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(54) Title: METHOD FOR TREATING COTTON

(57) Abstract: A method of treating cotton comprising: (i) contacting cotton with an aqueous wash liquor comprising water and an endo-β-1,3-glucanase enzyme; (ii) optionally rinsing the cotton; and (iii) drying the cotton. The wash liquor may contain surfactant. The cotton may be contacted with the aqueous wash liquor with agitation. The method may be for removal of callose from cotton. The method may be for the manufacture of a cotton-containing fabric having improved whiteness maintenance, soil repellency, reduced malodour, anti-wrinkle benefits, and/or improved drying.

METHOD FOR TREATING COTTON

REFERENCE TO A SEQUENCE LISTING

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

FIELD OF THE INVENTION

The present invention relates to a method for treating cotton with an endo-β-1,3-glucanase enzyme. The method of the invention is suitable for use in industrial and household treatments. The invention also relates to a method for making cotton-containing fabrics having improved whiteness maintenance and/or resistance to whiteness degradation. It is known to incorporate glucanase enzymes into cleaning compositions, for example as described in WO2005/003319. Such glucanase enzymes hydrolyse glucoside bonds. There are many different glucanase enzymes, for example endo-beta-1,3(4)-glucanase enzymes which hydrolyse both 1,3 and 1,4 linkages in beta glucans as well as endo-beta-1,3:1,4-glucanase enzymes, endo-beta-1,4-glucanase enzymes and endo-beta-1,3-glucanase enzymes. The present inventors have found that certain endo-beta-1,3-glucanase enzymes are particularly useful.

BACKGROUND OF THE INVENTION

Degradation of whiteness in fabrics, particularly white fabrics is a continuing problem. Efforts to promote the longevity of fabrics are on-going. Whilst there are many cleaning and treatment technologies and processes aimed at mitigating such problems however, it is a constant challenge to provide improved efficacy and especially to do so in an environmentally favourable manner.

The present inventors have found that natural impurities, particularly present in cotton fibres contribute to whiteness degradation and also play a role in adhering other soils which contact the cotton fibres during use or even during the washing process.

Thus, it is an object of the present invention to provide a method of treating cotton to counteract whiteness degradation.

SUMMARY OF THE INVENTION

The present invention provides a method of treating cotton comprising: (i) contacting cotton with an aqueous wash liquor comprising water and an endo-β-1,3-glucanase enzyme having

at least 60% sequence identity to one or more of the amino acid sequences selected from: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7; (ii) optionally rinsing the cotton; and (iii) drying the cotton. The cotton may be in the form of a fabric optionally in the form of a mixed fabric comprising cotton and one or more additional materials, preferably polyester.

The cotton may be contacted with the aqueous wash liquor at any temperature, but preferably at a temperature of 60°C or below, or more preferably at a temperature of 40°C or 35°C or below, most preferably at a temperature of 30°C or below.

The endo-β-1,3-glucanase is preferably from E.C. class 3.2.1.39.

Preferably the endo-β-1,3-glucanase enzyme has at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, sequence identity to one or more of the amino acid sequences selected from: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7. The endo-β-1,3-glucanase enzyme is preferably obtained from *Paenibacillus sp, Zobellia galactanivorans, Thermotoga petrophila* or *Trichoderma sp* micro-organism, preferably *Paenibacillus sp* or *Zobellia galactanivorans,* most preferably *Paenibacillus sp.*

The invention also relates to the use of an endo-β-1,3-glucanase enzyme or a method as described above for removal of callose from cotton. The invention also relates to the use of an endo-β-1,3-glucanase enzyme or a method as described above for manufacture of a cotton-containing fabric having improved whiteness maintenance, improved soil repellency, reduced malodour, anti-wrinkle benefits, and/or improved drying. As used herein, removal means partial or complete removal.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Parent or Parent endo-β-1,3-glucanase enzyme: The term "parent" or "parent endo-β-1,3-glucanase" means an endo-β-1,3-glucanase to which an alteration is made to produce the enzyme variants. The parent may be a naturally occurring (wild-type) polypeptide or a variant thereof. For example, the parent may be any of SEQ ID Nos: 1, 2, 3, 4, 5, 6 or 7 listed herein.

Sequence Identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity". For purposes of the present invention, the degree of sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol.

Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

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

Alternatively, the parameters used may be jgap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

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

Variant: The term "variant" means a polypeptide having endo-β-1,3-glucanase activity comprising an alteration/mutation, i.e., a substitution, insertion, and/or deletion, at one or more (e.g. several) positions relative to the parent endo-β-1,3-glucanase. A substitution means a replacement of an amino acid occupying a position with a different amino acid; a deletion means removal of an amino acid occupying a position; and an insertion means adding 1-3 amino acids adjacent to and immediately following an amino acid occupying a position.

Wild-Type Enzyme: The term "wild-type" endo-β-1,3-glucanase means an endo-β-1,3-glucanase expressed by a naturally occurring microorganism, such as a bacterium, yeast, or filamentous fungus found in nature.

Endo-β-1,3-glucanase enzyme

The endo-β-1,3-glucanase enzyme is an enzyme having activity for β-1,3 glucoside bonds in β-1,3-glucans and in addition preferably weak/no activity on β-1,3 glucoside bonds in mixed linkage glucans, having both 1,3- and 1,4- β- glucan bonds. Thus, the endo- β-1,3-glucanase enzyme herein does not include endo-1,3-1,4-β-D glucan-4-glucanohydrolases (licheninases) (E.C. class 3.2.1.73) or endo- β-1,3 (4)-glucanase ((E.C. class 3.2.1.6). Preferably the endo-β-1,3-glucanase enzyme is from E.C. class 3.2.1.39. Endo-β-1,3-glucanase activity can be confirmed by activity to pachyman, curdlan, callose, schizophyllan and/or scleroglucan. Preferably the endo-β-1,3-glucanase enzyme herein will have activity on one or more of pachyman, carboxymethyl curdlan, callose, schizophyllan and/or scleroglucan greater than or equal to the activity

demonstrated by the equivalent amount of active protein according to SEQ ID NO: 7. Preferably the endo-β-1,3-glucanase enzyme herein will have activity on carboxymethyl curdlan for example P-CMCUR (available from Megazyme International, Bray, Ireland) greater than or equal to the activity demonstrated by the equivalent amount of active protein according to SEQ ID NO: 7 (30 degrees C, pH 8.0 or pH of the cleaning composition). Preferably the endo-β-1,3-glucanase enzyme herein will the same or less activity on barley β-glucan (for example P-BGBM from Megazyme International, Bray, Ireland) than that demonstrated by the equivalent amount of active protein according to SEQ ID NO: 7 (30 degrees C, pH 8.0 or the pH of the cleaning composition).

The endo-β-1,3-glucanase enzyme useful in the invention having endo-β-1,3-glucanase enzyme activity is preferably microbial in origin, preferably bacterial or fungal (for example *Trichoderma sp*), most preferably bacterial. Preferably the endo-β-1,3-glucanase enzyme is obtainable from *Paenibacillus sp, Zobellia galactanivorans, Thermotoga petrophila* micro-organism, preferably *Paenibacillus sp or Zobellia galactanivorans, most preferably Paenibacillus sp.* Preferably the endo-β-1,3-glucanase enzyme is from glycosyl hydrolase (GH) family 16 or 64, preferably GH family 16. Preferably the endo-β-1,3-glucanase enzyme has a carbohydrate binding module CBM 6 or CBM 56.

