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(71) Applicant: **AB Enzymes GmbH**
64293 Darmstadt (DE)

(72) Inventors:
• **Besenmatter, Werner**
64293 Darmstadt (DE)

• **Quiros, Mauricio**
Plantation, 33324 (US)
• **Hellmuth, Hendrik**
64293 Darmstadt (DE)
• **Langfelder, Kim**
64293 Darmstadt (DE)

(74) Representative: **Espatent Oy**
Kaivokatu 10 D
00100 Helsinki (FI)

Remarks:

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(54) **A BLEACHING PROCESS**

(57) A bleaching process of pulp is disclosed comprising performing a delignification of the pulp by carrying out in a following sequence: an enzymatic treatment step comprising contacting the pulp with a glycoside hydrolase family GH11 xylanase enzyme at a pH of at least 9.5 and a temperature of at least 85 °C, an oxidation step wherein a bleaching agent is added, an alkaline extraction step wherein an alkaline agent is added, and recovering bleached pulp, wherein the GH11 xylanase enzyme has an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90°C and pH 10.5, compared to the same mixture without any xylanase enzyme.

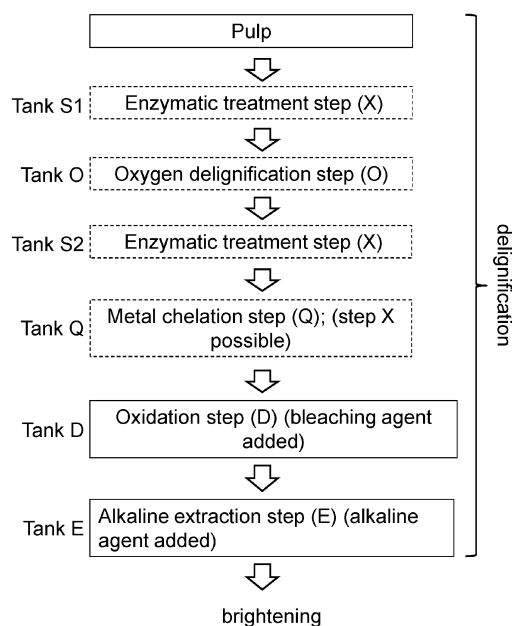


Fig. 1

Description**TECHNICAL FIELD**

[0001] The present disclosure generally relates to bleaching of pulp. The disclosure relates particularly, though not exclusively, to an improved process of delignification of pulp in the presence of GH11 xylanase enzyme at high pH and high temperature.

BACKGROUND

[0002] This section illustrates useful background information without admission of any technique described herein representative of the state of the art.

[0003] Bleached pulp is used to make, for example, paper, paperboard, tissue, dissolving pulp, fluff pulp, market pulp, and recently also new products defined as MFC (Micro Fibrillated Cellulose) and NCF (Nano Cellulose Fibers). Pulp is commonly processed by kraft pulping, wherein free cellulose fibers are obtained by dissolving the lignin in alkaline cooking liquor. The residual lignin and lignin derivatives attached to carbohydrate moieties of the pulp give the pulp undesired brown color, and the pulp is traditionally thereafter bleached.

[0004] The pulp bleaching process can be divided into two subsequent sections: delignification and brightening. In the delignification, the wood components lignin and hemicelluloses are at least partly dissolved and removed from the pulp via the use of bleaching chemicals. During delignification, the brightness of the pulp is also increased. In the subsequent brightening section, the delignified pulp is treated with bleaching chemicals that will react with chromophores leading to their elimination thereby producing bleached or white pulp.

[0005] Typically, the delignification has at least two stages, an oxidation step wherein a bleaching agent is added, and an alkaline extraction step, wherein an alkaline agent is added. Additionally, the oxidation step can be preceded by an oxygen-delignification step (O), wherein oxygen is mixed into the pulp, and wherein a large portion of the lignin within the pulp is removed.

[0006] The bleaching agent added in the oxidation step is further contributing to the lignin removal, and several bleaching agents can be employed in the process. Pulp has been traditionally bleached (whitened) with chemicals comprising chlorine, such as elemental chlorine, which enables the creation of chlorinated organic by-products having toxic and mutagenic effects in the environment. Therefore, elemental chlorine-free methods for pulp bleaching have been utilized, to avoid the release of chlorogenic compounds. A totally chlorine free bleaching utilizes bleaching agents, such as hydrogen peroxide.

[0007] Pulp comprises a complex mixture of polysaccharides, mainly cellulose, lignin and hemicellulose. Xylan is an important component of hemicellulose. The chemical composition of xylan comprises a polysaccharide main chain of β -1,4-linked xylose residues, and often side chains which can be, for example, acetyl, arabinosyl and/or glucuronosyl groups. As a component of pulp, xylan holds back substances such as lignin and hexenuronic acid, that consume bleaching chemicals. Such substances can be chemically bound to xylan and/or physically entrapped in xylan. Hexenuronic acid is formed in pulp material digesters.

[0008] Xylanases are enzymes that digest xylans resulting in mixtures of smaller xylan fragments. Xylanases that hydrolyze β -1,4-linked xylose residues, particularly endo-1,4- β -xylanases (EC 3.2.1.8) have been classified in glycoside hydrolase families GH5, GH8, GH10, GH11, GH30 and GH43 in the CAZy database. The xylanases of different glycoside hydrolase families differ structurally from each other, the GH11 xylanase enzymes having a β -jelly roll structure, the GH5, GH10 and GH30 xylanase enzymes having a $(\beta/\alpha)_8$ barrel structure, the GH8 xylanase enzymes having a $(\alpha/\alpha)_6$ barrel structure, and the GH43 xylanase enzymes having a 5-fold β -propeller structure. Many different xylanases are known, for example in GH10 and GH11 families there are 5949 and 2187 different enzymes, respectively. Additionally, many variants of xylanases found in nature have been engineered. In pulp bleaching processes, a xylanase shall depolymerize xylan, break the link between xylan and lignin, facilitate lignin reduction and improve the accessibility of bleaching chemicals to lignin, thereby contributing to delignification, bleaching efficiency and final brightness of the pulp. In pulp bleaching, the pulp entering the delignification stage is hot and alkaline, and therefore the xylanases used in such processes must possess exceptionally good stability and activity at biologically challenging conditions of very alkaline pH and high temperature.

[0009] There is an increasing demand for such xylanase enzymes, which can retain activity and function in a pulp bleaching process, particularly with regard to improving pulp brightness, reduction of bleaching chemical consumption and reduction of toxic chlorine-containing compound generation. Several xylanases with pronounced differences in pulp bleaching performance, pH- and temperature profile, enzyme stability, specific activity and other enzyme properties have been reported, but generally xylanases are not stable and do not perform under very high temperatures and alkaline conditions of pulp bleaching processes. Therefore, there is a need for xylanase enzymes which are both, very thermostable and alkaline-tolerant, and which are active and perform in industrial processes such as pulp bleaching. There-

fore, it is an object of the present disclosure to provide a bleaching process with a xylanase enzyme that is able to perform in the said process at high temperature and alkaline pH.

SUMMARY

[0010] The appended claims define the scope of protection. Any example and/or technical description of an apparatus, system, product and/or process in the description and/or drawing which is not covered by the claims is presented herein not as an embodiment of the invention but as an example useful for understanding the invention.

[0011] According to a first aspect is provided a bleaching process comprising:

providing pulp;
performing a delignification of the pulp by carrying out in the following sequence:

- a) an enzymatic treatment step (X) comprising contacting the pulp with a glycoside hydrolase family GH11 xylanase enzyme at a pH of at least 9.5 and a temperature of at least 85 °C;
- b) an oxidation step (D), wherein a bleaching agent is added;
- c) an alkaline extraction step (E), wherein an alkaline agent is added; and
- d) recovering bleached pulp;

wherein the GH11 xylanase enzyme has an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5, compared to the same mixture without any xylanase enzyme.

[0012] The inventors have surprisingly found, and show in the examples below, a suitable xylanase enzyme for the present bleaching process can be selected based on the molecular mechanism underlying pulp bleaching performance of the xylanase enzyme, rather than just xylanase enzyme activity or the release of xylan hydrolysis products from pulp. This is rationalized by a pre-bleaching effect of xylanases, being the result mainly of xylan depolymerization, rather than solubilization.

[0013] In an embodiment, GH11 xylanase enzymes that depolymerize xylan without solubilization of xylan are particularly advantageous in a pulp bleaching process, because xylan solubilization may lead to undesirable chemical oxygen demand (COD) increase and pulp yield loss. Therefore, the selection of suitable GH11 xylanase enzyme for pulp bleaching performance should be based on the molecular mechanism underlying the pulp bleaching performance, in addition to and/or instead of the xylanase enzyme activity or the release of xylan hydrolysis products from pulp. When polymers like xylan are depolymerized, the viscosity of a xylan-containing mixture in question is reduced. However, because xylan is only a minor component of pulp, any xylanase induced viscosity reduction in pulp is likely to be small and hardly measurable. It was surprisingly found that xylanase induced viscosity reduction indicating the depolymerization potential of xylanase enzymes, could be measured in xylan-containing mixtures comprising pure or purified xylan, rather than pulp. Furthermore, it was surprisingly found that xylanase induced viscosity reduction is connected to xylanase performance in a pulp bleaching process at high temperature and high pH.

[0014] The bleaching process provides an efficient way to produce delignified and bleached pulp in the presence of the GH11 xylanase enzyme at high temperature and high pH.

[0015] According to a second aspect is provided a pulp composition comprising:

- (a) pulp having a pH of at least 9.5; and
- (b) 0.01 - 200 g of a glycoside hydrolase family GH11 xylanase enzyme protein per 1000 kg of dry matter pulp;

wherein the GH11 xylanase enzyme protein has:

xylanase activity, and
an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5 compared to the same mixture without any xylanase enzyme, wherein the xylan-containing mixture comprises 0.07 g/ml or less of xylan and 3.0 µg or less of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes.

[0016] According to a further aspect is provided a bleaching process comprising:

providing pulp;
performing a delignification of the pulp by carrying out in the following sequence:

- a) an enzymatic treatment step (X) comprising contacting the pulp with the glycoside hydrolase family GH11

xylanase enzyme at a pH of 9.5 - 11.5 and a temperature of 85 - 100 °C, wherein the xylanase enzyme has a residual xylanase activity of at least 25 % after 20 min of step X;
 b) an oxidation step (D), wherein a bleaching agent is added;
 c) an alkaline extraction step (E), wherein an alkaline agent is added; and
 d) recovering delignified pulp.

[0017] According to a further aspect is provided a pulp composition comprising:

- (a) pulp having a pH of at least 9.5;
- (b) 0.25 - 4 wt-% of a bleaching agent of the total weight of dry matter pulp, the bleaching agent being selected from ClO₂, O₃, H₂O₂, and peroxides; and
- (c) 0.01 -200 g of a glycoside hydrolase family GH11 xylanase enzyme protein per 1000 kg of dry matter pulp;

wherein the GH11 xylanase enzyme protein has xylanase activity, and an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5 compared to the same mixture without any xylanase enzyme, wherein the xylan-containing mixture comprises 0.07 g/ml or less of xylan and 3.0 µg or less of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes.

