the bis-benzoylhydrazones of glyoxal and methylglyoxal, both of which had intense yellow color. The rate of reaction was accelerated by the catalytic influence of bismuth ions and the generation of the glyoxal and methylglyoxal derivatives was monitored at 405 nm and was proportional to the amount of reducing sugars concentration as calculated from a standard curve constructed from xylose calibration standards. The pulp filtrates were measured as it was for RS and as well after a secondary enzymatic hydrolysis with a beta-xylosidase (200 µL of pulp filtrate at buffered pH 3.5 with 0,002 mg EP/mL at 50°C for 60 min) to convert the small xylosaccharides (XOS) present in the filtrate into xylose and thereby increase the response of the reducing sugar assay. The ratio of the RS value with beta-xylosidase to the RS value without beta-xylosidase was an indication of the average degree of polymerization of the XOS present in the pulp filtrates.

Chemicals used as buffers and substrates were commercial products of at least reagent grade.

Enzymes used in the Examples

Xylanase family	Description
GH8 xylanase A	shown as the mature polypeptide of SEQ ID NO: 1 herein, also disclosed as the mature polypeptide of SEQ ID NO: 3 of WO2011/070101.
GH8 xylanase B	shown as SEQ ID NO: 2 and SEQ ID NO: 3 herein, also disclosed as SEQ ID NO: 2 and SEQ ID NO: 3 of WO 2019/122083.
GH8 xylanase C	shown as SEQ ID NO: 4 and SEQ ID NO: 5 herein, also disclosed as SEQ ID NO: 23 and SEQ ID NO: 24 of WO 2019/122083.
GH8 xylanase D	shown as SEQ ID NO: 6 herein, also disclosed as SEQ ID NO: 18 of WO 2019/122083.
GH8 xylanase E	shown as SEQ ID NO: 7 and SEQ ID NO: 8 herein, also disclosed as SEQ ID NO: 8 and SEQ ID NO: 9 of WO 2019/122083.
GH8 xylanase F	shown as SEQ ID NO: 9 and SEQ ID NO: 10 herein, also disclosed as SEQ ID NO: 5 and SEQ ID NO: 6 of WO 2019/122083.
GH10 xylanase A	shown as the mature polypeptide of SEQ ID NO: 11 herein, and also disclosed as the mature polypeptide of SEQ ID NO: 22 of WO2014197296.

GH10 xylanase B	shown as the mature polypeptide of SEQ ID NO: 12 herein, and also disclosed as the mature polypeptide of SEQ ID NO: 1 of WO2005118769.
GH11 xylanase A	shown as the mature polypeptide of SEQ ID NO: 13 herein, also disclosed as the mature polypeptide of SEQ ID NO: 14 of WO2019/055455.
GH11 xylanase B	shown as the mature polypeptide of SEQ ID NO: 14 herein, also disclosed as the mature polypeptide of SEQ ID NO: 2 of WO9743409.

EXAMPLE 1 Effect of a xylanase treatment of a market hardwood bleached pulp on pulp and filtrate properties

Bleached northern mixed hardwood kraft pulp in sheet form (dry lap market paper-grade
5 pulp) was teared by hand and soaked in distilled water overnight and then disintegrated in a pulp disintegrator at 10000 rpm. After disintegration, the pulp was filtered and crumbled before being used in the experiments. Typically, 20 g odp (oven-dry pulp; dry matter basis) was treated with a xylanase at 10% consistency, 45ºC and pH 4.5 (acetate buffer) for 4 hours using 10 mg enzyme protein (EP) / kg odp (oven-dry pulp; dry matter basis). The pulp suspension was
10 incubated in sealed polyethylene plastic bags immersed in a temperature controlled water bath. After incubation, the pulp was filtered and the filtrate collected. The pulp was then washed and filtered in three consecutive steps with 1 L of warm tap water. Control experiments were run in parallel under the same conditions except for the use of enzyme.

In Table 1, it is observed that the GH10 and GH11 endo-xylanase treatments led to a much
15 higher release of reducing sugars (aldehyde groups, CHO) in the filtrate as compared with the two GH8 endo-xylanases tested. As expected, with the beta-xylosidase treatment of the pulp filtrate, a higher amount of reducing sugars can be measured due to the enzymatic hydrolysis of the XOS. However, a surprisingly higher average degree of polymerization (DP) of the XOS was obtained in the filtrates from the pulps treated with the two GH8 xylanases (DP ca. 6) as
20 compared to the two GH10 (DP = 2.6) and GH11 (DP = 3.3) xylanases tested, as estimated by the CHO ratio.