Preferably the endo-β-1,3-glucanase enzyme has at least 50% identity or at least 60%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, sequence identity to one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7 listed herein. Thus, preferred endo-β-1,3-glucanase enzyme corresponds to the wild-type or is a variant of the wild-type of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6 or 7 listed herein.

When the endo-β-1,3-glucanase enzyme is a variant of a parent amino acid sequence, the parent endo-β-1,3-glucanase enzyme preferably has a sequence identity to the polypeptide of one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6 or 7 of at least 50 % or at least 60%, or at least 70% or at least 80%, such as at least 85%, at least 90%, e.g. at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99, or 100%, which has endo-β-1,3-glucanase enzyme activity. It may be preferred for the variant amino acid sequence to differ from the parent endo-β-1,3-glucanase by no more than ten amino acids, or no more than five amino acids, by four amino acids, by three amino acids, by two amino acids, and by one amino acid from the polypeptide of one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6 or 7.

The parent may be obtained from microorganisms of any genus. For purposes of the

present invention, the term "obtained from" as used herein in connection with a given source shall mean that the parent encoded by a polynucleotide is produced by the source or by a cell in which the polynucleotide from the source has been inserted. In one aspect, the parent is secreted extracellularly. Variants may be prepared using any mutagenesis procedure known in the art, such as site-directed mutagenesis, synthetic gene construction, semi-synthetic gene construction, random mutagenesis, shuffling, etc.

The endo-β-1,3-glucanase enzyme may be incorporated into the invention in the form of a substantially pure enzyme. Alternatively, in particular where the enzyme is a variant of a wild-type enzyme, the variant is not recovered, but rather a host cell expressing the enzyme is used as the source of the endo-β-1,3-glucanase enzyme.

The endo-β-1,3-glucanase enzyme may be incorporated in the form of a dry or solid composition. For instance, it may be added to water to form the aqueous wash liquor in the form of a granulate or a microgranulate. The endo-β-1,3-glucanase enzyme may be stabilized in accordance with methods known in the art.

The endo-β-1,3-glucanase enzyme is preferably present in the aqueous wash liquor in an amount form 1ppb to 100ppm, preferably from 10 ppb to 50ppm, or from 0.01ppm to 30ppm or from 0.1ppm to 25ppm.

The present inventors have found that the enzyme provides good soil breakdown, however the removal of the products of the breakdown of the substrates and soils containing them, particularly callose break-down products may be significantly improved by the presence of surfactant and/or agitation during contact of the cotton with the aqueous wash liquor. The aqueous wash liquor may be formed by addition of a composition comprising the endo-β-1,3-glucanase enzyme and optional cleaning adjuncts to water, or by adding the enzyme and further optional components separately. Where the enzyme is added in the form of a composition comprising additional optional ingredients, the composition preferably comprises endo-β-1,3-glucanase enzyme in an amount from 0.00005 to 5 wt% active protein, preferably from 0.0001 to 2 wt% active protein or from 0.0005 to 1 wt% active protein.

Surfactant

A preferred cleaning adjunct comprises surfactant, which is preferably present in the aqueous wash liquor in an amount from 0.0001g/l to 10g/l, or from 0.001 to 8g/l, or 0.001 to 5g/l based on the aqueous wash liquor.

Preferably the aqueous wash liquor comprises a surfactant and preferably the weight ratio of surfactant to active endo-β-1,3-glucanase enzyme protein is at least 500:1, preferably at least

1000:1 or at least 1500:1 or at least 2000:1.

The surfactant preferably comprises a surfactant system comprising a mixture of more than one surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic and/or ampholytic and/or amphoteric and/or semi-polar nonionic and/or mixtures thereof.

Preferably the composition comprises an anionic surfactant. Preferred anionic surfactants are sulfonate and sulfate surfactants, preferably alkylbenzene sulphonates and/or (optionally alkoxylated) alkyl sulfates. Particularly preferred anionic surfactant comprises linear alkylbenzenesulfonates (LAS). Preferred alkyl sulfates comprise alkyl ether sulfates, especially C-9-15 alcohol ether sulfates, especially those having an average degree of ethoxylation from 0.5 to 7, preferably from 1 to 5, C8-C16 ester sulfates and C10-C14 ester sulfates, such as mono dodecyl ester sulfates. In a preferred composition according to the invention the surfactant comprises anionic surfactant, preferably comprising alkyl benzene sulphonate and/or optionally ethoxylated alkyl sulfate, preferably having a degree of ethoxylation from 0 to 7, more preferably from 0.5 to 3. Isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ether sulfates (AES or AEOS or FES, also known as alcohol ethoxy sulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof are also suitable anionic surfactants. The aqueous wash liquor may comprise surfactant, preferably comprising anionic and/or nonionic surfactant. Where present anionic surfactant preferably comprises alkyl benzene sulphonate and/or optionally ethoxylated alkyl sulfate, preferably having a degree of ethoxylation from 0 to 7, more preferably from 0.5 to 3.

The anionic surfactants are used in the form of salts. Preferred cations are alkali metal ions, such as sodium and potassium. However, the salt form of the anionic surfactant may be formed in situ by neutralization of the acid form of the surfactant with alkali such as sodium hydroxide or an amine, such as mono-, di-, or tri-ethanolamine. Preferably the surfactant comprises

non-ionic surfactant. The aqueous wash liquor may comprise both anionic and nonionic surfactant, preferably in a weight ratio of anionic to nonionic of from 30:1 to 1:2, preferably from 20:1 to 2:3 or 1:1.

Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof. Alcohol ethoxylates are particularly preferred, preferably having a C9-18 alkyl chain, preferably from C12-15 and preferably having an average degree of ethoxylation 3 to 9, more preferably from 3 to 7. Commercially available nonionic surfactants include Plurafac ™, Lutensol™ and Pluronic™ from BASF, Dehypon™ series from Cognis and Genapol™ series from Clariant.

The preferred weight ratio of surfactant to active endo-β-1,3-glucanase enzyme protein is at least 500:1, preferably at least 1000:1 preferably no greater than 200000:1 or up to 100000:1 or 50000:1.

Cleaning Adjuncts

The wash liquor may comprise further cleaning/detergent adjunct in addition to surfactant. Typically any cleaning adjunct will be present in the composition in an amount from 1ppb to 5g/l based on the aqueous wash liquor. Suitable cleaning adjuncts comprise: builders, bleaches, bleach catalysts, colorants, bleach boosters, chelating agents, dye transfer agents, deposition aids, dispersants, additional enzymes, and enzyme stabilizers, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, optical brighteners, photoactivators, fluorescers, fabric hueing agents, fabric conditioners, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, filler salts, hydrotropes, brighteners, suds suppressors, structure elasticizing agents, fabric softeners, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, germicides, fungicides, anti-tarnish, anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids, pigments, dyes, perfumes and pH control agents, encapsulates, polymers. For example, these may include: bleach ingredients such as imine bleach boosters; sources of hydrogen peroxide such as percarbonate and/or perborate, especially percarbonate coated with material such as carbonate and/or sulphate salt, silicate salt,