[0018] The present bleaching process comprising the GH11 xylanase enzyme is advantageous in reducing the overall amount of bleaching agent used in the pulp bleaching process. The present bleaching process is advantageous in reducing the amount of ClO₂, O₃, H₂O₂ or peroxide consumption in the pulp bleaching process. The present bleaching process is advantageous in achieving higher pulp brightness and/ or higher bleaching product production with the same amount of bleaching agent used in the pulp bleaching process, when compared to a bleaching process without the GH11 xylanase enzyme. The present bleaching process is advantageous in achieving higher pulp brightness and/ or higher bleaching product production with reduced amount of bleaching agent used in the pulp bleaching process, when compared to a bleaching process without the GH11 xylanase enzyme. The present bleaching process is advantageous in reducing the production costs of bleached pulp. The present bleaching process allows more efficient bleaching of the pulp, resulting in more efficient utilization of wood raw material. The present bleaching process is advantageous in integrating the use of the GH11 xylanase enzyme with a high pH- and thermostability to a bleaching process, thereby reducing or avoiding the need to change existing process conditions and/or the setup/hardware commonly used in pulp bleaching. The present bleaching process is advantageous in reducing the corrosion, wear and erosion of the metal pulp bleaching tanks, due to reduced need for lowering pulp pH with acidic agents. The present bleaching process is advantageous in reducing fresh water consumption of the bleaching process, due to reduced need for additional water washes. The present bleaching process is advantageous in reducing the generation of AOX (Adsorbable Organic Halide also known as chloro-organic compounds) which are discharged with the wastewater bleaching effluents. The present bleaching process is advantageous in reducing the amount of energy needed to increase the temperature of pulp after being washed and before the oxygen delignification or before the oxidation step, due to reduced need for lowering pulp temperature for enzyme treatment.

[0019] Different non-binding example aspects and embodiments have been illustrated in the foregoing. The embodiments in the foregoing are used merely to explain selected aspects or steps that may be utilized in different implementations. Some embodiments may be presented only with reference to certain example aspects. It should be appreciated that corresponding embodiments may apply to other example aspects as well.

BRIEF DESCRIPTION OF THE FIGURES

[0020] Some example embodiments will be described with reference to the accompanying figure, in which:
 Fig. 1 shows a flow chart of the process steps of a delignification bleaching process of pulp according to an example embodiment. The process steps X, O, Q inside dashed line boxes indicate optional and/or alternative process steps.

SEQUENCE LISTING

[0021]

SEQ ID NO: 1	AM24, amino acid sequence of the truncated form of <i>Thermopolyspora flexuosa</i> Xyn11A protein (AM35).The AM24 contains the amino acids D44-L263 of the full-length wild-type AM35
SEQ ID NO: 2	Amino acid sequence of the AM24 variant C31-4 comprising the mutations T3C, A23S, S28I, T30C
SEQ ID NO: 3	AM35, amino acid sequence of the full length mature <i>Thermopolyspora flexuosa</i> Xyn11A protein

DETAILED DESCRIPTION

[0022] The invention relates to a bleaching process utilizing a glycoside hydrolase family GH11 xylanase enzyme in a delignification step of the process, said GH11 xylanase enzyme having stability, activity and performance at biologically challenging conditions of high temperature and alkaline pH, resulting in improved bleaching of pulp.

[0023] By the term "bleaching process" is meant a chemical treatment process where the brightness or whiteness of the pulp is increased by removing chemical elements such lignin and chromophoric double bonds that give color to the pulp.

[0024] By the term "delignification" is meant the removal and extraction of lignin contained within pulp. Kappa number can be used as a measure of lignin content in pulp.

[0025] By the term "alkaline extraction step" is meant a bleaching step wherein pH of the pulp is increased to a level where oxidized lignin is extracted from the pulp.

[0026] By the term "glycoside hydrolase" is meant a hydrolase that is capable of hydrolyzing glycosidic bonds between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety.

[0027] The term "glycoside hydrolase family 11" or "GH11" refers to GH11 xylanases according to the definition of the CAZy database, <http://www.cazy.org/GH11.html>, the GH11 xylanases having a β -jelly roll structure, the GH11 xylanase enzymes thereby differing structurally from other xylanases.

[0028] The term "xylan" refers to matrix polysaccharides or heteropolymers, composed of a repeating β -1,4-linked xylose residue backbone, with various side-chain groups. Xylan polysaccharides can be categorized into classes O-acetylglucuronoxylans (AcGX), O-acetyl-arabinoxylans (AcAX), O-acetylglucuronarabinoxylans (AcGAX), and arab-inoglucuronoxylans (AGX), based on the substituted side-chain groups. Xylan-containing materials comprise plant-based or plant-originating material.

[0029] As used herein, the term "xylanase" denotes a xylanase enzyme defined according to that known in the art as endo-1,4- β -xylanase, or 4- β -D-xylan xylanohydrolase, known to catalyze the endohydrolysis of (1 $\rightarrow$ 4)- β -D-xylosidic linkages in xylans. Xylanases have the alternative names endo-(1 $\rightarrow$ 4)- β -xylan 4-xylanohydrolase, endo-1,4-xylanase, xylanase, β -1,4-xylanase, endo-1,4-xylanase, endo- β -1,4-xylanase, endo-1,4- β -D-xylanase, 1,4- β -xylan xylanohydro-lase, β -xylanase, β -1,4-xylan xylanohydrolase, endo-1,4- β -xylanase and β -D-xylanase. Xylanases are classified accord-ing to the Enzyme Nomenclature as EC 3.2.1.8.

[0030] As used herein, the term "GH11 xylanase enzyme protein" refers to pure GH11 xylanase enzyme protein. According to some embodiments, the GH11 xylanase enzyme protein can be added at an enzymatic treatment step X as one component of a composition, but in such embodiment also, the term GH11 xylanase enzyme protein refers to pure GH11 xylanase enzyme protein as one of the components of the composition. In some embodiments the amount of the GH11 xylanase enzyme protein refers to a dry weight (grams) of the GH11 xylanase enzyme protein, which dry-weight can be determined according to the test procedure described in the examples.

[0031] As used herein, the term "pulp" means water-comprising fibrous material originally obtained from plants. The pulp can originate from wood, fiber crops, bagasse, straw, waste paper or rags, or any combination thereof. Therefore, in the context of this application "pulp" can refer to wood pulp originating from wood material, and/or to non-wood pulp, originating from non-wood material.

[0032] As used herein, the term "wood pulp" means water-comprising fibrous material originating from wood material and produced by digesting hard or softwood chips at temperatures above about 120 °C with a solution of sodium hydroxide and sodium sulfide.

[0033] As used herein, the term "non-wood pulp" means water-comprising fibrous material originating from non-wooden materials, produced by digesting said non-wooden materials, such as grasses, cereal straws, corn stalks, bamboo, kenaf, or bagasse, or any combination thereof.

[0034] As used herein, the term "xylan-containing pulp" means wood or non-wood pulp containing xylan.

[0035] As used herein, the term "hardwood pulp" means wood pulp originating from wood fibers of broadleaved trees such as oak, beech, birch, aspen, eucalyptus and/or poplar.

[0036] As used herein, the term "softwood pulp" means wood pulp originating from wood fibers of needle-bearing trees such as pines, spruce, fir, and/or hemlock.

[0037] As used herein, the term "kraft pulp" means wood pulp produced by the kraft process, also known as the sulfate process, where wood chips are treated with sodium sulfide (Na₂S) and sodium hydroxide (NaOH).

[0038] As used herein, the term "thermostable enzyme" or "temperature tolerant enzyme" refers to an enzyme with a good ability to withstand and resist structural changes leading to enzyme inactivation, caused by high temperature such as temperature of 85 °C or higher.

[0039] As used herein, the term "pH stability of an enzyme" or "pH tolerance of an enzyme" describes the enzyme's property to withstand and resist structural changes leading to enzyme inactivation, caused by high pH such as pH of 9.5 or higher, or alternatively, caused by low pH.

[0040] By the term "process water" is meant any water that is used in the pulp bleaching process.

[0041] By the term "process wash water" is meant any water that is used for the purpose of washing pulp in the pulp bleaching process.

[0042] As used herein, the term "viscosity" or "relative viscosity" means the viscosity of the indicated material, such as a xylan-containing mixture, a suspension, or a solution. As used herein, a viscosity reduction of a mixture containing GH11 xylanase enzyme and xylan is an indicator of the xylan depolymerization activity of said GH11 xylanase enzyme. In an embodiment, the lower the viscosity of a xylan-containing mixture is reduced over time, the higher is the xylan depolymerization activity of the GH11 xylanase enzyme. The viscosity is measured with a rolling-ball viscometer.

[0043] As used herein, the term "xylan-containing mixture" refers to a mixture which contains xylan and which mixture can refer to a solution, suspension or a mixture of liquid and solids. In an embodiment, the xylan-containing mixture contains also xylanase enzyme, such as GH11 xylanase enzyme. The xylan-containing mixture is suitable for assessing the xylan-degrading activity of a xylanase enzyme. Therefore, the xylan-containing mixture does not contain any substances, which would distort or falsify the assessment of the xylan degrading activity of a xylanase enzyme. For example, the xylan-containing mixture must be suitable for assessing the effect the GH11 xylanase enzyme has on the viscosity of the xylan-containing mixture.

[0044] As used herein, the term "Adsorbable Organic Halides (AOX)" means a measure of organic halogens chlorine, bromine, and iodine load in a sample, such as water sample.

[0045] As used herein, the term "chemical oxygen demand (COD)" means a measure of the amount of oxygen that can be consumed by reactions in a measured solution. COD indicates the amount of oxygen needed to break down organic matter completely into CO₂ in a solution. The COD measurement is also used to determine amount of oxidizable pollutants, and hence, toxicity to biological life, wherein a high COD correlates with a high toxicity of the organic matter.

[0046] As used herein, the term "sequence identity" means the percentage of exact matches of amino acid residues between two optimally aligned sequences over the number of positions where there are residues present in both sequences. When one sequence has a residue with no corresponding residue in the other sequence, the used alignment program allows a gap in the alignment, and that position is not counted in the denominator of the identity calculation.

[0047] As used herein, the term "corresponding positions" or "corresponding amino acid position" means aligning at least two amino acid sequences according to identified regions of similarity or identity as pairwise alignment or as multiple sequence alignment, thereby pairing up the corresponding amino acids.

[0048] As used herein, the term "xylanase activity" refers to the xylan hydrolyzing activity.

[0049] By the term "residual xylanase activity" is meant a xylanase enzyme activity which is not the initial activity of said enzyme, but which has been changed, optionally decreased, due to process conditions the enzyme in question has been exposed to. Therefore, residual xylanase activity refers to the amount, for example the percentage amount, of initial activity left after exposure to specific process conditions.

[0050] The term "functional fragment" means a fragment or portion of the current GH11 xylanase enzyme polypeptide, which retains the same or substantially the same enzymatic function or effect as the entire GH11 xylanase enzyme polypeptide.

[0051] The term "stability" in context of enzyme or xylanase stability, describes the enzyme's property to withstand and/or function in process conditions that are challenging for the activity and functioning of the enzyme in question, such process conditions being, for example, high temperature, pH or radiation, a certain concentration of inorganic salt or an organic solvent, or a specific reaction mixture composition comprising e.g. proteases, stabilizers, builders, surfactants etc. The term "stability" reflects the stability of the xylanase according to the disclosure as a function of time, e.g., how much activity is retained when the xylanase is exposed to process conditions that are challenging for the activity and functioning of the enzyme in question.

[0052] As used herein, Xyn11A denotes a *Thermopolyspora flexuosa* xylanase from the glycoside hydrolase family 11 (GH11), also named as AM35. As used herein, AM35 denotes the wild-type mature GH11 xylanase, which is the parent polypeptide used for the AM24 polypeptide. The amino acids of the mature AM35 protein correspond to the amino acid sequence of SEQ ID NO: 3.

[0053] As used herein, the AM24 (also called AM24 protein herein) is a truncated form of the xylanase AM35. The AM24 protein comprises the catalytic module (core) and lacks the carbohydrate binding module (CBM) and part of the linker region between core and CBM. The amino acids of the mature polypeptide of AM24 correspond to the amino acids of the sequence SEQ ID NO: 1, comprising the amino acids D1-L220. The "core polypeptide" of AM24 comprises the amino acids D1 - G191 of SEQ ID NO:1, whereas the "inner core polypeptide" of AM24 comprises the amino acids T3 - S180 of SEQ ID NO:1.