Regarding the amount of reducing end groups (CHO content) in the pulps, the treatment with xylanases increased its amount due to the enzymatic hydrolysis of pulp xylan. However, a surprisingly high amount of CHO groups was attained with the GH8 xylanases (ca. 34 mmol/kg
25 odp) comparable to the amount reached with the GH11 xylanase (ca. 35 mmol/kg odp). This was an important finding as the GH8 xylanases led to a very low amount of solubilized xylan in the filtrate as measured by the RS (CHO) measurements in the filtrates. This was further supported by the amount of COD generated by the xylanases (Table 2), as the GH10 xylanase and GH11 xylanase generated a much higher amount of COD compared to the GH8 xylanases.

The same tendency was also seen in Absorbance at 280 nm which thus indicated a lower release of aromatic structures associated with xylan by the GH8 xylanases. However, in terms of brightness gain, all tested xylanases could increase the brightness of the pulp to similar extent (Table 3). Therefore, the main advantage with the use of GH8 xylanases was that they produced
5 a more controlled (less severe) hydrolysis of xylan and thereby had a more xylan retaining modification of pulp xylan as compared to the other xylanase families while still improving pulp properties, such as brightness. This had a significant importance as xylan loss needed to be avoided in paper pulp production.

In terms of strength properties, it can be seen in Table 3 that tensile and burst indexes
10 were increased by the xylanases, being highest with the GH11, followed by the GH8 and then the GH10 xylanase. A positive impact of the GH8 xylanases was thus achieved on strength development while preserving pulp yield and minimizing COD impact as opposed to the GH10 and GH11 xylanases. This again highlights the advantage of using GH8 xylanases since the GH10 and GH11 xylanases may lead to a too severe xylan hydrolysis and thus creating more
15 harm than benefit in the pulp and paper production process.

Table 1. Evaluation of reducing end groups in the pulps that were treated with xylanases belonging to GH8, GH10 and GH11 families and in the resulting pulp filtrates: amount of reducing sugars in the pulp filtrates with and without a secondary hydrolysis with a beta-xylosidase (BX).
20 CHO ratio between the measurements with and without BX treatment is an estimate of the average degree of polymerization of the XOS in the filtrate; amount of reducing end groups (aldehydes) in the treated pulps given as mmol/kg odp.

Enzyme	Xylose eq. as CHO content in filtrate (mg/mL)		CHO ratio in filtrate	CHO content in pulp (mmol/kg odp)
	A: Without secondary hydrolysis with BX	B: With secondary hydrolysis with BX	B/A (estimate of the size of released XOS)	
Control	0.006	0.134	---	23.3
GH8 xylanase A	0.061	0.365	6.0	33.9
GH8 xylanase B	0.058	0.333	5.7	33.7
GH10 xylanase A	0.570	1.501	2.6	42.6
GH11 xylanase A	0.466	1.542	3.3	34.8

Table 2. Absorbance and COD of the filtrates from the treated pulps

Enzyme	Absorbance at 280 nm	COD (mg/L)
Control	0.05	4671±8

GH8 xylanase A	0.15	4968±32 (+6.4%)
GH8 xylanase B	0.15	4503±18 (-3.6%)
GH10 xylanase A	0.34	5925±2 (+26.8%)
GH11 xylanase A	0.36	6201±20 (+32.8%)

Table 3. Brightness and strength properties of the treated pulps

Enzyme	ISO brightness (%)	Tensile index (Nm/g)	Burst index (kPa.m^2/g)
Control	88.0	25.4	1.25
GH8 xylanase A	88.3	26.1 (+2.6%)	1.37 (+9.2%)
GH8 xylanase B	88.6	26.6 (+4.7%)	1.33 (+5.8%)
GH10 xylanase A	88.7	25.7 (+1.2%)	1.28 (+1.8%)
GH11 xylanase A	88.8	28.3 (+11.3%)	1.40 (+12.0%)

EXAMPLE 2 Effect of xylanase treatment in the pre-bleaching of an unbleached pulp on bleach
5 boosting performance

An unbleached eucalypt kraft pulp was submitted to a sequence of treatments in the following order: xylanase (X-stage), followed by chlorine dioxide bleaching (D-stage) and then an alkaline extraction reinforced with hydrogen peroxide (Ep-stage) with pulp washing in between each stage. Thirty grams of pulp (as oven-dry pulp) were treated at 50°C, pH 6
10 (phosphate buffer), for 120 min with a dosage of xylanase of 10 mg EP/kg odp. The chlorine dioxide treatment (D-stage) was done using 1.12 % odp ClO_2 (100% dosage) or 0.95% odp ClO_2 (85% dosage), 70ºC, initial pH of 3,0 (adjusted with sulfuric acid) for 60 min. The Ep-stage was performed at 75°C, using 1.35 % odp NaOH and 0.20% odp H_2O_2 for 60 min. All pulp treatments were run at 10% consistency in sealed polyethylene plastic bags immersed in a temperature
15 controlled water bath.