borosilicate, and any mixture thereof; pre-formed peracid, including pre-formed peracid in encapsulated form; transition metal catalysts; suds suppressors or suppressor systems such as silicone based suds suppressors and/or fatty acid based suds suppressors;; fabric-softeners such as clay, silicone and/or quaternary ammonium compounds; flocculants such as polyethylene oxide; dye transfer inhibitors such as polyvinylpyrrolidone, poly 4-vinylpyridine N-oxide and/or co-polymer of vinylpyrrolidone and vinylimidazole; fabric integrity components such as oligomers produced by the condensation of imidazole and epichlorhydrin; soil dispersants and soil anti-redeposition aids such as alkoxylated polyamines and ethoxylated ethyleneimine polymers; anti-redeposition components such as polyesters; carboxylate polymers such as maleic acid polymers or co-polymers of maleic and acrylic acid; perfumes such as perfume microcapsules, starch encapsulated accords, perfume spray-on; soap rings; aesthetic particles; dyes; fillers such as sodium sulphate and/or citrus fibres, although it may be preferred for the composition to be substantially free of fillers; silicate salt such as sodium silicate, including 1.6R and 2.0R sodium silicate, or sodium metasilicate; co-polyesters of di-carboxylic acids and diols; cellulosic polymers such as methyl cellulose, carboxymethyl cellulose, hydroxyethoxycellulose, or other alkyl or alkylalkoxy cellulose**;** solvents such as 1,2 propanediol, monoethanolamine; diethylene glycol, ethanol, and any mixture thereof; hydrotropes such as sodium cumene sulphonate, sodium xylene sulphonate, sodium toluene sulphonate, and any mixtures; organic acids such as citric acid; and any combination thereof. The composition may be such that the cleaning adjunct comprises one or more selected from the group consisting of (i) perfume microcapsule; (ii) fabric hueing agent; (iii) protease; (iv) amphiphilic cleaning polymer; (v) lipase, or (vi) mixtures thereof.

The wash liquor preferably comprises cleaning composition may comprise one or more additional enzymes. Suitable additional enzymes comprise one or more additional enzymes preferably selected from the group consisting of aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, haloperoxidase, invertase, laccase, lipase, mannanase, mannosidase, oxidase, pectinolytic enzyme, peptidoglutaminase, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, xylanase, xanthan lyase, xanthanase and mixtures thereof. Preferably the composition comprises additional enzymes selected from xanthan lyase, xanthanase, mannanase and mixtures thereof. Mannanase is particularly preferred. Xanthan lyase and xanthanase and mixtures thereof are also particularly preferred. The additional enzyme(s)

may be produced, for example, by a microorganism belonging to the genus *Aspergillus*, *e.g.*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, or *Aspergillus oryzae*; *Fusarium*, *e.g.*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sulphureum*, *Fusarium toruloseum*, *Fusarium trichothecioides*, or *Fusarium venenatum*; *Humicola*, *e.g.*, *Humicola insolens* or *Humicola lanuginosa*; or *Trichoderma*, *e.g.*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride*.

Preferably the composition comprises a protease or mixtures of more than one protease, a lipase or mixtures of more than one lipase, a peroxidase or mixtures of more than one peroxidase, one or more amylolytic enzymes, e.g., an alpha-amylase, glucoamylase, maltogenic amylase, preferably an additional alpha amylase, one or mixtures of more than one CGTase and/or a cellulase or mixtures of more than one cellulase, mannanase (such as MANNAWAY™ from Novozymes, Denmark) or mixtures of more than one mannanase, pectinase, pectate lyase, cutinase, and/or laccase or mixtures of more than one of one or more of these.

In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (i.e., pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts. Preferably, the product of the invention comprises at least 0.01 mg, preferably from about 0.05 to about 10, more preferably from about 0.1 to about 6, especially from about 0.2 to about 5 mg of active further enzyme/ g of composition.

Proteases: Suitable proteases for use in combination with the variant proteases of the invention include metalloproteases and serine proteases, including neutral or alkaline microbial serine proteases, such as subtilisins (EC 3.4.21.62). Suitable proteases include those of animal, vegetable or microbial origin. In one aspect, such suitable protease may be of microbial origin. The suitable proteases include chemically or genetically modified mutants of the aforementioned suitable proteases. In one aspect, the suitable protease may be a serine protease, such as an alkaline microbial protease or/and a trypsin-type protease. Examples of suitable neutral or alkaline proteases include:

(a) subtilisins (EC 3.4.21.62), especially those derived from Bacillus, such as Bacillus sp., B. lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, B. pumilus , B. gibsonii, and B. akibaii described in WO2004067737, WO2015091989, WO2015091990,

WO2015024739, WO2015143360, US 6,312,936 B1, US 5,679,630, US 4,760,025, DE102006022216A1, DE102006022224A1, WO2015089447, WO2015089441, WO2016066756, WO2016066757, WO2016069557, WO2016069563, WO2016069569 and WO2016174234. Specifically, mutations S9R, A15T, V66A, A188P, V199I, Q239R, N255D (Savinase numbering system).

(b) subtilisins from *B. pumillus* such as the ones described in WO2019048486, WO2019048488, and WO2019048495 including variants comprising amino acid substitutions at positions 29, 48, 101, 130, 131, 133, 144, 224, 252, 271; and variants comprising a substitution at position 271 in combinations with one or more substitutions at the following positions; 18, 61, 92, 99, 137, 149, 156, 159, 162, 172, 192, 199, 217, 265.

(c) S8 proteases from Bacillus sp. NN018132, Bacillus borgouniensis and Paenibacillus dendritiformis such as the ones described in US20180340162.

(d) trypsin-type or chymotrypsin-type proteases, such as trypsin (e.g., of porcine or bovine origin), including the *Fusarium* protease described in WO 89/06270 and the chymotrypsin proteases derived from *Cellumonas* described in WO 05/052161 and WO 05/052146.

(e) metalloproteases, especially those derived from *Bacillus amyloliquefaciens* decribed in WO07/044993A2; from *Bacillus, Brevibacillus, Thermoactinomyces, Geobacillus, Paenibacillus, Lysinibacillus* or *Streptomyces spp.* Described in WO2014194032, WO2014194054 and WO2014194117; from *Kribella alluminosa* described in WO2015193488; and from *Streptomyces* and *Lysobacter* described in WO2016075078.

(f) protease having at least 90% identity to the subtilase from Bacillus sp. TY145, NCIMB 40339, described in WO92/17577 (Novozymes A/S), including the variants of this Bacillus sp TY145 subtilase described in WO2015024739, WO2015014790, WO2016066757 and US20190040376

(g) Halotolerant proteases such as the one described in WO2019105675.

Especially preferred additional proteases for the detergent of the invention are polypeptides demonstrating at least 90%, preferably at least 95%, more preferably at least 98%, even more preferably at least 99% and especially 100% identity with the wild-type enzyme from Bacillus lentus, comprising mutations at one or more, preferably two or more and more preferably three or more of the following positions, using the BPN' numbering system: 9, 15, 68, 76, 78, 87,

99, , X101, 103, 104, 118, 118, 128, 129, 130, 167, 170, 194, 205, 206, 209, 222, 245. Most preferably the additional proteases for the detergent invention comprise one or more, preferably two or more and more preferably three or more of the following mutations using the BPN' numbering system and amino acid abbreviations as illustrated in WO00/37627 which is incorporated herein by reference: S9R, A15T, V68A, N76D, N87S, S99D, S99E, S99SD, S99A, S101G, S101M, S103A, V104N/I, G118V, G118R, S128L, P129Q, S130A, Y167A, R170S, A194P, V205I, Q206L/D/E, Y209W, M222S, and/or Q245R.

Most preferably the additional protease is selected from the group of proteases comprising the below mutations (BPN' numbering system) versus either the PB92 wild-type (SEQ ID NO:2 in WO 08/010925) or the subtilisin 309 wild-type (sequence as per PB92 backbone, except comprising a natural variation of N87S).