[0054] As used herein, the C31-4 refers to a variant of AM24 comprising the substitutions T3C, A23S, S28I, T30C. The amino acids of the mature polypeptide of C31-4 correspond to the amino acids of the sequence SEQ ID NO: 2 comprising the amino acids D1-L220. The "core polypeptide" of C31-4 comprises the amino acids D1 - G191 of SEQ ID NO:2, whereas the "inner core polypeptide" of C31-4 comprises the amino acids C3 - S180 of SEQ ID NO:2.

[0055] As used herein, the term "carbohydrate binding domain" or "CBM" refers to specific domain or module in a polypeptide, which is capable in facilitating the binding of the polypeptide to a carbohydrate.

[0056] As used herein, the term "mature polypeptide" means any polypeptide wherein at least one signal sequence or signal peptide or signal peptide and a putative pro-peptide is cleaved off. For example, the "mature polypeptide" of AM24 comprises the amino acids D1 - L220 of SEQ ID NO:1 and the "mature core polypeptide" of AM24 comprises the amino acids D1 - G191 of SEQ ID NO:1.

[0057] As used herein, a "peptide" and a "polypeptide" are amino acid sequences including a plurality of consecutive polymerized amino acid residues. For purpose of this disclosure, peptides are molecules including up to 20 amino acid residues, and polypeptides include more than 20 amino acid residues. The peptide or polypeptide may include modified amino acid residues, naturally occurring amino acid residues not encoded by a codon, and non-naturally occurring amino acid residues. As used herein, a "protein" may refer to a peptide or a polypeptide of any size. A protein may be an enzyme, a protein, an antibody, a membrane protein, a peptide hormone, regulator, or any other protein.

[0058] As used herein, the term "variant" means a sequence or a fragment of a sequence (nucleotide or amino acid) inserted, substituted or deleted by one or more nucleotides/amino acids, or which is chemically modified. The term variant may in some embodiments also include a variant polypeptide of xylanase, a fusion protein including a variant polypeptide of xylanase, or a recombinant xylanase enzyme.

[0059] The term "xylanase variant" and "variant xylanase" and "variant of xylanase" means any xylanase molecule obtained by site-directed or random mutagenesis, insertion, substitution, deletion, recombination and/or any other protein engineering method, which leads to xylanases that differ in their amino acid sequence from the parent xylanase, the parent xylanase being a wild-type xylanase or a xylanase variant itself. The terms "wild type xylanase", "wild type enzyme", "wild type", or "wt" in accordance with the disclosure, describe a xylanase enzyme with an amino acid sequence found in nature or a fragment thereof. The variant encoding gene can be synthesized or the parent gene be modified using genetic methods, e.g. by site-directed mutagenesis, a technique in which one or more than one mutations are introduced at one or more defined sites in a polynucleotide encoding the parent polypeptide.

[0060] As used herein, the term "disulfide bridge" refers to a bond formed between the sulfur atoms of cysteine residues in a polypeptide or a protein. Disulfide bridges can be naturally occurring, or non-naturally occurring, and, for example, introduced by way of amino acid substitution(s).

[0061] The term "catalytic domain" or "catalytic module" or "core" or "catalytic core domain" denotes a domain of an enzyme, which may or may not have been modified or altered, but which retains at least part of its original activity.

[0062] By the term "linker" or "spacer" is meant a polypeptide comprising at least two amino acids which may be present between the domains of a multidomain protein, for example an enzyme comprising a catalytic domain and a binding domain such as a carbohydrate binding module (CBM), or any other enzyme hybrid, or between two proteins or polypeptides produced as a fusion polypeptide, for example a fusion protein comprising two core enzymes. For example, the fusion protein of a catalytic domain with a CBM is provided by fusing a DNA sequence encoding the catalytic domain, a DNA sequence encoding the linker and a DNA sequence encoding the CBM sequentially into one open reading frame and expressing this construct.

[0063] As used herein, the term "dry matter" or "dry matter pulp" or "O.D. pulp" refers to pulp dried with the oven-drying method, wherein the dry matter pulp is moisture-free, i.e., free of water. The dry matter content of oven-dried pulp can be determined according to ISO 638:2008.

[0064] As used herein, "amino acid substitution" means an amino acid residue replacement with an amino acid residue that is different than the original amino acid in that specific replacement position. The term "amino acid substitution" can refer to both, conservative amino acid substitutions and non-conservative amino acid substitutions, which means the amino acid residue is replaced with an amino acid residue having a similar side chain (conservative), or a different side chain (non-conservative), as the original amino acid residue in that place. Substitutions are described using of the following nomenclature: amino acid residue in the protein scaffold; position; substituted amino acid residue(s). According to this nomenclature the substitution of, for instance, a single residue of alanine to serine residue at position 23 is indicated as Ala23Ser or A23S.

[0065] As used herein, the term "comprising" includes the broader meanings of "including", and "containing", as well as the narrower expressions "consisting of" and "consisting only of". The words "comprise", "include", and "contain" are each used as open-ended expressions with no intended exclusivity.

[0066] In an embodiment, material originating from wood material or non-wood material is bleached in the bleaching process. In an embodiment, prior to the bleaching process, the wood or non-wood material is digested in a digester, for obtaining pulp. In an embodiment, the pulp is treated with chemicals to dissolve lignin in the pulp.

[0067] In an embodiment, the pulp is hardwood pulp or softwood pulp, or mixture thereof. In an embodiment, the pulp is non-wood pulp. In an embodiment, the pulp is xylan-containing pulp. In an embodiment, the pulp is kraft pulp cooked in alkaline liquor. In an embodiment, the bleaching process comprises multiple steps and one or more water washes in between the steps, or alternatively, each or some of the stages comprises a water wash as a final step of the stage.

[0068] In an embodiment, the bleaching process comprises providing pulp and performing a delignification of the pulp by carrying out in the following sequence: a) an enzymatic treatment step (X) comprising contacting the pulp with a glycoside hydrolase family GH11 xylanase enzyme at a pH of at least 9.5 and a temperature of at least 85 °C; b) an

oxidation step (D), wherein a bleaching agent is added; c) an alkaline extraction step (E), wherein an alkaline agent is added; and d) recovering bleached pulp. In an embodiment, the GH11 xylanase enzyme used in the bleaching process, has an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5, compared to the same mixture without any xylanase enzyme.

[0069] In an embodiment, the GH11 xylanase enzyme is a glycoside hydrolase family 11 xylanase enzyme.

[0070] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 25 %, preferably at least 30 %, more preferably at least 40 % and most preferably at least 50 % reduction in viscosity of the xylan-containing mixture, compared to the same mixture without any xylanase enzyme.

[0071] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 17 %, preferably at least 20 %, more preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, even more preferably at least 50 %, most preferably at least 60 % reduction in viscosity of a xylan-containing mixture at 85 - 100 °C and at pH 9.5 - 11.5, compared to the same mixture without any xylanase enzyme.

[0072] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 10 %, preferably at least 20 %, more preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, even more preferably at least 60 %, most preferably at least 70 % reduction in viscosity of a xylan-containing mixture at 85 - 90 °C and at pH 8 - 11, compared to the same mixture without any xylanase enzyme. In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 50 %, preferably at least 55 %, more preferably at least 60 %, more preferably at least 65 %, even more preferably at least 70 %, most preferably at least 75 %, reduction in viscosity of a xylan-containing mixture at 85 - 90 °C and at pH 8 - 11, compared to the same mixture without any xylanase enzyme.

[0073] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 10 %, preferably at least 20 %, more preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, even more preferably at least 50 %, most preferably at least 60 %, reduction in viscosity of a xylan-containing mixture at 90 °C and at pH 10 - 11, compared to the same mixture without any xylanase enzyme. In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 50 %, preferably at least 55 %, more preferably at least 60 %, more preferably at least 65 %, even more preferably at least 70 %, most preferably at least 75 %, reduction in viscosity of a xylan-containing mixture at 90 °C and at pH 10 - 11, compared to the same mixture without any xylanase enzyme.

[0074] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 20 %, preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, even more preferably at least 60 %, most preferably at least 69 % reduction in viscosity of a xylan-containing mixture at 90 °C and at pH 10.5, compared to the same mixture without any xylanase enzyme.

[0075] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 25 % reduction in viscosity of a xylan-containing mixture at 90 °C and at pH 10.5, compared to the same mixture without any xylanase enzyme.

[0076] In an embodiment, the GH11 xylanase enzyme used in the step X has the ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 85 - 100 °C and pH 9.5 - 11.5, compared to the same mixture without any xylanase enzyme. In an embodiment, the GH11 xylanase enzyme used in the step X has the ability to cause at least 10 %, preferably at least 20 %, more preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, even more preferably at least 60 %, most preferably at least 70 % reduction in viscosity of a xylan-containing mixture at 85 - 100 °C and at pH 9.5 - 11.5, compared to the same mixture without any xylanase enzyme. In an embodiment, the ability of the GH11 xylanase enzyme to cause a strong reduction in viscosity of a xylan-containing mixture is an indicator of the enzyme's xylan depolymerisation activity. Therefore, a strong reduction in viscosity of a xylan-containing mixture indicates the GH11 xylanase enzyme used has a high xylan depolymerisation activity. In an embodiment, the stronger the viscosity reduction of the xylan-containing mixture in the presence of the GH11 xylanase enzyme, the higher is the xylan depolymerisation activity of the GH11 xylanase enzyme. In an embodiment, the xylan depolymerisation activity of the GH11 xylanase is an indicator of the thermostability and pH stability of the said enzyme. In an embodiment, the xylan depolymerisation activity of the GH11 xylanase at 90 °C and pH 10.5 is an indicator of the thermostability and pH stability of the said enzyme. A high xylan depolymerisation activity indicates the GH11 xylanase in question is suitable for pulp bleaching with high pH and temperature, such as pH 9 - 12 and a temperature of 85 - 100 °C, preferably pH 9.5 - 11.5 and a temperature of 85 - 100 °C.

[0077] In an embodiment, the xylan depolymerization activity of the GH11 xylanase enzyme is tested in a xylan-containing mixture which is not wood pulp.

[0078] In an embodiment, the xylan-containing mixture comprises: not more than 0.07 g xylan per ml of said mixture, and not more than 3.0 µg of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes, preferably within 10 minutes, more preferably within 5 minutes and most preferably within 2 minutes. In an embodiment, the xylan-containing mixture comprises: 0.07 g/ml or less of xylan, and 3.0 µg or less of the GH11 xylanase enzyme protein per gram of said mixture. In an embodiment, the reduction in viscosity of the xylan-containing mixture takes place within 20 minutes preferably within 10 minutes, more preferably within 5 minutes and most preferably within 2 minutes.

[0079] In an embodiment, the xylan-containing mixture comprises ≤ 0.07 g/ml, ≤ 0.06 g/ml, ≤ 0.05 g/ml, ≤ 0.04 g/ml,

≤ 0.03 g/ml, ≤ 0.02 g/ml, ≤ 0.01 g/ml or less of xylan. In an embodiment, the concentration of GH11 xylanase enzyme in the xylan-containing mixture is ≤ 3.0 , ≤ 2.9 , ≤ 2.5 , ≤ 2.0 , ≤ 1.9 , ≤ 1.5 , ≤ 1.0 , ≤ 0.5 , ≤ 0.3 , or ≤ 0.1 μ g xylanase enzyme protein per gram of said mixture. In an embodiment, the viscosity of the xylan-containing mixture is reduced with the GH11 xylanase enzyme within 20 min, 15 min, 10 min, 5 min, 2 min or less.

[0080] In an embodiment, the step X comprises contacting the pulp with the GH11 xylanase enzyme at a pH 9.5 - 11.5 and a temperature of 85 - 100 °C.

[0081] In an embodiment, the step X comprises contacting the pulp with the GH11 xylanase enzyme at a pH of at least 9.5 and a temperature of at least 85 °C. In an embodiment, the step X comprises a pH of 9.5 - 11.5 and a temperature of 85 - 100 °C. In an embodiment, the pH of the pulp during the step X is 9.5 - 11.5. In an embodiment, the temperature of the pulp during the step X is 85 - 100 °C.