After each stage the pulp was filtered and washed as described in Example 1. The pulp filtrate was collected to measure the COD.

The use of GH8 and GH10 xylanases in the pre-bleaching of the eucalypt kraft pulp allowed to save ca. 15% ClO_2 while the GH11 xylanase could deliver higher savings (Table 4).
20 However, the higher chlorine dioxide savings were at the cost of a severe loss in pulp yield as indicated by the much higher increase in COD of the filtrates of the GH11 treated pulps (Table 5). From a selectivity point of view, the GH8 xylanases revealed a much lower COD impact and thus retained more xylan in the pulp and preserved yield while still enabling ca. 15% ClO_2 savings.

25

Table 4. Brightness of the X-D-Ep bleached pulps. D stages with 100% or 85% ClO_2 as indicated.

Enzyme	ISO brightness (%)
Control with 100% ClO_2	77.3
Control with 85% ClO_2	76.0
GH8 xylanase A (with 85% ClO_2)	77.1
GH8 xylanase B (with 85% ClO_2)	76.9
GH10 xylanase A (with 85% ClO_2)	77.3
GH11 xylanase A (with 85% ClO_2)	78.4

Table 5. COD of the filtrates from the treated pulps

Enzyme	COD (mg/L)			
	X	D	Ep	Sum (X-D-Ep)
Control with 100% ClO_2	3984	1053	971	6008
Control with 85% ClO_2		1058	1028	6069
GH8 xylanase A (with 85% ClO_2)	3990 (+0.2%)	1314 (+24.3%)	974 (-5.3%)	6278 (+3.4%)
GH8 xylanase B (with 85% ClO_2)	4230 (+6.2%)	1338 (+26.5%)	1064 (+3.5%)	6632 (+9.3%)
GH10 xylanase A (with 85% ClO_2)	5016 (+25.9%)	1731 (+63.7%)	1050 (+2.2%)	7797 (+28.5%)
GH11 xylanase A (with 85% ClO_2)	5244 (+31.6%)	1698 (+60.6%)	1055 (+2.6%)	7997 (+31.8%)

EXAMPLE 3 Effect of xylanase treatment in the post-bleaching of a never dried bleached
5 eucalypt kraft pulp

A bleached never dried eucalypt kraft pulp was treated with GH8 and GH11 xylanases as a post-bleaching step. 20 g odp (oven-dry pulp; dry matter basis) was treated with xylanases at 10% consistency, 55ºC and pH 6.0 (phosphate buffer) for 2 hours using 1.5, 4.0 and 10 mg enzyme protein (EP) / kg odp (oven-dry pulp; dry matter basis). The pulp was treated and
10 handled as described in Example 1. Control experiments were run in parallel under the same conditions except for the use of enzyme.

The results shown in Table 6 reveal that all enzyme treatments were able to reach an ISO brightness of ca. 91% and thus ca. 1.0 brightness point increase. It is once again highlighted the much lower organic load released into the pulp filtrate, as indicated by both the COD and CHO
15 content in the filtrates, attained with the GH8 xylanases at all dosage levels compared to the GH11 xylanase. It was again confirmed this xylan preservation effect in the pulp by the GH8 xylanase treatments while still benefiting in terms of a brightness gain to the same level as with the GH11 xylanases.

Table 6. Brightness of the treated pulps and COD and reducing sugars in the pulp filtrates

Enzyme and dosage (mg EP/kg odp)		Pulp	Filtrate			
		ISO brightness (%)	COD (mg/L)	Xylose eq. as CHO content (mg/mL)		
				A: Without BX hydrolysis	B: With BX hydrolysis	Ratio B/A
Control	0	90.1	407	0.007	0.273	---
GH8 xylanase A	1.5	91.0	1315	0.086	0.603	7.0
	4.0	91.3	1466	0.122	0.717	5.9
	10	91.0	2316	0.186	0.858	4.6
GH8 xylanase B	1.5	90.9	1415	0.112	0.648	5.8
	4.0	91.1	1676	0.170	0.810	4.8
	10	91.1	2445	0.254	1.056	4.2
GH11 xylanase A	1.5	91.5	1875	0.291	1.027	3.5
	4.0	91.3	2711	0.499	1.339	2.7
	10	91.1	3605	0.596	1.997	3.3