(i) G118V + S128L + P129Q + S130A

(ii) S101M + G118V + S128L + P129Q + S130A

(iii) N76D + N87R + G118R + S128L + P129Q + S130A + S188D + N248R

(iv) N76D + N87R + G118R + S128L + P129Q + S130A + S188D + V244R

(v) N76D + N87R + G118R + S128L + P129Q + S130A

(vi) V68A + N87S + S101G + V104N

(vii) S99AD

(viii) S99E

(ix) S9R+A15T+V68A+N218D+Q245R

Suitable commercially available additional protease enzymes include those sold under the trade names Alcalase®, Savinase®, Primase®, Durazym®, Polarzyme®, Kannase®, Liquanase®, Liquanase Ultra®, Savinase Ultra®, Ovozyme®, Neutrase®, Everlase®, Coronase®, Blaze®, Blaze Ultra® and Esperase® by Novozymes A/S (Denmark); those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Properase®, Purafect®, Purafect Prime®, Purafect Ox®, FN3®, FN4®, Excellase®, Ultimase® and Purafect OXP® by Dupont; those sold under the tradename Opticlean® and Optimase® by Solvay Enzymes; and those available from

Henkel/Kemira, namely BLAP (sequence shown in Figure 29 of US 5,352,604 with the following mutations S99D + S101 R + S103A + V104I + G159S, hereinafter referred to as BLAP), BLAP R (BLAP with S3T + V4I + V199M + V205I + L217D), BLAP X (BLAP with S3T + V4I + V205I) and BLAP F49 (BLAP with S3T + V4I + A194P + V199M + V205I + L217D); and KAP (Bacillus alkalophilus subtilisin with mutations A230V + S256G + S259N) from Kao.

Especially preferred for use herein in combination with the variant protease of the invention are commercial proteases selected from the group consisting of Properase®, Blaze®, Ultimase®, Everlase®, Savinase®, Excellase®, Blaze Ultra®, BLAP and BLAP variants.

Preferred levels of protease in the product of the invention include from about 0.05 to about 10, more preferably from about 0.5 to about 7 and especially from about 1 to about 6 mg of active protease/g of composition.

Lipases: The composition preferably comprises a lipase. The presence of oils and/or grease can further increase the resiliency of stains comprising mannans and other polysaccharides. As such, the presence of lipase in the enzyme package can further improve the removal of such stains. Suitable lipases include those of bacterial or fungal or synthetic origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g., from *H. lanuginosa* (*T. lanuginosus*) or from *H. insolens*, a *Pseudomonas lipase*, e.g., from *P. alcaligenes* or *P. pseudoalcaligenes*, *P. cepacia P. stutzeri, P. fluorescens, Pseudomonas* sp. strain SD 705, *P. wisconsinensis* , a *Bacillus* lipase, e.g., from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* or *B. pumilus* .

The lipase may be a "first cycle lipase" such as those described in U.S. Patent 6,939,702 B1 and US PA 2009/0217464. In one aspect, the lipase is a first-wash lipase, preferably a variant of the wild-type lipase from *Thermomyces lanuginosus* comprising T231R and N233R mutations. The wild-type sequence is the 269 amino acids (amino acids 23 – 291) of the Swissprot accession number Swiss-Prot O59952 (derived from *Thermomyces lanuginosus (Humicola lanuginosa)).* Preferred lipases include those sold under the tradenames Lipex®, Lipolex® and Lipoclean®.

Other suitable lipases include: Liprl 139, e.g. as described in WO2013/171241; TfuLip2, e.g. as described in WO2011/084412 and WO2013/033318; Pseudomonas stutzeri lipase, e.g. as described in WO2018228880; *Microbulbifer thermotolerans* lipase, e.g. as described in WO2018228881; *Sulfobacillus acidocaldarius* lipase, e.g. as described in EP3299457; LIP062 lipase e.g. as described in WO2018209026; PinLip lipase e.g. as described in WO2017036901 and

Absidia sp. lipase e.g. as described in WO2017005798.

A suitable lipase is a variant of SEQ ID NO:5 comprising:

(a) substitution T231R

and

(b) substitution N233R or N233C

and

(c) at least three further substitutions selected from E1C, D27R, N33Q, G38A, F51V, G91Q, D96E, K98L, K98I, D111A, G163K, H198S, E210Q, Y220F, D254S, I255A, and P256T;

where the positions correspond to the positions of SEQ ID NO:5 and wherein the lipase variant has at least 90% but less than 100% sequence identity to the polypeptide having the amino acid sequence of SEQ ID NO: 5 and wherein the variant has lipase activity.

One preferred lipase is a variant of SEQ ID NO: 5 comprising the following substitutions: T231R, N233R, D27R, G38A, D96E, D111A, G163K, D254S and P256T

One preferred lipase is a variant of SEQ ID NO: 5 comprising the following substitutions: T231R, N233R, N33Q, G91Q, E210Q, I255A.

Suitable lipases are commercially available from Novozymes, for example as Lipex Evity 100L, Lipex Evity 200L (both liquid raw materials) and Lipex Evity 105T (a granulate). These lipases have different structures to the products Lipex 100L, Lipex 100T and Lipex Evity 100T which are outside the scope of the invention.

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium*, e.g., the fungal cellulases produced from *Humicola insolens, Myceliophthora thermophila* and *Fusarium oxysporum*. disclosed in US 4,435,307 , US 5,648,263 , US 5,691,178 , US 5,776,757 and US 5,691,178 .

In one aspect, preferred enzymes include microbial-derived endoglucanases exhibiting endo-beta-1,4-glucanase activity (E.C. 3.2.1.4), prefrebaly selected from the group comprising:

(a) a bacterial polypeptide endogenous to a member of the genus Bacillus which has a sequence of at least 90%, 94%, 97% and even 99% identity to the amino acid sequence SEQ ID NO:2 in US 7,141,403B2, preferred substitutions compriseone or more positions corresponding to positions 292, 274, 266, 265,

255, 246, 237, 224 and 221 of the mature polypeptide of SEQ ID NO: 2, and t he variant has cellulase activity.;

(b) a glycosyl hydrolase having enzymatic activity towards both xyloglucan and amorphous cellulose substrates, wherein the glycosyl hydrolase is selected from GH families 5, 7, 12, 16, 44 or 74;

(c) a glycosyl hydrolase having a sequence of at least 90%, 94%, 97% and even 99% identity to the amino acid sequence SEQ ID NO:3 in WO09/148983;

(d) Variants exhibiting at least 70% identity with SEQ ID NO: 5 in WO2017106676. Preferred substitutions comprise one or more positions corresponding to positions 4, 20, 23, 29, 32, 36, 44, 51, 77, 80, 87, 90, 97, 98, 99, 102, 112, 116, 135, 136, 142, 153, 154, 157, 161, 163, 192, 194, 204, 208, 210, 212, 216, 217, 221, 222, 225, 227, and 232;

(e) and mixtures thereof.

Suitable endoglucanases are sold under the tradenames Celluclean® and Whitezyme® (Novozymes A/S, Bagsvaerd, Denmark). Examples include Celluclean® 5000L, Celluclean® Classic 400L, Celluclean® Classic 700T, Celluclean® 4500T, Whitezyme® 1.5T, Whitezyme® 2.0L.