[0082] In an embodiment, the GH11 xylanase enzyme is added to the pulp with a pH selected from ≥ 9.5 , ≥ 10 , ≥ 10.5 , ≥ 11 , and 11.5. In an embodiment, the GH11 xylanase enzyme is added to the pulp with a temperature selected from ≥ 85 °C, ≥ 90 °C, ≥ 95 °C, and 100 °C.

[0083] In an embodiment, lowering of alkaline pH of the pulp and/or lowering of process temperature prior to addition of the GH11 xylanase enzyme is/are omitted. In an embodiment, an alkaline pH of the pulp is not lowered with acid and/or acid washing step(s) and/or the temperature of the pulp is not reduced with water prior to addition of the GH11 xylanase enzyme, as the GH11 xylanase enzyme tolerates the high delignification pH and temperature of the bleaching process. In an embodiment, since lowering of process temperature with excess water prior to addition of the GH11 xylanase enzyme is not necessary, the water consumption of the bleaching process is reduced. In an embodiment, as lowering of process pH with acid prior to addition of the GH11 xylanase enzyme is not necessary, acid consumption of the bleaching process and the bleaching process hardware wear is reduced.

[0084] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and at pH 10.5, compared to the same mixture without any xylanase enzyme, wherein the xylan-containing mixture comprises: ≤ 0.07 g/ml of xylan, and ≤ 3.0 μ g of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes.

[0085] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 20 %, preferably at least 25 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, even more preferably at least 60 %, most preferably at least 65 % reduction in viscosity of said xylan-containing mixture within 20 minutes at a pH of 10.5 and at a temperature of 90 °C, when compared to the xylan-containing mixture without any xylanase enzyme, wherein the GH11 xylanase enzyme protein concentration is ≤ 3.0 μ g per g of xylan-containing mixture.

[0086] In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 10 % reduction in viscosity of xylan-containing mixture within 20 minutes at a pH of 11 and at a temperature of 90 °C, when compared to the xylan-containing mixture without any xylanase enzyme, wherein the GH11 xylanase enzyme concentration is 1.9 μ g xylanase protein per g of xylan-containing mixture. In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 53 % reduction in viscosity of xylan-containing mixture within 20 minutes at a pH of 11 and at a temperature of 90 °C, when compared to the xylan-containing mixture without any xylanase enzyme, wherein the GH11 xylanase enzyme concentration is 1.9 μ g xylanase protein per g of xylan-containing mixture. In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 17 % reduction in viscosity of xylan-containing mixture within 20 minutes at pH of 10 and at a temperature of 90 °C, when compared to the xylan-containing mixture without any xylanase enzyme, wherein the GH11 xylanase enzyme concentration is 2.9 μ g xylanase protein per g of xylan-containing mixture. In an embodiment, the GH11 xylanase enzyme has an ability to cause at least 58 % reduction in viscosity of xylan-containing mixture within 20 minutes at pH of 10 and at a temperature of 90 °C, when compared to the xylan-containing mixture without any xylanase enzyme, wherein the GH11 xylanase enzyme concentration is 1.9 μ g xylanase protein per g of xylan-containing mixture.

[0087] In an embodiment, the GH11 xylanase enzyme has a residual xylanase activity of at least 25 %, preferably at least 50 % and more preferably at least 75 % after 10 min, preferably after 20 min, more preferably after 30 min of the step X. In an embodiment, the GH11 xylanase enzyme has a residual xylanase activity of at least 25 %, preferably at least 50 %, more preferably at least 75 % after 20 min of the step X, wherein the step X comprises a pH 9.5 - 11.5 and a temperature of 85 - 100 °C.

[0088] In an embodiment, the GH11 xylanase enzyme has a residual xylanase activity of at least 25 % after 30 min, preferably after 40 min, more preferably after 50 min of the step X, wherein the step X comprises a pH 9.5 - 11.5 and a temperature of 85 - 100 °C.

[0089] In an embodiment, the xylanase enzyme retains at least 25 % of its xylanase activity after 20 min at 85 °C and at pH 9.5.

[0090] In an embodiment, the xylanase enzyme retains at least 25 % of its xylanase activity after 20 min at 100 °C and at pH 11.5.

[0091] In an embodiment, the GH11 xylanase enzyme has a residual xylanase activity of at least 75 %, preferably at least 80 %, more preferably at least 85 % after at least 30 min at pH 10.9 and a temperature of 85 °C. In an embodiment,

the GH11 xylanase enzyme in a hardwood pulp bleaching process water has a residual xylanase activity of at least 75 %, preferably at least 80 %, more preferably at least 85 % after at least 30 min at pH 10.9 and a temperature of 85 °C.

[0092] In an embodiment, the xylanase enzyme has a residual xylanase activity of at least 75 %, preferably at least 80 %, more preferably at least 85 % after at least 30 min at pH 10.5 and a temperature of 85 °C. In an embodiment, the GH11 xylanase enzyme in a softwood pulp bleaching process water has a residual xylanase activity of at least 75 %, preferably at least 80 %, more preferably at least 85 % after at least 30 min at pH 10.5 and a temperature of 85 °C.

[0093] In an embodiment, the bleaching process comprises: providing pulp; performing a delignification of the pulp, by carrying out in the following sequence: (a) an enzymatic treatment step (X) comprising contacting the pulp with the glycoside hydrolase family GH11 xylanase enzyme at a pH of 9.5 - 11.5 and a temperature of 85 - 100 °C, wherein the xylanase enzyme has a residual xylanase activity of at least 25 % after 20 min of step X; (b) an oxidation step (D), wherein a bleaching agent is added; (c) an alkaline extraction step (E), wherein an alkaline agent is added; and (d) recovering delignified pulp.

[0094] In an embodiment, the pulp is provided to the delignification of the bleaching process from a digester.

[0095] In an embodiment, at least 0.01 g, preferably at least 0.1 g, more preferably at least 0.2 g, most preferably at least 0.5 g of the GH11 xylanase enzyme protein is added per 1000 kg of dry matter pulp at the step X.

[0096] In an embodiment, the GH11 xylanase enzyme is added to the step X at a concentration of 0.01 - 200 g, or 0.1 - 20 g, or 0.2 - 10 g, or 0.5 - 5 g of the GH11 xylanase enzyme protein per 1000 kg of dry matter pulp. In an embodiment, the concentration of the GH11 xylanase enzyme depends on the type of pulp used.

[0097] In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the glycoside hydrolase family GH11 xylanase enzyme and at least one further enzyme selected from the group consisting of protease, amylase, cellulase, β -glucosidase, lipase, xylanase, mannanase, cutinase, esterase, α -glucuronidase, phytase, nuclease, pectinase, pectinolytic enzyme, pectate lyase, carbohydrase, arabinase, galactanase, xanthanase, xyloglucanase, polysaccharide monooxygenase, laccase, peroxidase and oxidase with or without a mediator, or a combination thereof.

[0098] In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a cellulase enzyme. In an embodiment, the enzymatic treatment step X comprises contacting the pulp with the GH11 xylanase enzyme and a mannanase enzyme. In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a peroxidase enzyme. In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a lignin peroxidase enzyme (EC 1.11.1.14). In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a manganese peroxidase enzyme (EC 1.11.1.13). In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a versatile peroxidase enzyme (EC 1.11.1.16). In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a dye-decolorizing peroxidase enzyme (EC 1.11.1.19). In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a haem peroxidase enzyme. In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a copper-based laccase enzyme (EC 1.10.3.2).

[0099] In an embodiment, the bleaching process comprises contacting the pulp with a surfactant, surface-active agent, anti-foaming agent, defoamer, emulsifier, dispersant and/or detergent. In an embodiment, the enzymatic treatment step (X) comprises contacting the pulp with the GH11 xylanase enzyme and a surfactant, surface-active agent, anti-foaming agent, defoamer, emulsifier, dispersant and/or detergent.

[0100] In an embodiment, the enzymatic treatment step (X) comprises contacting pulp with an enzyme composition which comprises one or more enzymatic activities, possibly isolated and purified, from one or more species of a microorganism. In an embodiment, the enzymes of the enzyme composition may originate from different species, preferably fungal or bacterial species, or they are present in a fermentation product of a one microorganism which acts as a host cell for enzyme production, such as in the production of the GH11 xylanase enzyme, when the microorganism simultaneously produces other enzymes in addition to the GH11 xylanase enzyme.

[0101] In an embodiment, the enzymatic treatment step (X) comprises contacting pulp with an enzyme composition comprising the GH11 xylanase enzyme protein.

[0102] In an embodiment, the pulp is an aqueous mixture, comprising at least 0.5 - 35 wt-% of pulp fibers, wherein the pulp fibers are wood fibers and/or non-wood fibers. In an embodiment, the pulp fibers contain xylan, the pulp therefore being xylan-containing pulp.

[0103] In an embodiment, the delignification is done in the presence of the GH11 xylanase enzyme, the GH11 xylanase enzyme having high pH and thermostability.

[0104] In an embodiment, the contacting of the pulp with the GH11 xylanase enzyme in the step X means the GH11 xylanase enzyme is contacted with the xylan comprised by the pulp.

[0105] In an embodiment, the delignification further comprises an oxygen-delignification step (O) before the oxidation step D, wherein oxygen is added at the step O; and wherein the step X is before or after the step O.

[0106] In an embodiment, oxygen is added as O₂ gas in the oxygen-delignification step, wherein the step is called O₂

step.

[0107] In an embodiment, the duration of the enzymatic treatment step X is at least 2 min, at least 5 min, at least 10 min, at least 15 min, at least 20 min, at least 30 min, at least 1 h, at least 1.5 h, at least 2 h, at least 2.5 h, or at least 3 h. In an embodiment, the duration of the enzymatic treatment step X is 2 min - 3 h, 5 min - 2 h, 15 - 60 min, or 20 - 30 min. In an embodiment, the temperature of the step X is at least 80 °C, at least 85 °C, at least 90 °C, at least 95 °C, or at least 100 °C. In an embodiment, the pH of the pulp during the step X is at least 8.5, at least 9.0, at least 9.5, at least 10, at least 10.5, at least 11, at least 11.5 or at least 12.

[0108] In an embodiment, the duration of the step O is at least 0.5 h, at least 1h, at least 1.5 h, at least 2 h, at least 2.5 h, or at least 3 h. In an embodiment, the temperature of the step O is at least 75 °C, at least 80 °C, at least 85 °C, at least 90 °C, at least 95 °C, at least 105 °C, at least 110 °C, at least 115 °C, at least 120 °C, at least 125 °C, or at least 130 °C. In an embodiment, the pH of the pulp during the step O is at least 9.5, at least 10, at least 10.5, at least 11, at least 11.5, or at least 12. In an embodiment, the pH and the temperature of the pulp can change during the step O.

[0109] In an embodiment, the pH of the pulp during the step O is 10 - 12 and/or the temperature is 75 - 130 °C.

[0110] In an embodiment, the xylanase enzyme is added to the pulp before the step O as its own enzymatic treatment step X. In an embodiment, the xylanase enzyme is added to the pulp before the step O, and the xylanase enzyme retains at least 25 % residual xylanase activity during at least 20 min of the enzymatic treatment step X comprising a pH of 9.5 - 11.5 and a temperature of 85 - 100 °C. The step O releases high amounts of lignin from the pulp. Therefore, in the embodiments wherein the bleaching process comprises the step X prior to step O, washing the pulp between the step X and the step O is not necessary. In an embodiment, wherein the GH11 xylanase enzyme is added to the pulp before the step O, the GH11 xylanase enzyme in the pulp retains some of its activity in the step O.

[0111] In an embodiment, the xylanase enzyme is added to the pulp after the step O and before the step D as its own enzymatic treatment step X. In an embodiment, the xylanase enzyme is added to the pulp after the step O, and the xylanase enzyme retains at least 25 % residual xylanase activity during at least 20 min of the enzymatic treatment step X comprising a pH of 9.5 - 11.5 and a temperature of 85 - 100 °C. In an embodiment, the GH11 xylanase enzyme can be added at any stage prior to step D, as long as the step X is done in the presence of the GH11 xylanase enzyme having a residual xylanase activity of at least 25 % after at least 20 min at pH of 9.5 - 11.5 and temperature of 85 - 100 °C. In an embodiment, the GH11 xylanase enzyme has activity at any pH between 5.6 - 11.5 and/or at any temperature between 55 - 100 °C.