EXAMPLE 4 Effect of xylanase treatment in the post-bleaching of a never dried bleached hardwood kraft pulp

A bleached never dried hardwood kraft pulp was treated with GH8, GH10 and GH11
5 xylanases as a post-bleaching step. 10 g odp (oven-dry pulp; dry matter basis) was treated with xylanases at 10% consistency, 45ºC and pH 6.0 (phosphate buffer) for 3 and 20 hours using 2.0 mg enzyme protein (EP) / kg odp (oven-dry pulp; dry matter basis). The pulp was treated and handled as described in Example 1. Control experiments were run in parallel under the same conditions except for the use of enzyme.

10 The results shown in Table 7 reveal that all enzyme treatments were able to reach a gain in ISO brightness of at least 0.6-0.8 brightness points with the GH8 xylanases after 3 hours while the GH10 and G11 xylanases reached a gain of 0.7 and 1.2, respectively. Similarly, to the previous examples, the brightness gains of the GH8 xylanases were achieved at a lower release of COD into the filtrate and thus preserving more the yield. At an extended time of 20 hours, the
15 GH8 xylanases again confirmed a much lower release of organic load into the filtrate as measured by the COD and reducing sugars. This is important as in full-scale context the pulp can be in contact with the xylanase for a long time in case of stops in the process of pulp production and/or papermaking. The GH8 xylanases show once again a surprisingly higher average degree of polymerization of the released XOS compared to the GH10 and GH11
20 xylanases after a long incubation time of 20h.

Table 7. Brightness of the treated pulps and COD and reducing sugars in the pulp filtrates at two different treatment times

Enzyme	Pulp		Filtrate				
	ISO brightness (%)		COD (mg/L)		Xylose eq. as CHO content at 20 hours (mg/mL)		
	3 hours	20 hours	3 hours	20 hours	A: Without BX hydrolysis	B: With BX hydrolysis	Ratio B/A
Control	86.9	87.5	68	255	0.085	0.402	---
GH10 xylanase A	87.7	87.8	507	1304	0.456	1.115	2.4
GH11 xylanase A	88.2	88.1	1009	1882	0.680	1.916	2.8
GH8 xylanase C	87.7	88.3	412	827	0.227	0.792	3.5
GH8 xylanase D	87.5	87.9	336	594	0.115	0.571	5.0
GH8 xylanase E	87.6	87.8	306	611	0.122	0.605	5.0

EXAMPLE 5 Effect of xylanase treatment in the post-bleaching of a never dried bleached eucalypt kraft pulp

A bleached never dried hardwood kraft pulp was treated with GH8, GH10 and GH11
5 xylanases as a post-bleaching step. 5 g odp (oven-dry pulp; dry matter basis) was treated with xylanases at 10% consistency, 65ºC and pH 6.0 (phosphate buffer) for 20 hours using 1.0 mg enzyme protein (EP) / kg odp (oven-dry pulp; dry matter basis). The pulp was treated and handled as described in Example 1. Control experiments were run in parallel under the same conditions except for the use of enzyme.

10 All tested enzymes increased brightness as seen in Table 8. All GH8 xylanases released less COD than the tested GH10 and GH11 xylanases. At the same ISO brightness level, e.g. 89.5±0.1%, a much lower amount of COD was released by the action of the GH8 xylanases.

Table 8. Brightness of the treated pulps and COD in the resulting pulp filtrates

Enzyme	Pulp	Filtrate
	ISO brightness (%)	COD (mg/L)
Control	88.9	88
GH10 xylanase A	89.5	930
GH10 xylanase B	89.1	310
GH11 xylanase B	89.5	898
GH8 xylanase A	89.2	203
GH8 xylanase C	89.2	189
GH8 xylanase D	89.1	159
GH8 xylanase E	89.2	173
GH8 xylanase F	89.4	218

15 **EXAMPLE 6** Effect of a xylanase treatment using a bleached eucalypt kraft market paper-grade pulp

A bleached eucalypt market kraft paper-grade pulp (dried pulp) was treated with GH8, GH10 and GH11 xylanases. 5 g odp (oven-dry pulp; dry matter basis) was treated with xylanases at 10% consistency, 65ºC and pH 6.0 (phosphate buffer) for 23 hours using 1.0 mg enzyme protein (EP) / kg odp (oven-dry pulp; dry matter basis). The pulp was treated and handled as
5 described in Example 1. Control experiments were run in parallel under the same conditions except for the use of enzyme.