Other commercially available cellulases include Celluzyme®, Carezyme®, Carezyme® Premium (Novozymes A/S), Clazinase®, Puradax HA®, Revitalenz® 1000, Revitalenz® 2000 (Genencor International Inc.), KAC-500(B)® (Kao Corporation), Biotouch® FCL, Biotouch® DCL, Biotouch® DCC, Biotouch® NCD, Biotouch® FCC, Biotouch® FLX1 (AB Enzymes)

Amylases: Preferably the composition of the invention comprise an amylase. Suitable alpha-amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included. A preferred alkaline alpha-amylase is derived from a strain of Bacillus, such as *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus stearothermophilus, Bacillus subtilis*, or other *Bacillus sp.*, such as Bacillus sp. NCBI 12289, NCBI 12512, NCBI 12513, DSM 9375 (USP 7,153,818) DSM 12368, DSMZ no. 12649, KSM AP1378 (WO 97/00324), KSM K36 or KSM K38 (EP 1,022,334). Preferred amylases include:

(a) variants described in USP 5,856,164 and WO99/23211, WO 96/23873, WO00/60060, WO06/002643 and WO2017/192657, especially the variants with one or more substitutions in the following positions versus the AA560 enzyme listed as SEQ ID No. 12 in WO 06/002643:
26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 202, 214, 231, 246, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 339,

345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 461, 471, 482, 484, preferably that also contain the deletions of D183* and G184*.

(b) variants exhibiting at least 85%, preferably 90% identity with SEQ ID No. 4 in WO06/002643, the wild-type enzyme from Bacillus SP722, especially variants with deletions in the 183 and 184 positions and variants described in WO 00/60060, WO2011/100410 and WO2013/003659, particularly those with one or more substitutions at the following positions versus SEQ ID No. 4 in WO06/002643 which are incorporated herein by reference:
51, 52, 54, 109, 304, 140, 189, 134, 195, 206, 243, 260, 262, 284, 347, 439, 469, 476 and 477.

(c) variants exhibiting at least 90% identity with the wild-type enzyme from Bacillus sp.707 (SEQ ID NO:7 in US 6,093,562), especially those comprising one or more of the following mutations M202, M208, S255, R172, and/or M261. Preferably said amylase comprises one or more of M202L, M202V, M202S, M202T, M202I, M202Q, M202W, S255N and/or R172Q. Particularly preferred are those comprising the M202L or M202T mutations. Additional relevant mutations/deletions based on SP707 backbone include W48, A51, V103, V104, A113, R118, N125, V131, T132, E134, T136, E138, R142, S154, V165, R182, G182, H183, E190, D192, T193, I206, M208, D209, E212, V213, V214, N214, L217, R218, N219, V222, T225, T227, G229, I235, K242, Y243, S244, F245, T246, I250, S255, A256, H286, V291, T316, V317, V318, N417, T418, A419, H420, P421, I428, M429, F440, R443, N444, K445, Q448, S451, A465, N470, S472.

(d) variants described in WO 09/149130, preferably those exhibiting at least 90% identity with SEQ ID NO: 1 or SEQ ID NO:2 in WO 09/149130, the wild-type enzyme from *Geobacillus Stearophermophilus* or a truncated version thereof.

(e) variants described in WO10/115021, especially those exhibiting at least 75%, or at least 85% or at least 90% or at least 95% with SEQ ID NO:2 in WO10/115021, the alpha-amylase derived from *Bacillus sp*. TS-23.

(f) variants exhibiting at least 89% identity with SEQ ID NO:1 in WO2016091688, especially those comprising deletions at positions H183+G184 and additionally one or more mutations at positions 405, 421, 422 and/or 428.

(g) variants described in WO2014099523, especially those exhibiting at least 60% amino acid sequence identity with the "PcuAmyl α-amylase" from *Paenibacillus curdlanolyticus* YK9 (SEQ ID NO:3 in WO2014099523).

(h) variants described in WO2014099523, especially those exhibiting at least 60% amino acid sequence identity with the “CspAmy2 amylase” from *Cytophaga sp.* (SEQ ID NO:1 & 6 in WO2014164777. Especially those comprising one of more of the following deletions and/or

mutations based on SEQ ID NO:1 in WO2014164777: R178*, G179*, T38N, N88H, N126Y, T129I, N134M, F153W, L171R, T180D, E187P, I203Y, G476K, G477E, Y303D.

(i) variants exhibiting at least 85% identity with AmyE from *Bacillus subtilis* (SEQ ID NO:1 in WO2009149271).

(j) variants exhibiting at least 90% identity with the wild-type amylase from Bacillus sp. KSM-K38 with accession number AB051102.

(k) variants described in WO2016180748, especially those exhibiting at least 80% identity with the mature amino acid sequence of AAI10 from *Bacillus sp* in SEQ ID NO: 7 in WO2016180748; those exhibiting at least 80% identity with the mature amino acid sequence of *Alicyclobacillus sp.* amylase in SEQ ID NO: 8 in WO2016180748, and those exhibiting at least 80% identity with the mature amino acid sequence of SEQ ID NO: 13 in WO2016180748, especially those comprising one or more of the following mutations H*, N54S, V56T, K72R, G109A, F113Q, R116Q, W167F, Q172G, A174S, G184T, N195F, V206L, K391A, P473R, G476K.

(l) variants described in WO2018060216, especially those exhibiting at least 70% identity with the mature amino acid sequence of SEQ ID NO: 4 in WO2018060216, the fusion molecule of *Bacillus amyloliquefaciens* and *Bacillus licheniformis*. Especially those comprising one or more substitutions at positions H1, N54, V56, K72, G109, F113, R116, T134, W140, W159, W167, Q169, Q172, L173, A174, R181, G182, D183, G184, W189, E194, N195, V206, G255, N260, F262, A265, W284, F289, S304, G305, W347, K391, Q395, W439, W469, R444, F473, G476, and G477.

Preferably the amylase is an engineered enzyme, wherein one or more of the amino acids prone to bleach oxidation have been substituted by an amino acid less prone to oxidation. In particular it is preferred that methionine residues are substituted with any other amino acid. In particular it is preferred that the methionine most prone to oxidation is substituted. Preferably the methionine in a position equivalent to 202 in SEQ ID NO:11 is substituted. Preferably, the methionine at this position is substituted with threonine or leucine, preferably leucine.

Suitable commercially available alpha-amylases include DURAMYL®, LIQUEZYME®, TERMAMYL®, TERMAMYL ULTRA®, NATALASE®, SUPRAMYL®, STAINZYME®, STAINZYME PLUS®, FUNGAMYL®, ATLANTIC®, ACHIEVE ALPHA®, AMPLIFY® PRIME, INTENSA® and BAN® (Novozymes A/S, Bagsvaerd, Denmark), KEMZYM® AT 9000 Biozym Biotech Trading GmbH Wehlistrasse 27b A-1200 Wien Austria, RAPIDASE® , PURASTAR®, ENZYSIZE®, OPTISIZE HT PLUS®, POWERASE®, PREFERENZ S® series

(including PREFERENZ S1000® and PREFERENZ S2000® and PURASTAR OXAM® (DuPont., Palo Alto, California) and KAM® (Kao, 14-10 Nihonbashi Kayabacho, 1-chome, Chuo-ku Tokyo 103-8210, Japan).

Preferably, the product of the invention comprises at least 0.01 mg, preferably from about 0.05 to about 10, more preferably from about 0.1 to about 6, especially from about 0.2 to about 5 mg of active amylase/ g of composition.

Preferably, the protease and/or amylase of the composition of the invention are in the form of granulates, the granulates comprise more than 29% of sodium sulfate by weight of the granulate and/or the sodium sulfate and the active enzyme (protease and/or amylase) are in a weight ratio of between 3:1 and 100:1 or preferably between 4:1 and 30:1 or more preferably between 5:1 and 20:1.

Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g., from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

Commercially available peroxidases include GUARDZYME® (Novozymes A/S).