[0112] In an embodiment, adding the GH11 xylanase enzyme to the pulp before or after the step O, and before the step D, allows performing the step X in a pre-existing storage tank or a storage tower of a pulp processing facility, without the need to build separate processing containers/tanks when integrating the enzymatic treatment step X into a bleaching process of pulp, which process did not contain an enzymatic treatment step X previously.

[0113] In an embodiment, each step of the delignification of the bleaching process takes place in a separate tank, as indicated in the Fig. 1. In an embodiment, the optional oxygen-delignification step (O) is performed in a separate tank O. In an embodiment, the enzymatic treatment step (X) can be performed in a separate storage tank S1 and/or S2, before and/or after the step O, respectively. In an embodiment, performing the step X in a separate storage tank S1 and/or S2 allows an adequate retention time for the enzymatic treatment of the pulp. However, depending on the process, the presence of storage tank S1 and/or S2 may not be necessary, as the enzymatic treatment step X can be performed in another process tank preceding in the bleaching process sequence the tank D, wherein the oxidation step D is performed.

[0114] In an embodiment, the oxidation step D is performed in a tank D (Fig. 1). In an embodiment, wherein the bleaching process does not comprise the step O, the pulp is directed from the digester (not shown) directly, or via other tanks, such as storage tanks S1 and/or S2, to the oxidation step in the tank D. In an embodiment, wherein the bleaching process comprises the step O, the oxygen-treated pulp is directed from the tank O directly, or via other tanks, such as storage tank S2, to the tank D. Preferably, the pulp is washed in between the tanks O and D, to remove dissolved lignin. In an embodiment, wherein the bleaching process includes a process sequence comprising the steps O, X, D, and a water washing step of the pulp is performed after the step X, at least part of the wash water comprising the GH11 xylanase enzyme is recycled back to the step O. In an embodiment, wherein the bleaching process includes a process sequence comprising the steps O, X, D, and a water washing step of the pulp is performed between the steps O and X, washing the pulp between the steps X and D is optional. However, in this case also if a water washing step of the pulp is performed after the step X, at least part of the wash water comprising the GH11 xylanase enzyme is recycled back to the step O. In an embodiment, the GH11 xylanase enzyme in the pulp retains some of its activity in the step D, if no washing step is performed between the steps X and D.

[0115] In an embodiment, the bleaching agent at the oxidation step D comprises an oxygen-containing oxidizing agent, preferably the bleaching agent is selected from a group consisting of ClO_2 , O_3 , H_2O_2 , and peroxides. Examples for non-oxygen-containing oxidizing agents are Cl_2 and other halogens. In an embodiment, the bleaching agent is a chemical compound comprising the element oxygen. In an embodiment, the bleaching agent is ClO_2 . In an embodiment, the bleaching agent is H_2O_2 . In an embodiment, the bleaching agent is an organic peroxide. In an embodiment, the bleaching

agent is an inorganic peroxide. In an embodiment, the bleaching agent is O_3 . In an embodiment, the bleaching agent is ClO_2 and the oxidation step is called step D₀. In an embodiment, the pulp is directed into the tank D for the step D and the bleaching agent is added in the tank D.

[0116] In an embodiment, both the GH11 xylanase enzyme and the bleaching agent participate in bleaching the pulp during the bleaching process, and therefore the amount of bleaching agent required in the bleaching process is reduced when compared to a bleaching process without any xylanase enzyme. In an embodiment, the pulp comprises the GH11 xylanase enzyme with residual activity in the step D, both the GH11 xylanase enzyme and the bleaching agent participating in bleaching the pulp simultaneously. In an embodiment, the pulp comprises the GH11 xylanase enzyme with residual activity in the step D, and the bleaching agent in the step D is preferably H_2O_2 . In another embodiment, the pulp no longer comprises the GH11 xylanase enzyme at the step D, as the enzyme has been removed in a washing step prior to the step D. In such embodiment also, the required amount of bleaching agent in the oxidation step is reduced when compared to a bleaching process without any xylanase enzyme, as the pulp entering the step D is already bleached to some degree in the enzymatic treatment step X. In an embodiment, at least 5 %, preferably at least 7,5 %, more preferably at least 10 % less of the bleaching agent is used than in a corresponding process without the GH11 xylanase enzyme, for achieving a specific pulp brightness. In an embodiment, the reduction of the amount of bleaching agent leads to reduction in the amount of Adsorbable Organic Halides (AOX) in a water effluent of the bleaching process and reduced need for effluent water treatment afterwards.

[0117] In an embodiment, the GH11 xylanase enzyme used in the bleaching process is highly tolerant to high pH and temperature. In an embodiment, the use of temperature and pH tolerant xylanase enzyme in the bleaching process results in higher pulp brightness when compared to a bleaching process without a xylanase enzyme, or when compared to a process comprising less temperature and pH tolerant xylanase enzyme. On the other hand, the use of temperature and pH tolerant xylanase enzyme in the bleaching process results in a reduced need of bleaching agent, when a specific pulp brightness is desired.

[0118] In an embodiment, the bleaching agent used is H_2O_2 , a metal chelation step (Q) for removal of metals is performed prior to the oxidation step D, and the GH11 xylanase enzyme is added to the pulp before, during or after the chelation step Q. In an embodiment, the step Q comprises washing the pulp with a chelating agent, such as EDTA or DTPA, for removing redox-active metal ions from the pulp. In an embodiment, the duration of the step Q is the same as the duration of the step X.

[0119] In an embodiment, the step Q takes place in a separate metal chelation tank Q, prior to the step D in the bleaching process sequence (Fig. 1). The purpose of the step Q is to remove metals from the pulp which reduce the bleaching activity of H_2O_2 , such metals being, for example, copper, manganese and iron. In an embodiment, the enzymatic treatment step X of the pulp is performed in the same tank Q as the metal chelation step. In an embodiment, the enzymatic treatment step X of the pulp is performed in the same tank Q as the metal chelation step, before, after or during the metal chelation step. Alternatively, the step X is performed in another tank prior to and/or after the step Q. In an embodiment, the step Q takes place after the oxygen-delignification is performed to the pulp in the tank O. In an embodiment, the pulp is directed from metal chelation in the tank Q directly, or via other tanks to the oxidation step in the tank D. In an embodiment, the pulp is directed from the tank Q to a storage tank (not shown), wherein the enzymatic treatment step X is performed, after which the pulp is directed to the oxidation step in the tank D. Preferably, the pulp is washed in between the steps Q and D, to remove the dissolved metals from the pulp prior to the oxidation step.

[0120] In an embodiment the GH11 xylanase enzyme can be added to the pulp before, during or after the step Q. In an embodiment, wherein the xylanase enzyme is added to the pulp before or during the step Q, and the process comprises a water washing step of the pulp between the step Q and the oxidation step in the tank D. In this case, at least part of the wash water comprising the GH11 xylanase enzyme with residual activity can be recycled back to the metal chelation in the tank Q, preferably, at least part of the said wash water is recycled back to a pulp washer upstream from the tank Q. In an embodiment, wherein the pulp is washed with water between the steps Q and D, the GH11 xylanase enzyme can be first added to the pulp in the tank D or in a separate storage tank (not shown) after the washing step, before the addition of the bleaching agent H_2O_2 . In such embodiment, the GH11 xylanase enzyme in the pulp can retain some of its activity in the step D.

[0121] In an embodiment, the duration of the delignification oxidation step in tank D is at least 0.5 h, at least 1 h, at least 1.5 h, at least 2 h, at least 2.5 h, or at least 3 h. In an embodiment, the temperature of the delignification oxidation step is at least 50 °C, at least 60 °C, at least 70 °C, at least 80 °C, at least 90 °C, or at least 100 °C. In an embodiment, the pH of the pulp during the delignification oxidation step is 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, or 1 or less. In an embodiment, the bleaching agent added in the step D reduces the pH of the pulp after oxidation.

[0122] In an embodiment, after the step D, the pulp is directed to the alkaline extraction step E in the tank E (Fig. 1). In an embodiment, the bleaching process also includes a water washing step of the pulp between the oxidation step in the tank D and the alkaline extraction step in the tank E. In such embodiments, the wash water optionally comprising the GH11 xylanase enzyme is recycled back to a water washing step before the step D. In an embodiment, wherein the

bleaching agent at the step D is H_2O_2 , the xylanase enzyme in the wash water being recycled back into a water washing step still has residual activity and is able to contribute to xylan degradation in the tank D.

[0123] In an embodiment, the alkaline agent is added into the alkaline extraction step E in the tank E. In an embodiment, the alkaline agent of the alkaline extraction step E is selected from NaOH, MgO, or combinations thereof. In an embodiment,

[0124] In an embodiment, the reduction in the amount of needed ClO_2 as a bleaching agent results into reduced amount of NaOH as an alkaline agent required in the step E. In an embodiment, the reduction in the amount of NaOH as an alkaline agent results into reduction in the chemical oxygen demand (COD) measure of the bleaching process effluent water.

[0125] In an embodiment, the GH11 xylanase enzyme has a higher activity in a delignification process water than in normal tap water, when the process conditions including temperature and pH are the same. In an embodiment, the process water at the delignification stage induces the enzyme stability.

[0126] In an embodiment, the duration of the delignification alkaline extraction step E is at least 0.5 h, at least 1 h, at least 1.5 h, at least 2 h, at least 2.5 h, or at least 3 h. In an embodiment, the temperature of the step E is at least 70 °C, at least 80 °C, at least 90 °C, or at least 100 °C. In an embodiment, the pH of the pulp during the step E is at least 8, at least 9, at least 9.5, at least 10, at least 10.5, or at least 11. In an embodiment, the alkaline agent increases the pH of the pulp, the pH of the pulp being alkaline at the beginning of the alkaline extraction step, and less alkaline at the end of the alkaline extraction step.

[0127] In an embodiment, at least 1 unit, preferably at least 2 units, more preferably at least 2.5 units higher final brightness of the pulp can be achieved with the current bleaching process, than with a corresponding process without the GH11 xylanase enzyme.

[0128] In an embodiment, the delignification of the bleaching process is followed by a brightening section of the delignified pulp, which can also comprise several process steps. The brightening section can comprise at least one or more brightening steps (Db, P), wherein a bleaching agent is added, and/or one or more brightening alkaline extraction steps (Eb), wherein an alkaline agent is added. In an embodiment, the main purpose of the brightening section is not to remove remaining lignin from the pulp but to decolorize remaining lignin, thereby obtaining a higher final brightness.

[0129] In an embodiment, the glycoside hydrolase family GH11 xylanase enzyme used in the bleaching process according to the invention can be of various origin. In an embodiment, the xylanase enzyme used in the bleaching process according to the invention, is a wild type xylanase enzyme. In an alternative embodiment, the xylanase enzyme used in the bleaching process according to the invention, is a modified xylanase enzyme, and/or a variant of a wild type xylanase enzyme.

[0130] In an embodiment, the GH11 xylanase enzyme originates from a thermophilic bacteria. In an embodiment, the GH11 xylanase enzyme originates from bacteria, preferably from Actinobacteria, more preferably from *Streptosporangiales*, more preferably from *Streptosporangiaceae*, even more preferably from *Thermopolyspora* species, most preferably from *Thermopolyspora flexuosa*. In an embodiment, the xylanase enzyme originates from the group of terrabacteria. In an embodiment, the xylanase enzyme originates from a phylum of Actinobacteria. In an embodiment, the xylanase enzyme originates from class of *Actinomycetia*. In an embodiment, the xylanase enzyme originates from the order of *Streptosporangiales*. In an embodiment, the xylanase enzyme originates from the family of *Streptosporangiaceae*. In an embodiment, the xylanase enzyme originates from the genus of *Thermopolyspora*.