In Table 9 it can be seen that the GH8 xylanases achieved higher brightness at lower COD release which confirmed once again a more xylan-retaining modification of the pulp in comparison with the GH10 and GH11 xylanases that led to higher losses of xylan and thus a
10 higher content of COD in the filtrate.

Table 9. Brightness of the treated pulps and COD in the resulting pulp filtrates

Enzyme	Pulp	Filtrate
	ISO brightness (%)	COD (mg/L)
Control	90.2	147
GH10 xylanase A	90.4	394
GH10 xylanase B	90.4	226
GH11 xylanase B	90.6	317
GH8 xylanase B	90.4	153
GH8 xylanase C	90.7	183
GH8 xylanase D	90.6	163
GH8 xylanase E	90.6	149
GH8 xylanase F	90.7	192

CLAIMS

1. A method for treating a paper pulp, comprising contacting the paper pulp with a GH8 xylanase.

2. The method of claim 1, wherein the GH8 xylanase is an endo-beta-1,4-xylanase.

3. The method of claim 1 or 2, wherein the GH8 xylanase is a member of DPSY clade; preferably GH8 xylanase is a member of at least one of the following clades as defined herein: SMDY clade, ALWNW clade, and WFAAAL clade.

4. The method of any of claims 1 to 3, wherein the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

5. The method of any of claims 1 to 4, wherein the pulp is wood pulp.

6. The method of any of claims 1 to 5, comprises a sequence of treatments selected from the group consisting of:

a) X-B;
b) B-X;
c) XB;
wherein,
X is a GH8 xylanase treatment stage,
B is a bleaching stage, preferably a chlorine dioxide bleaching stage, and
XB is a GH8 xylanase treatment together with a bleaching stage.

7. The method of any of claims 1 to 6, which further comprises contacting the pulp with one or more additional enzyme(s) having protease, lipase, xylanase, cutinase, oxidoreductase, cellulase, endoglucanase, amylase, mannanase, steryl esterase, polysaccharide monooxygenase (LPMO) and/or cholesterol esterase activity, preferably a cellulase.

8. The method of any of claims 1 to 7, wherein an average degree of polymerization of the xylosaccharides in a filtrate from the pulp obtained from the method is 3.5-8, preferably 4-7.

9. The method of any of claims 1 to 8, wherein the method results in an improved benefit/harm balance with regard to a positive effect on strength and/or brightness development and/or dewatering of the pulp while minimizing a negative effect on pulp yield, and/or COD of the resulting filtrate/effluent, compared with a method with the GH8 xylanase treatment replaced by a GH10 xylanase treatment or GH11 xylanase treatment.

10. A paper pulp prepared by the method of any one of claims 1 to 9.

11. A pulp composition, comprising a paper pulp and a GH8 xylanase.

12. The pulp composition of claim 10 or 11, wherein the GH8 xylanase is an endo-beta-1,4-xylanase; or wherein the GH8 xylanase is a member of DPSY clade; preferably GH8 xylanase is a member of at least one of the following clades as defined herein: SMDY clade, ALWNW clade, and WFAAAL clade.

13. The pulp composition of any of claims 10 to 12, wherein the GH8 xylanase is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; preferably the polypeptide has at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a mature polypeptide of any of SEQ ID NO: 1 to SEQ ID NO: 10; and

(b) a polypeptide derived from (a) by substitution, deletion, and/or insertion of one or more (several) amino acids; and

(c) a fragment of the polypeptide of (a), or (b) that has GH8 xylanase activity.

14. Use of a GH8 xylanase for treating a paper pulp.

15. The use of claim 14, wherein GH8 xylanase improves the brightness and/or strength and/or dewatering of the paper pulp.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/070900

A. CLASSIFICATION OF SUBJECT MATTER
INV. D21C5/00 D21C9/10 D21C9/14 D21H17/00
ADD.

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)
D21C D21H

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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/018908 A1 (NOVOZYMES AS [DK]) 12 February 2015 (2015-02-12) claims 1-15; examples 1-9	1-15
X	WO 03/074780 A1 (IOGEN BIO PRODUCTS CORP [CA]; TOLAN JEFFREY S [CA] ET AL.) 12 September 2003 (2003-09-12) claims 1-20; example 2	1-15
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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