Pectate lyase: suitable pectate lyases include those sold under the tradenames Pectawash®, Pectaway®, X-Pect®, (all Novozymes A/S, Bagsvaerd, Denmark) Preferenz® F1000 (DuPont Industrial Biosciences).

Mannanases. Preferably the composition comprises a mannanase. As used herein, the term "mannanase" or "galactomannanase" denotes a mannanase enzyme defined according to that known in the art as mannan endo-1,4-beta-mannosidase and having the alternative names beta-mannanase and endo-1,4-mannanase and catalysing hydrolysis of 1,4-beta-D-mannosidic linkages in mannans, galactomannans, glucomannans, and galactoglucomannans. Mannanases are classified according to the Enzyme Nomenclature as EC 3.2.1.78. Commercially available mannanases include those sold under the tradenames Mannaway® (Novozymes A/S, Bagsvaerd, Denmark), Effectenz® M1000, Mannastar® 375, Preferenz M100 and Purabrite® (all DuPont Industrial Biosciences, Palo Alto, California). Preferred mannanases include: those having at least 85% sequence identity to residues 27- 331 of SEQ ID NO: 8. SEQ ID NO: 8 corresponds to the full-length amino acid sequence of the Man7 mannanase endogenous to *Bacillus hemicellulosilyticus* including a signal sequence. Particularly preferred mannanases have at least 90% sequence identity to residues 27-331 of SEQ ID NO: 3, optionally comprising at least one substitution at positions 123, 158, 180, 272, 285, or 307 or a combination thereof; and mannanase

from the the glycoside hydrolase family 26 that catalyze the hydrolysis of 1,4-3-D-mannosidic linkages in mannans, galactomannans and glucomannans. Suitable examples are described in WO2015040159.

Xanthan-degrading enzymes: Preferably the composition comprises a xanthan-degrading enzyme. Suitable enzymes for degradation of xanthan soils such as xanthan gum include combinations of xanthan endoglucanase and xanthan lyase. As used herein, the term xanthan endoglucanase denotes an enzyme exhibiting endo-beta-1,4-glucanase activity that is capable of catalysing hydrolysis of the 1,4-linked β-D-glucose polymeric backbone of xanthan gum in conjunction with a suitable xanthan lyase enzyme. Preferred xanthan endoglucanases have endo-beta-1,4-glucanase activity and a polypeptide having at least 60% identity to SEQ ID NO: 9. SEQ ID NO: 9 corresponds to the amino acid sequence of a xanthan endoglucanase endogenous to Paenibacillus sp-62047. The xanthan endoglucanase may be a variant with at least 61 %, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71 %, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 9. The xanthan endoglucanase may have substitutions at one or more of positions 17, 20, 51 , 53, 55, 56, 60, 63, 79, 87, 186, 192, 302, 31 1 , 313, 387, 388, 390, 403, 408, 410, 416, 448, 451 , 471 , 472, 476, 489, 507, 512, 515, 538, 598, 599, 602, 605, 609, 676, 688, 690, 694, 697, 698, 699, 711, 719, 754, 756, 760, 781 , 786, 797, 833, 834, 835 and 1048 of SEQ ID NO: 9. The xanthan endoglucanase may have substitutions at one or more of positions S17A, F20P, F20N, F20G, F20Y, K51Q, K51H, E53P, E53G, Y55M, V56M, Y60F, S63F, T87R, A186P, K192N, I320D, I302H, I302V, I302M, H311N, S313D, I387T, K388R, K390Q, I403Y, E408D, E408S, E408P, E408A, E408G, E408N, P410G, Q416S, Q416D, A448E, A448W, A448S, K451S, G471S, S472Y, D476R, Q489P, K507R, K512P, S515V, S538C, Y579W, S598Q, A599S, I602T, I602D, V603P, S605T, G609E, D676H, A688G, Y690F, T694A, T697G, R698W, T699A, T711V, T711Y, W719R, K754R, V756H, V756Y, S760G, T781M, N786K, T797S, A824D, N833D, Q834E, S835D and F1048W. As used herein, the term “xanthan lyase” denotes an enzyme that cleaves the β-D-mannosyl-β-D-1 ,4-glucuronosyl bond of xanthan and have been described in the literature. Xanthan lyases are classified according to the Enzyme Nomenclature as EC 4.2.2.12, and are known to be produced by many xanthan-degrading bacteria including Bacillus, Corynebacterium and Paenibacillus

species. The xanthan lyase in accordance with the invention has xanthan lyase activity and comprises a polypeptide having at least 60% identity to SEQ ID NO: 10. SEQ ID NO: 10 corresponds to the amino acid sequence of a xanthan lyase endogenous to a Paenibacillus sp. The xanthan lyase may be a variant with at least 61 %, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71 %, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 10. The xanthan lyase may be a variant with alterations at one or more positions selected from the group consisting of positions: 9, 15, 46, 58, 66, 89, 95, 100, 106, 109, 183, 188, 190, 203, 204, 221 , 229, 234, 238, 240, 242, 243, 257, 258, 291 , 293, 316, 320, 324, 329, 333, 339, 341 , 352, 354, 360, 377, 399, 400, 419, 440, 450, 451 , 454, 458, 481 , 492, 567, 568, 578, 579, 582, 664, 672, 703, 728, 843, 855, 887, 892, 1008 and 1016 of SEQ ID NO: 10. The xanthan lyase may be a variant with alterations at one or more positions selected from the group consisting of positions 624, 631, 635, 649, 656, 752, 752, 754, 757, 769, 775, 777, 800, 801, 875, 911, and 915 of SEQ ID NO: 10. The xanthan lyase may be a variant with one or more substitutions selected from the group consisting of: K9R, N15T, L46D, A58L, S66H, Q89Y, K95E, S100D, N106Y, Q109R, Q109D, Q109F, Q109K, Q109A, K183Q, K183R, V188I, A190Q, A203P, K204R, A221 P, E229N, E229S, I234V, I238W, I238L, I238M, I240W, N242S, G243V, Y257W, R258E, K291R, A293G, A293P, K316R, K320R, L324Q, K329R, K333R, L339M, 1341P, V352I, S354P, K360G, K360R, F377Y, N399K, K400R, F419Y, N440K, D450P, K451E, K451 R, A454V, D458S, K481R, A492L, A492H, K567R, G568A, S578K, S578R, S579R, S579K, S582K, A624E, T631N, S635E, T649K, I656V, T664K, N672D, I703L, M728V, G738L, P752K, P752R, G753E, S754E, S754R, S757D, A769D, L775A, D777R, V800P, D801G, A843P, K855R, K875T, K887R, N892Y, N892W, N892F, A911V, T915A, N1008D and K1016T of SEQ ID NO: 10.

Preferably the composition comprises a nuclease such as a RNase or DNase or mixtures thereof. The nuclease enzyme is an enzyme capable of cleaving the phosphodiester bonds between the nucleotide sub-units of nucleic acids. The nuclease enzyme herein is preferably a deoxyribonuclease or ribonuclease enzyme or a functional fragment thereof. By functional fragment or part is meant the portion of the nuclease enzyme that catalyzes the cleavage of phosphodiester linkages in the DNA backbone and so is a region of said nuclease protein that

retains catalytic activity. Thus it includes truncated, but functional versions, of the enzyme and/or variants and/or derivatives and/or homologues whose functionality is maintained.