[0131] In an embodiment, the GH11 xylanase enzyme originates from *Thermopolyspora flexuosa*. *Thermopolyspora flexuosa* is meant synonymous to *Nonomuraea flexuosa*, *Nonomuria flexuosa*, *Nocardia flexuosa*, *Microtetraspora flexuosa*, *Acetomadura flexuosa* and/or *Actinomadura flexuosa*.

[0132] In an embodiment, the glycoside hydrolase family GH11 xylanase enzyme used in the bleaching process comprises a catalytic core domain and a carbohydrate binding module (CBM), linked together by a linker. In an embodiment, the glycoside hydrolase family GH11 xylanase enzyme used in the bleaching process is a truncated enzyme, comprising a catalytic core domain and a linker domain, or a catalytic core domain and part of a linker domain, whereas a CBM is absent. In yet another embodiment, the glycoside hydrolase family GH11 xylanase enzyme used in the bleaching process comprises only a catalytic core domain, whereas a linker domain and a CBM are absent. In an embodiment, the GH11 xylanase enzyme used in the bleaching process comprises a mature catalytic core domain.

[0133] In an embodiment, the GH11 xylanase enzyme is a polypeptide, wherein the polypeptide, or a functional fragment of the polypeptide, has a molecular mass of 15 - 100 kDa, preferably 18 - 70 kDa, more preferably 19 - 40 kDa, even more preferably 20 - 33 kDa. In an embodiment, the GH11 xylanase enzyme has a molecular mass of 19 - 40 kDa, preferably 20 - 30 kDa, more preferably 23 - 25 kDa, most preferably 24 kDa, calculated from its amino acid sequence without glycosylation. In an embodiment, the GH11 xylanase enzyme has a β -jelly roll structure.

[0134] In an embodiment, the GH11 xylanase enzyme is a polypeptide, wherein the polypeptide or a functional fragment of the polypeptide has a molecular mass of 31 kDa. In an embodiment, the GH11 xylanase enzyme is a polypeptide, wherein the polypeptide or a functional fragment of the polypeptide has a molecular mass of 27 kDa. In an embodiment, the GH11 xylanase enzyme is a polypeptide, wherein the polypeptide or a functional fragment of the polypeptide has a

molecular mass of 24 kDa. The molecular mass of the GH11 xylanase enzyme polypeptide or a functional fragment of the polypeptide can be determined by SDS-PAGE. In an embodiment, the molecular mass of the GH11 xylanase enzyme polypeptide or a functional fragment of the polypeptide is calculated from its amino acid sequence without glycosylation. In an embodiment, the GH11 xylanase enzyme polypeptide or a functional fragment of the polypeptide has a molecular mass of 24 kDa, calculated from its amino acid sequence without glycosylation. In an embodiment, the GH11 xylanase enzyme lacks xylosidase and cellulase activity.

[0135] In an embodiment, the GH11 xylanase enzyme is a variant polypeptide. In an embodiment, the GH11 xylanase enzyme is a wild type polypeptide.

[0136] In an embodiment, the GH11 xylanase enzyme has at least one, or at least two disulfide bridges between two cysteine residues in its amino acid sequence. In an embodiment, the at least one disulfide bridge stabilizes the GH11 xylanase enzyme, thereby increasing the pH stability and thermostability of the GH11 xylanase enzyme.

[0137] In an embodiment the GH11 xylanase enzyme is a polypeptide comprising residues 3C and 30C, the positions of these residues corresponding to the positions 3 and 30 of the SEQ ID NO: 2, and a disulfide bridge being formed between the residues 3C and 30C. In an embodiment the GH11 xylanase enzyme is polypeptide comprising at least one disulfide bridge between two cysteine residues in the 1 - 191 amino acid region of the polypeptide, the position of at least one of the two cysteine residues being different from the positions corresponding to positions 3 and 30 of the SEQ ID NO: 2. In an embodiment the GH11 xylanase enzyme is polypeptide comprising at least one disulfide bridge between cysteine residues in the 1 - 30 amino acid region of the polypeptide, the positions of the amino acid region corresponding to positions of the SEQ ID NO: 2.

[0138] In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having amino acids corresponding to the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof; the GH11 xylanase enzyme having at least one disulfide bridge between two cysteine residues of the polypeptide. In an embodiment, the GH11 xylanase enzyme is polypeptide comprising an amino acid sequence having amino acids corresponding to the amino acids 1 - 191 of SEQ ID NO: 2; or functional fragments thereof, the GH11 xylanase enzyme having at least one disulfide bridge between two cysteine residues of the polypeptide. In an embodiment, the at least one disulfide bridge stabilizes the GH11 xylanase enzyme, thereby increasing the pH stability and thermostability of the GH11 xylanase enzyme compared to a GH11 xylanase enzyme without a disulfide bridge. In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having amino acids corresponding to the amino acids 1 - 191 of SEQ ID NO: 1; the GH11 xylanase enzyme having at least one disulfide bridge between two cysteine residues of the polypeptide, and one or two amino acid substitution(s) at a position/positions corresponding to the position(s) 23 and/or 28 of SEQ ID NO: 1.

[0139] In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with amino acids 1 - 191 of SEQ ID NO: 1.

[0140] In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 93 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, or at least 99% sequence identity with amino acids 1 - 191 of SEQ ID NO: 1.

[0141] In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof. In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with amino acids 1 - 191 of SEQ ID NO: 2, or functional fragments thereof.

[0142] In an embodiment the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 93 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, or at least 99% sequence identity with amino acids 3 - 180 of SEQ ID NO: 2.

[0143] In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with SEQ ID NO: 2. In an embodiment, the GH11 xylanase enzyme comprises the inner core polypeptide of C31-4. In an embodiment, the GH11 xylanase enzyme comprises the core polypeptide of C31-4. In an embodiment, the GH11 xylanase enzyme comprises the mature polypeptide of C31-4. In an embodiment, the GH11 xylanase enzyme is a variant polypeptide having the amino acid sequence SEQ ID NO: 2.

[0144] In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof; wherein the amino acid sequence has at least one disulfide bridge between two cysteine residues of the polypeptide. In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at

least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with amino acids 1 - 191 of SEQ ID NO: 2; wherein the amino acid sequence has at least one disulfide bridge between two cysteine residues of the polypeptide.

[0145] In an embodiment, the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 % amino acid sequence identity with amino acids 3 - 180 of SEQ ID NO: 2, wherein the amino acid sequence has at least one disulfide bridge between two cysteine residues of the polypeptide.

[0146] In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with amino acids 3 - 180 of SEQ ID NO: 1, and wherein the amino acid sequence has at least one disulfide bridge between two cysteine residues of the polypeptide, and an amino acid substitution at the position 23 or 28, or at the positions 23 and 28, the positions corresponding to the positions 23 and 28 of the SEQ ID NO: 1.

[0147] In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with SEQ ID NO: 1, and an amino acid substitution at the positions 3, 23 and 30, the amino acid positions corresponding to the positions of the SEQ ID NO: 1. In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with SEQ ID NO: 1, and an amino acid substitution at the positions 3, 28 and 30, the amino acid positions corresponding to the positions of the SEQ ID NO: 1. In an embodiment, the GH11 xylanase enzyme is a variant polypeptide comprising an amino acid sequence having at least 79 %, but less than 100 % amino acid sequence identity with SEQ ID NO: 1, and an amino acid substitution at the positions 3 and 30, the amino acid positions corresponding to the positions of the SEQ ID NO: 1.

[0148] In an embodiment, is provided a pulp composition comprising: pulp having a pH of at least 9.5, and 0.01 - 200 g, preferably 0.1 - 20 g, more preferably 0.2 - 10 g of glycoside hydrolase family GH11 xylanase enzyme protein per 1000 kg of dry matter pulp; wherein the GH11 xylanase enzyme protein has xylanase activity. In an embodiment the GH11 xylanase enzyme, which is part of the pulp composition, has an ability to cause at least 20 %, preferably at least 25%, more preferably at least 30%, even more preferably at least 40%, most preferably at least 50% reduction in viscosity of a xylan-containing mixture at 90°C and pH 10.5 compared to the same mixture without any xylanase enzyme, wherein the xylan-containing mixture comprises 0.07 g/ml or less of xylan and 3.0 µg or less of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes, preferably within 10 minutes, more preferably within 5 minutes and most preferably within 2 minutes.

[0149] In an embodiment, the pulp composition has a pH of 9.5 - 11.5 and/or a temperature of 85 - 100 °C. In an embodiment, the pulp composition has a pH of at least 9.5, at least 10, at least 10.5, at least 11, or at least 11.5. In an embodiment, the pulp composition has a temperature of at least 85 °C, at least 90 °C, at least 95 °C, or at least 100 °C.

[0150] In an embodiment, the pulp composition comprises an enzyme composition, comprising the GH11 xylanase enzyme and at least one further enzyme selected from the group consisting of protease, amylase, cellulase, β-glucosidase, lipase, xylanase, mannanase, cutinase, esterase, α-glucuronidase, phytase, nuclease, pectinase, pectinolytic enzyme, pectate lyase, carbohydrase, arabinase, galactanase, xanthanase, xyloglucanase, polysaccharide monooxygenase, laccase, peroxidase and oxidase with or without a mediator, or a combination thereof.

[0151] In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a cellulase enzyme. In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a mannanase enzyme. In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a laccase enzyme. In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a peroxidase enzyme. In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a lignin peroxidase enzyme (EC 1.11.1.14). In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a manganese peroxidase enzyme (EC 1.11.1.13). In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a versatile peroxidase enzyme (EC 1.11.1.16). In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a dye-decolorizing peroxidase enzyme (EC 1.11.1.19). In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a haem peroxidase enzyme. In an embodiment, the pulp composition comprises an enzyme composition comprising the GH11 xylanase enzyme and a copper-based laccase enzyme (EC 1.10.3.2).

[0152] In an embodiment, the pulp composition further comprises a surfactant, a surface-active agent, an anti-foaming agent, a defoamer, an emulsifier, a dispersant a detergent, or any combination thereof.

[0153] In an embodiment, the pulp composition comprises 0.25 - 4 wt-% of a bleaching agent of the total weight of dry matter pulp, the bleaching agent being selected from ClO₂, O₃, H₂O₂, and peroxides.

EXAMPLES

[0154] The xylanases and xylanase variants were designed, made and tested for xylanase enzyme stability, as described in the application EP 20217335. All the xylanases were expressed in *Trichoderma reesei*.

[0155] Xylanase enzyme activity was measured as described by Bailey, M.J. and Poutanen, K. 1989 in Appl. Microbiol. Biotechnol. 30:5-10, but instead of 10 minutes at 50°C and pH 5.3, after 5 minutes at 70°C and pH 7 the amount of reducing carbohydrates released from beech xylan was determined spectrophotometrically using dinitrosalicylic acid and compared to xylose standard solutions. One thermoxylanase unit, abbreviated as TXU, is defined as the amount of xylanase enzyme that produces reducing carbohydrates having a reducing power corresponding to one nmol xylose from beech xylan in one second at pH 7 and 70°C (1 TXU = 1 nkat).

[0156] Protein amounts of purified enzyme samples were measured by absorption at 280nm. Calculated from amino acid compositions, the xylanases AM24 and C31-4 have a molecular mass without glycosylation of approximately 24 kDa and an absorption of 2.852 at 280 nm, 1 cm cuvette and 1 g pure protein per liter.

Example 1. Viscosity measurement to select enzymes suitable for pulp bleaching process

[0157] Viscous xylan suspensions were prepared by weighting 7 g beech wood xylan into a 100 ml volumetric flask, adding aqueous pH buffer, stirring and heating until the suspensions were boiling for approximately 20 minutes. For pH 10.5 a buffer containing 7.5 g/l glycine (which equals 0.1 M glycine) and sodium hydroxide was used. For pH 7.0 a 0.1 M KH_2PO_4 - K_2HPO_4 buffer was used.