Preferably the nuclease enzyme is a deoxyribonuclease, preferably selected from any of the classes E.C. 3.1.21.x, where x=1, 2, 3, 4, 5, 6, 7, 8 or 9, E.C. 3.1.22.y where y=1, 2, 4 or 5, E.C. 3.1.30.z where z= 1 or 2, E.C. 3.1.31.1 and mixtures thereof.

DNase: Suitable DNases include wild-types and variants of DNases defined by SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8 and 9 in WO2017162836 (Novozymes), and variants of the Bacillus cibi DNase including those described in WO2018011277 (Novozymes), incorporated herein by reference. Preferred DNases are as claimed in co-pending European Patent Application No. EP18202967.

RNase: suitable RNases include wild-types and variants of DNases defined by SEQ ID NOS: 3, 6, 9, 12, 15, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 72 and 73 in WO2018178061 (Novozymes), incorporated herein by reference.

Galactanase: Preferably the composition comprises a galactanase, ie. an extracellular polymer-degrading enzyme that includes an endo-beta-1,6-galactanase enzyme. The term "endo-beta-1,6-galactanase" or "a polypeptide having endo-beta-1,6-galactanase activity" means a endo-beta-1,6-galactanase activity (EC 3.2.1.164) from the glycoside hydrolase family 30 that catalyzes the hydrolytic cleavage of 1,6-3-D-galactooligosaccharides with a degree of polymerization (DP) higher than 3, and their acidic derivatives with 4-O-methylglucosyluronate or glucosyluronate groups at the non-reducing terminals. For purposes of the present disclosure, endo-beta-1,6-galactanase activity is determined according to the procedure described in WO 2015185689 in Assay I. Suitable examples from class EC 3.2.1.164 are described in WO 2015185689, such as the mature polypeptide SEQ ID NO: 2.

The wash liquor may comprise a fabric hueing agent (sometimes referred to as shading, bluing or whitening agents) and/or optical brightener. Typically the hueing agent provides a blue or violet shade to fabric. Hueing agents can be used either alone or in combination to create a specific shade of hueing and/or to shade different fabric types. This may be provided for example by mixing a red and green-blue dye to yield a blue or violet shade. Hueing agents may be selected from any known chemical class of dye, including but not limited to acridine, anthraquinone (including polycyclic quinones), azine, azo (e.g., monoazo, disazo, trisazo, tetrakisazo, polyazo), including premetallized azo, benzodifurane and benzodifuranone, carotenoid, coumarin, cyanine, diazahemicyanine, diphenylmethane, formazan, hemicyanine, indigoids, methane, naphthalimides, naphthoquinone, nitro and nitroso, oxazine, phthalocyanine, pyrazoles, stilbene,

styryl, triarylmethane, triphenylmethane, xanthenes and mixtures thereof.

Suitable fabric hueing agents include dyes, dye-clay conjugates, and organic and inorganic pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct, Basic, Reactive or hydrolysed Reactive, Solvent or Disperse dyes. Examples of suitable small molecule dyes include for example small molecule dyes selected from the group consisting of Colour Index (Society of Dyers and Colourists, Bradford, UK) numbers Direct Violet dyes such as 9, 35, 48, 51, 66, and 99, Direct Blue dyes such as 1, 71, 80 and 279, Acid Red dyes such as 17, 73, 52, 88 and 150, Acid Violet dyes such as 15, 17, 24, 43, 49 and 50, Acid Blue dyes such as 15, 17, 25, 29, 40, 45, 75, 80, 83, 90 and 113, Acid Black dyes such as 1, Basic Violet dyes such as 1, 3, 4, 10 and 35, Basic Blue dyes such as 3, 16, 22, 47, 66, 75 and 159, Disperse or Solvent dyes such as those described in EP1794275 or EP1794276, or dyes as disclosed in US 7,208,459 B2,and mixtures thereof. In another aspect, suitable small molecule dyes include small molecule dyes selected from the group consisting of Colour Index numbers Acid Violet 17, Acid Violet 50 or 51, Direct Blue 71, Direct Violet 51, Direct Blue 1, Acid Red 88, Acid Red 150, Acid Blue 29, Acid Blue 113 or mixtures thereof.

Preferred are polymeric dyes include polymeric dyes selected from the group consisting of polymers containing covalently bound (sometimes referred to as conjugated) chromogens, (dye-polymer conjugates), for example polymers with chromogens co-polymerized into the backbone of the polymer and mixtures thereof. Polymeric dyes include those described in WO2011/98355, WO2011/47987, US2012/090102, WO2010/145887, WO2006/055787 and WO2010/142503.

Preferred polymeric dyes comprise alkoxylated, preferably ethoxylated azo or anthraquinone or triarylmethane dyes. Ethoxylated thiophene azo dyes are especially preferred, for example polymeric dyes selected from the group consisting of fabric-substantive colorants sold under the name of Liquitint® (Milliken, Spartanburg, South Carolina, USA), dye-polymer conjugates formed from at least one reactive dye and a polymer selected from the group consisting of polymers comprising a moiety selected from the group consisting of a hydroxyl moiety, a primary amine moiety, a secondary amine moiety, a thiol moiety and mixtures thereof. In still another aspect, suitable polymeric dyes include polymeric dyes selected from the group consisting of Liquitint® Violet CT, carboxymethyl cellulose (CMC) covalently bound to a reactive blue, reactive violet or reactive red dye such as CMC conjugated with C.I. Reactive Blue 19, sold by Megazyme, Wicklow, Ireland under the product name AZO-CM-CELLULOSE, product code S-

ACMC, alkoxylated triphenyl-methane polymeric colourants, alkoxylated thiophene polymeric colourants, and mixtures thereof.

Preferred hueing dyes include the alkoxylated thiophene azo whitening agents found in US2008/0177090 which may be optionally anionic, such as those selected from Examples 1-42 in Table 5 of WO2011/011799. Other preferred dyes are disclosed in US 8138222.

Suitable pigments include pigments selected from the group consisting of Ultramarine Blue (C.I. Pigment Blue 29), Ultramarine Violet (C.I. Pigment Violet 15) and mixtures thereof. Pigments and/or dyes may also be added to add colour for aesthetic reasons. Preferred are organic blue, violet and green pigments.

The wash liquor may comprise an enzyme stabilizer, preferably selected from the group consisting of (a) inorganic salts selected from the group consisting of calcium salts, magnesium salts and mixtures thereof; (b) carbohydrates selected from the group consisting of oligosaccharides, polysaccharides and mixtures thereof; (c) mass efficient reversible protease inhibitors selected from the group consisting of phenyl boronic acid and derivatives thereof; and (d) mixtures thereof.

In another embodiment, the wash liquor comprises: (1) reversible protease inhibitors such as a boron containing compound; (2) 1-2 propane diol; (3) calcium formate and/or sodium formate; and (4) any combination thereof.

Polymers - The wash liquor may comprise one or more polymers. Examples are carboxymethylcellulose, poly(vinyl-pyrrolidone), poly (ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid co-polymers and amphiphilic polymers.