[0158] After the xylan-containing mixtures cooled to 25 °C, the volumetric flask was filled up with buffer to the calibration mark and stirred overnight. The next day these mixtures were centrifuged, and the supernatants transferred to a baker and put into an ultrasonic bath for 15 minutes.

[0159] Afterwards, 9 g of these supernatants were weighed into each 15 ml test tube, which test tubes were then sealed with rubber plugs and put into a water bath that was preheated to a temperature 5 °C above the measurement temperature. Then, 1 g enzyme solution, or 1 g water as a reference without enzyme addition, was added to the preheated supernatants and briefly mixed and quickly injected into the viscometer chamber, which had been preheated to the measurement temperature. Viscosity was measured with a falling sphere viscometer from Anton Paar, model DMA 4100M, over a period of 20 minutes.

[0160] The viscosity of mixtures without enzyme addition remained high, whereas the viscosity of mixtures with specific enzymes added reduced quickly within the first minutes until approximately constant viscosity end values were reached within 20 minutes.

[0161] The following tables summarize viscosity end values reached within 20 minutes (average values of at least duplicate measurements) at the temperature and the pH indicated for each of the tables, respectively.

Table 1. High performance difference of enzymes applied at biologically challenging conditions of 90°C and pH 10.5 was measured.

90°C and pH 10.5	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.31	100 %	No viscosity reduction, reference
AM24	1.84	80 %	
C31-4	0.72	31 %	Strong viscosity reduction
inactivated C31-4	2.34	101 %	No viscosity reduction, control

[0162] The xylanase enzyme concentrations of AM24 and C31-4 used in these experiments were approximately 29 and 19 µg of xylanase enzyme protein per g of applied enzyme solutions, respectively. Because 1 g enzyme solution was mixed with 9 g xylan supernatant, the mixtures injected into the viscosimeter resulted in ten times diluted enzyme concentrations; and the xylan concentration was diluted from 7 g per 100 ml to 6.3 g per 100 ml.

[0163] As a control experiment inactivated C31-4 was prepared by heating enzyme solution C31-4 to boiling for 2 hours, then removing precipitated protein. As described above, 1 g of this solution was mixed with 9 g supernatant for viscosity measurement. As expected, viscosity of this mixture was not decreased, indicating that viscosity reduction was caused by active enzyme.

Table 2. Almost no performance difference between enzymes AM24 and C31-4 applied at conditions of 80°C and pH 7.0 was measured.

80°C and pH 7.0	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.83	100 %	No viscosity reduction, reference
AM24	0.68	24 %	Strong viscosity reduction
C31-4	0.62	22 %	Strong viscosity reduction

Example 2. Further viscosity measurements

[0164] The following experiments were done as described in example 1. For pH 10 and 11 a buffer containing 7.5 g/l glycine (which equals 0.1 M glycine) and sodium hydroxide was used. For pH 8 a 0.1 M Tris buffer containing 12.1 g/l 2-amino-2-(hydroxymethyl)propane-1,3-diol and hydrochloric acid was used. For pH 9 a 0.1 M NaHCO₃ - Na₂CO₃ buffer was used. At approximately 60 °C pH was adjusted to the desired values.

[0165] The following tables summarize viscosity end values reached within 20 minutes at the temperature and the pH indicated for each of the tables, respectively.

Table 3. High performance difference of enzymes applied at conditions of 90°C and pH 11 was measured.

90°C and pH 11.0	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.09	100 %	No viscosity reduction, reference
AM24	1.88	90 %	
C31-4	0.99	47%	Strong viscosity reduction

Table 4. High performance difference of enzymes applied at conditions of 90°C and pH 10 was measured.

90°C and pH 10.0	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.34	100 %	No viscosity reduction, reference
AM24	1.95	83 %	
C31-4	0.99	42 %	Strong viscosity reduction

Table 5. Performance of enzymes applied at 90°C and pH 8 was measured.

90°C and pH 8.0	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.41	100 %	No viscosity reduction, reference
AM24	1.15	48 %	
C31-4	0.58	24 %	Strong viscosity reduction

Table 6. Almost no performance difference of enzymes applied at 85°C and pH 8 was measured.

85°C and pH 8.0	Viscosity [mPa·s]	Relative viscosity	Comments
without enzyme	2.53	100 %	No viscosity reduction, reference
AM24	0.93	37 %	Strong viscosity reduction
C31-4	0.80	32 %	Strong viscosity reduction

Example 3. High temperature and high pH application of xylanase in Kraft pulp from Eucalyptus

[0166] Short bleaching sequence XDE (step X, enzyme treatment; step D chlorine dioxide (ClO₂) treatment; step E,

alkaline extraction with sodium hydroxide) was used to compare the efficiency in bleaching of pre-treatments with AM24 and C31-4 xylanases at high temperature of 90°C. The pre-treatments were performed at two pH values, pH 8.3 and pH 10.5. The pulp used was oxygen delignified Eucalyptus Kraft pulp from an Asian mill with kappa number 15.5 and brightness 38.0 %-ISO. A constant ClO₂ dosage (0.2 X kappa number) was used. Bleaching trials were carried out in bleaching reactors. An Anchor type Kemu reactor (batch size 0.15-1.0 kg, T max 90 °C, consistency 25 %) was used in X- and step E and an air heated Hellsten reactor (batch size 0.3-0.8 kg, T max 85 °C, consistency 12 %) in step D₀. [0167] Approximately 2 g of enzyme protein per ton of pulp was used. Enzyme solutions of AM24 and C31-4 were applied in equal amounts. The enzyme treatment time was 60 minutes.

[0168] After the enzyme step X the enzyme was deactivated by addition of hot water and immediately adjusting the pH of the pulp suspension to pH value 2.5-3. Brightness and kappa number were analyzed after the step E.

Table 7. Results of XDE bleaching using eucalyptus pulp. In the REF samples, no enzyme was added. The enzyme pre-treatments were performed at 90 °C using 60 min reaction time. The aCl denotes active chlorine; ΔBr denotes difference in Brightness; and Δkappa denotes a difference in Kappa number.

		pH 8.3			pH 10.5		
		REF	AM24	C31-4	REF	AM24	C31-4
X	Initial pH	8.3	8.3	8.6	11.0	10.6	10.6
	End pH	8.3	8.3	8.3	10.4	10.4	10.5
	Kappa number	15.1	14.4	14.7	14.8	16.6	16.4
	Brightness, %-ISO	41.5	42.2	41.9	42.0	42.6	42.4
D	ClO ₂ cons., kg aCl/t	27.9	27.9	27.9	27.9	27.9	27.9
	Initial pH	2.7	2.6	2.3	2.6	2.6	2.5
	End pH	1.8	1.6	1.6	1.9	1.6	1.6
	Brightness, %-ISO	55.6	56.1	57.0	56.1	55.3	57.8
E	Initial pH	10.8	11.0	10.9	11.0	11.0	10.9
	End pH	10.9	10.9	10.9	11.0	10.9	10.9
	Kappa number	6.7	6.4	5.7	6.2	6.4	5.9
	Brightness, %-ISO	56.0	57.8	58.8	57.1	56.8	59.8
aCl cons./ΔBr, kg/t*		1.53	1.41	1.34	1.46	1.49	1.28
aCl cons./Δkappa, kg/t*		3.14	3.06	2.86	3.00	3.07	2.91

[0169] Pre-treatment with both the xylanases, AM24 and C31-4 improved the brightness of eucalyptus pulp at pH 8.3. Pulp pre-treated with C31-4 at pH 8.3 had 2.8 units higher brightness than the reference pulp and had 1.0 units higher brightness than the pulp treated with AM24. The active chlorine (aCl) used at pH 8.3 per Δkappa was 0.2 kg/t and per ΔBr 0.07 kg/t lower in the C31-4 treated pulp than in AM24 treated pulp and 0.28 kg/t and 0.19 kg/t, respectively, lower than in the reference pulp (Table 7).

[0170] When the xylanase pre-treatments were performed at pH 10.5 and 90°C, C31-4 treated pulp had 2.7 units higher brightness and the active chlorine (aCl) use per Δkappa was 0.09 kg/t and per ΔBr 0.18 kg/t lower than in the reference pulp (Table 7).

[0171] The measured brightness (%-ISO) increase was significant and revealed that the xylanase enzyme C31-4 application in Kraft pulp was beneficial, and effective at 90°C and pH 10.5.

Example 4. High temperature and high pH application of xylanase in Kraft pulp from softwood

[0172] The efficiency of the xylanase enzyme C31-4 in bleaching of softwood pulp at high temperature and high pH was tested by using short bleaching sequence XDE (X, enzyme; D, ClO₂ (chlorine dioxide); E, extraction with sodium hydroxide) with constant ClO₂ dosage (0.2 X kappa number). Bleaching trials were carried out in bleaching reactors with whole sequence. An Anchor type Kemu reactor (batch size 0.15-1.0 kg, T max 90 °C, consistency 25 %) was used in step X and step E and an air heated Hellsten reactor (batch size 0.3-0.8 kg, T max 85 °C, consistency 12 %) in step D₀.

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[0173] The pulp used in the trial was oxygen delignified SW Kraft from Scandinavian mill with following properties: kappa number 25.6, viscosity 1140 ml/g and brightness 26.8 %-ISO. The dosing of C31-4 enzyme preparation was approximately 2 g of enzyme protein per ton of pulp. The conditions used in different stages of bleaching are described in Tables 8 - 10.

[0174] The pulp was washed three times after the step X. The first wash was done using water at reaction temperature (90 °C), followed by two washes with cold (4°C) water. After the washing steps a centrifuge treatment (2600 rpm, 10 s) was performed to remove water and about 10 - 15 g (measured as oven dry) pulp samples were collected for analysis. In addition, dry matter analysis was done from the C31-4 treated samples using IR-dryer for better adjustment of pulp amount. Kappa number and brightness were analyzed from the pulp samples.

[0175] The results from the trial are shown in detail in Table 11.

Table 8. Conditions at step X. The pH of pulp was measured in the beginning (Target/Initial pH) and after the enzyme treatment (End pH).

Pre-treatment	Enzyme dosage (g/t)	Pulp consistency (%)	Temperature (°C)	Target / Initial pH	Reaction time (min)	End pH
No enzyme	0	10	90	10.5 / 10.6	60	10.7
C31-4	2	9.2	90	10.5 / 10.8	60	10.6

Table 9. Bleaching conditions at step D. The amount of pulp was measured as oven dry.

Pre-treatment	Target / used pulp (g)	Dosage target / used (active. Cl % of pulp)	Pulp consistency target / used (%)	Temperature (°C)	Initial pH / end pH	Reaction time (min)	Act Cl consumption (% of pulp)
No enzyme	215 / 200.3	5.1 / 5.5	9 / 8.4	70	2.6 / 2.1	30	5.5
C31-4	200 / 178.2	5.1 / 5.7	9 / 8.0	70	3.1 / 2.1	30	5.6

Table 10. Bleaching condition at step E.

Pre-treatment	Target / used pulp amount, g (as o.d.)	NaOH dosage target / used (% of pulp)	Pulp consistency, target / used, %	Temperature (°C)	Target Initial pH / end pH	Reaction time, min
No enzyme	200 / 185.2	2.3 / 2.5	10/9.3	80	10.8/ 10.7	90
C31-4	185 / 159.7	2.3/2.7	10 / 8.6	80	10.7/10.7	90

Table 11. Results from the bleaching trial.

Pre-treatment	Kappa after E-stage	Δ XDE kappa	Δ kappa/ kg used act. Cl	Brightness after E-stage, %-ISO	Δ XDE Brightness	Δ Brightness %-ISO / kg used act. Cl	Corrected end kappa number with the same 5.4 % act. Cl consumption	Corrected end Brightness with the same 5.4% act. Cl consumption
No enzyme	3.30	22.28	0.41	55.79	29.0	0.53	3.7	55.3
C31-4	2.52	23.06	0.41	59.38	32.6	0.58	3.5	58.0

[0176] Softwood Kraft pulp pretreated with C31-4 at pH 10.5 had 2.7 units higher corrected end brightness with the same 5.4 % active chlorine consumption compared to reference pulp (no enzyme used).

Example 5. High temperature and high pH further application of xylanase in Kraft pulp from softwood

[0177] The bleaching test using softwood pulp and conditions like in Example 4 was repeated with the AM24 and C31-4 xylanases. The pulp was washed at pH 11 and 60 °C for about 30 minutes before its characterization and experiments. After the washing step the Kappa number of the pulp was 16.7, viscosity 1050 ml/g and brightness 32.6 %-ISO. The dosing of both enzymes (C31-4 and AM24) was 2 g of enzyme protein per ton of pulp. The conditions used in the xylanase treatment, bleaching and extraction stages are described in Tables 12 - 14. In the xylanase treatment stage (X-stage) the two enzymes were applied at different pH and temperature because the AM24 xylanase is assumed to be inactive at such pulp mill conditions while the C31-4 variant was expected, basing on previously done experiments, to be able to work at these conditions (pH 10.5/90 °C).

Table 12. Conditions of step X. The pH of pulp was measured in the beginning (Target/Initial pH) and after the enzyme treatment (End pH).

Enzyme	Enzyme dosage, g/t	Pulp consistency, %	Temperature, °C	Target / Initial pH	Reaction time, min	End pH
1. No enzyme	-	10	90	10.5/10.4	60	10.4
2. AM24	2	10	80	7/7.1	60	7.1
3. C31-4	2	10	90	10.5/10.7	60	10.6

Table 13. Bleaching conditions of step D

Enzyme	D dosage*, as act. Cl % on pulp	Pulp consistency, %	Temperature, °C	Initial pH/end pH	Reaction time, min	Act Cl consumption, % on pulp
1. No enzyme	3.3	9	70	2.8/2.1	30	3.3
2. AM24	3.3	9	70	2.5/1.6	30	3.3
3. C31-4	3.3	9	70	2.7/1.7	30	3.3

Table 14. Conditions at step E

Enzyme	NaOH dosage, % on pulp	Pulp consistency, %	Temperature, °C	Target Initial pH / end pH	Reaction time, min
1. No enzyme	1.5	10	80	10.6/10.6	90
2. AM24	1.5	10	80	10.5/10.4	90
3. C31-4	1.5	10	80	10.7/10.6	90

[0178] The results from the experiments are included in Table 15.

Table 15. Results after step E

Enzyme	Kappa number after E-stage	Δ XDE kappa number	Δ kappa number / kg used act. Cl	Brightness after E-stage, %-ISO	Δ XDE Brightness, %-ISO	Δ Brightness % / kg used act. Cl
1. No enzyme	3.0	13.7	0.41	62.3	29.7	0.89
2. AM24	2.9	13.8	0.41	63.5	30.8	0.92
3. C31-4	2.5	14.2	0.43	65.1	32.4	0.97

[0179] Scandinavian Softwood Kraft pulp pretreated with C31-4 at pH 10.5/90 °C had a 2.8 units higher brightness compared to the reference pulp (no enzyme used) at identical conditions throughout the XDE bleaching sequence. The results confirm that C31-4 improves brightness at high pH and temperature conditions. The AM24 xylanase showed a brightness gain of 1.2 units, at adjusted conditions in the X-stage (pH 7/80 °C) and identical conditions as used for C31-4 in the D and E-stages.

[0180] The foregoing description has provided by way of non-limiting examples of particular implementations and embodiments a full and informative description of the best mode presently contemplated by the inventors for carrying out the invention. It is however clear to a person skilled in the art that the invention is not restricted to details of the embodiments presented in the foregoing, but that it can be implemented in other embodiments using equivalent means or in different combinations of embodiments without deviating from the characteristics of the invention.

Claims

1. A bleaching process comprising:

providing pulp;
performing a delignification of the pulp by carrying out in the following sequence:

- an enzymatic treatment step (X) comprising contacting the pulp with a glycoside hydrolase family GH11 xylanase enzyme at a pH of at least 9.5 and a temperature of at least 85 °C;
- an oxidation step (D), wherein a bleaching agent is added;
- an alkaline extraction step (E), wherein an alkaline agent is added; and
- recovering bleached pulp;

wherein the GH11 xylanase enzyme has an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5, compared to the same mixture without any xylanase enzyme.

2. The process of claim 1, wherein the GH11 xylanase enzyme has an ability to cause at least 25 %, preferably at least 30 %, more preferably at least 40 % and most preferably at least 50 % reduction in viscosity of the xylan-containing mixture, compared to the same mixture without any xylanase enzyme.

3. The process of claim 1 or 2, wherein the xylan-containing mixture comprises: not more than 0.07 g xylan per ml of said mixture, and not more than 3.0 μ g of the GH11 xylanase enzyme protein per gram of said mixture; and wherein the reduction in viscosity takes place within 20 minutes, preferably within 10 minutes, more preferably within 5 minutes and most preferably within 2 minutes.

4. The process of any preceding claim, wherein the step X comprises contacting the pulp with the GH11 xylanase enzyme at a pH 9.5 - 11.5 and a temperature of 85 - 100 °C.

5. The process of any preceding claim, wherein at least 0.01 g, preferably at least 0.1 g, more preferably at least 0.2 g, most preferably at least 0.5 g of the GH11 xylanase enzyme protein is added per 1000 kg of dry matter pulp at the step X.

6. The process of any preceding claim, the delignification further comprising an oxygen-delignification step (O) before

the oxidation step D, wherein oxygen is added at the step O; and wherein the step X is before or after the step O.

7. The process of any preceding claim, wherein the bleaching agent at the oxidation step D comprises an oxygen-containing oxidizing agent, preferably the bleaching agent is selected from a group consisting of ClO_2 , O_3 , H_2O_2 , and peroxides.
8. The process of any preceding claim, wherein the bleaching agent used is H_2O_2 , a metal chelation step (Q) for removal of metals is performed prior to the oxidation step D, and the GH11 xylanase enzyme is added to the pulp before, during or after the chelation step Q.
9. The process of any preceding claim, wherein the alkaline agent of the alkaline extraction step E is selected from NaOH, MgO, or combinations thereof.
10. The process of any preceding claim, wherein the GH11 xylanase enzyme originates from bacteria, preferably from Actinobacteria, more preferably from *Streptosporangiales*, more preferably from *Streptosporangiaceae*, even more preferably from *Thermopolyspora* species, most preferably from *Thermopolyspora flexuosa*.
11. The process of any preceding claim, wherein the GH11 xylanase enzyme has a molecular mass of 19 - 40 kDa, preferably 20 - 30 kDa, more preferably 23 - 25 kDa, most preferably 24 kDa, calculated from its amino acid sequence without glycosylation.
12. The process of any preceding claim, wherein the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having amino acids corresponding to the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof;
the GH11 xylanase enzyme having at least one disulfide bridge between two cysteine residues of the polypeptide.
13. The process of any preceding claim, wherein the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof.
14. The process of any preceding claim, wherein the GH11 xylanase enzyme is a polypeptide comprising an amino acid sequence having at least 79 %, preferably at least 85 %, more preferably at least 90 %, even more preferably at least 95 % amino acid sequence identity with the amino acids 3 - 180 of SEQ ID NO: 2, or functional fragments thereof,
wherein the amino acid sequence has at least one disulfide bridge between two cysteine residues of the polypeptide.
15. A pulp composition comprising:
 - (a) pulp having a pH of at least 9.5; and
 - (b) 0.01 - 200 g of a glycoside hydrolase family GH11 xylanase enzyme protein per 1000 kg of dry matter pulp;

wherein the GH11 xylanase enzyme protein has:

xylanase activity, and
an ability to cause at least 20 % reduction in viscosity of a xylan-containing mixture at 90 °C and pH 10.5 compared to the same mixture without any xylanase enzyme, wherein the xylan-containing mixture comprises 0.07 g/ml or less of xylan and 3.0 µg or less of the GH11 xylanase enzyme protein per gram of said mixture;
and wherein the reduction in viscosity takes place within 20 minutes.

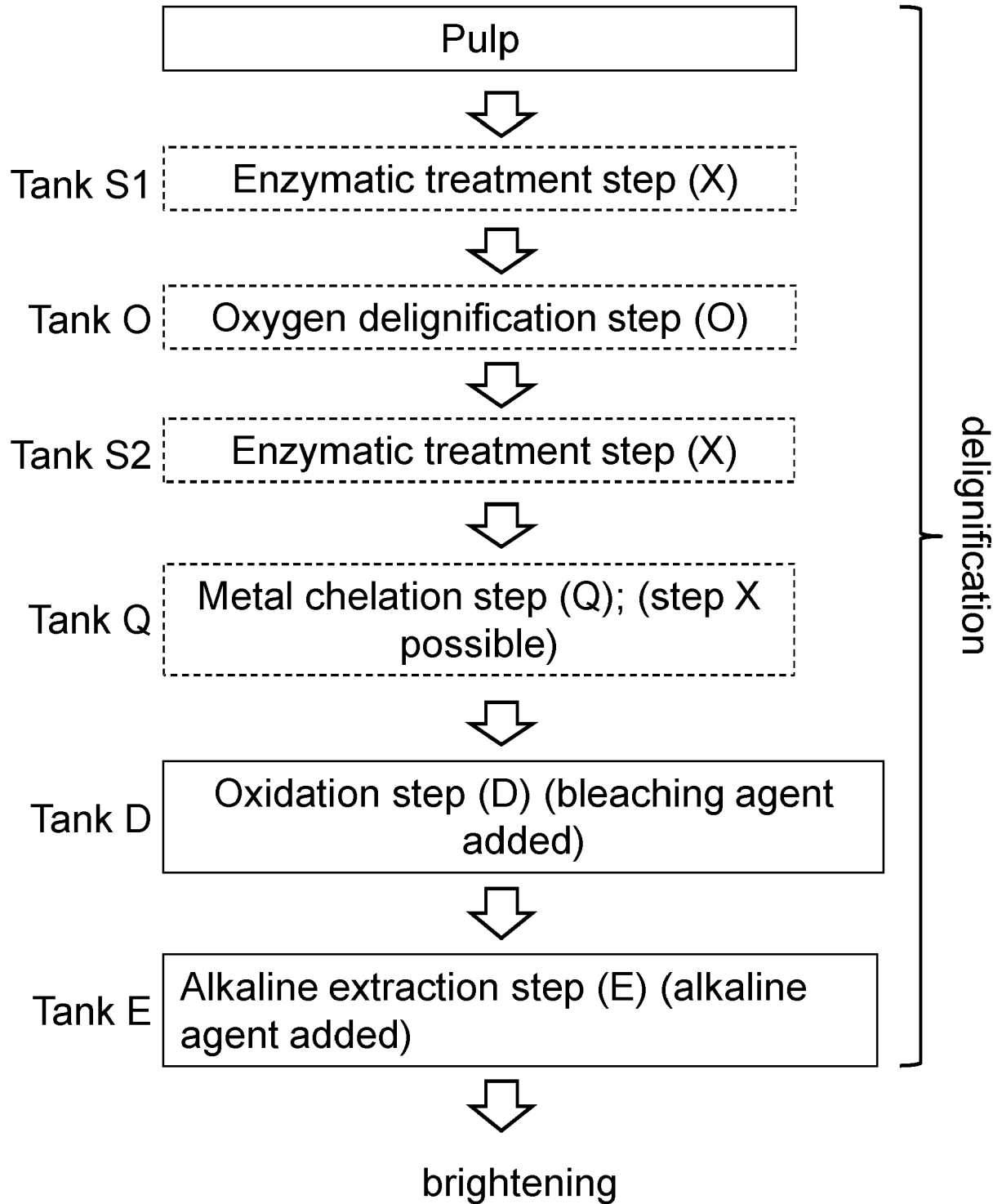


Fig. 1



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