Bleach - The wash liquor may contain a bleaching system, which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine or nonanoyloxybenzenesulfonate. Alternatively, the bleaching system may comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

Chelating Agents - The wash liquor may comprise a chelating agent. Suitable chelating agents include copper, iron and/or manganese chelating agents and mixtures thereof. When a chelating agent is used, the subject consumer product may comprise from about 0.005% to about 15% or even from about 3.0% to about 10% chelating agent by weight of the subject consumer product. Suitable chelants (complexing agents) include DTPA (Diethylene triamine pentaacetic acid), HEDP (Hydroxyethane diphosphonic acid), DTPMP (Diethylene triamine penta(methylene

phosphonic acid)), 1,2-Dihydroxybenzene-3,5-disulfonic acid disodium salt hydrate, ethylenediamine, diethylene triamine, ethylenediaminedisuccinic acid (EDDS), N-hydroxyethylethylenediaminetri-acetic acid (HEDTA), triethylenetetraaminehexaacetic acid (TTHA), N-hydroxyethyliminodiacetic acid (HEIDA), dihydroxyethylglycine (DHEG), ethylenediaminetetrapropionic acid (EDTP), methyl-glycine-diacetic acid (MGDA), glutamic-N,N- diacetic acid (GLDA), iminodisuccinic acid (IDS), carboxy methyl inulin; and salts derivatives thereof and mixtures thereof. Preferred chelants are selected from the group consisting of methyl-glycine-diacetic acid (MGDA), its salts and derivatives thereof, glutamic-N,N- diacetic acid (GLDA), its salts and derivatives thereof, iminodisuccinic acid (IDS), its salts and derivatives thereof, carboxy methyl inulin, its salts and derivatives thereof and mixtures thereof. Especially preferred complexing agent for use herein is selected from the group consisting of MGDA and salts thereof, especially preferred for use herein is the three-sodium salt of MGDA.

Enzyme stabilizer - The enzymes used in the present invention may be stabilized using conventional stabilizing agents, and/or protease inhibitors e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, salts such as sodium chloride and potassium chloride, lactic acid, formic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, or a peptide aldehyde such as di-, tri- or tetrapeptide aldehydes or aldehyde analogues (either of the form B1-B0-R wherein, R is H, CH3, CX3, CHX2, or CH2X (X=halogen), B0 is a single amino acid residue (preferably with an optionally substituted aliphatic or aromatic side chain); and B1 consists of one or more amino acid residues (preferably one, two or three), optionally comprising an N-terminal protection group, or as described in WO09118375, WO98/13459) or a protease inhibitor of the protein type such as RASI, BASI, WASI (bifunctional alpha-amylase/subtilisin inhibitors of rice, barley and wheat) or CI2 or SSI. In some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of zinc (II), calcium (II) and/or magnesium (II) ions in the finished compositions that provide such ions to the enzymes, as well as other metal ions (e.g., barium (II), scandium (II), iron (II), manganese (II), aluminum (III), Tin (II), cobalt (II), copper (II), Nickel (II), and oxovanadium (IV)).

Preferably in the wash liquor the endo-β-1,3-glucanase enzyme and other optional enzymes may be added in an amount corresponding to 0.001-100 mg of enzyme protein per liter of wash liquor, preferably 0.005-5 mg of enzyme protein per liter of wash liquor, more preferably 0.01-1 mg of enzyme protein per liter of wash liquor and in particular 0.1-1 mg of enzyme protein per liter of wash liquor.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Drying of cotton may be accomplished by any one of the common means employed either in domestic or industrial settings. The drying air may be heated from about 15°C to about 400°C, from about 25°C to about 200°C, from about 35°C to about 100°C, or even from about 40°C to about 85°C and used in the dryer to dry a surface and/or a fabric. The wash liquor preferably has a pH of from about 4 to about 10.5. The enzyme and optional cleaning adjuncts may be employed at total concentrations of from about 500 ppm to about 15,000 ppm in solution. The water temperatures typically range from about 5 °C to about 90 °C. The water to fabric ratio is typically from about 1:1 to about 30:1.

EXAMPLES

Example 1: Wash performance comprising an endo-β-1,3-glucanase

The wash performance of an endo-β-1,3-glucanase on cotton was determined in the context of a liquid laundry detergent as follows:

5cm x 5cm swatches of knitted cotton supplied by Warwick Equest Ltd, Consett, U.K. were contacted with an aqueous wash liquor comprising endo-β-1,3-glucanase using a Tergotometer Detergent Tester, supplied by Copley Scientific Ltd, Nottingham, U.K. Two pots were filled with 400mL tap water (6.5 grains per US gallon) heated to 40°C, and 1.04mL Ariel liquid (purchased from Asda Stores Ltd in the UK in February 2019, product code Product Code: 6016650) added to each pot.

The pots were then agitated and 0.4 mg active endo-β-1,3-glucanese enzyme (CZ0861, batch 18011, from NZYTech, Lisbon, Portugal) added via a pipette to one of the two pots resulting in a wash concentration of 1ppm active endo-β-1,3-glucanese. This enzyme is described by the supplier as belonging to Glycosyl Hydrolase family 16 and endogenous to *Paenibacillus* sp., and was confirmed by analysis to have a polypeptide sequence of greater than 98% identity to SEQ ID NO: 1. The temperature was maintained at 40°C for the duration of the test. After 30 minutes, the

wash water was drained, and the fabrics rinsed twice for 5 minutes in 400mL cold tap water (6.5 grains per US gallon), before placing the washed stain swatches flat on a rack to dry under ambient conditions.

Example 2: Wash performance of liquid detergent composition comparing endo-β-glucanase enzymes

The above procedure was repeated but using two stain swatches, each comprising five stains, image analysed for CIELab values (DigiEye, VeriVide, Leicester, U.K.) before testing. The tests were repeated using each of: 0.4 mg active endo-β-1,3-glucanese enzyme (CZ0861, batch 18011, from NZYTech, Lisbon, Portugal); 0.4mg active endo-β-1,3(4)-glucanase (E-LICACT from *Clostridium thermocellum*, batch 160201b from Megazyme, Bray Co. Wicklow, Ireland); and 0.4mg active endo-β-1,4-glucanase (Celluclean 5000L, batch CEN010785 from Novozymes A/S, Bagsvaerd, Denmark).

For each enzyme, the process was carried out four times, with rotation of the treatments between the two pots. This resulted in a total of 40 washed stains per treatment, i.e. 4 external replicates, each comprising 2 swatches of 5 stains. The washed stains were analysed for CIELab as before, and Stain Removal Index (SRI) calculated using the following equation:

$$SRI = \left(\frac{\Delta E_{pre-wash} - \Delta E_{post-wash}}{\Delta E_{pre-wash}}\right) x\ 100$$

Where

$$\Delta E_{pre-wash} = \sqrt{[(\mathrm{L}_{Clean} - \mathrm{L}_{Stained})^2 + (a_{Clean} - a_{Stained})^2 + (\mathrm{b}_{Clean} - \mathrm{b}_{Stained})^2]}$$

And

$$\Delta E_{post-wash} = \sqrt{[(\mathrm{L}_{Clean} - \mathrm{L}_{Washed})^2 + (a_{Clean} - a_{Washed})^2 + (\mathrm{b}_{Clean} - \mathrm{b}_{Washed})^2]}$$

Treatment	SRI	CI (95%)
Nil	57	3.4
Endo-β-1,3-Glucanase	74	2.0
Endo-β-1,3(4)-Glucanase	57	1.6
Endo-β-1,4-Glucanase	56	1.8

The results show that only the endo-β-1,3-glucanase in accordance with the invention significantly improves the removal of makeup stains from cotton, increasing removal from 57 to 74%. This improvement is highly significant and noticeable to the eye.

DETERGENT EXAMPLES

Addition of any of the following detergent examples to water is suitable for forming the aqueous wash liquor herein.

Examples 1-6. Granular laundry detergent compositions designed for hand washing or top-loading washing machines.

	1 (wt %)	2 (wt %)	3 (wt %)	4 (wt %)	5 (wt %)	6 (wt %)
Linear alkylbenzenesulfonate	20	22	20	15	20	20
C12-14 Dimethylhydroxyethyl ammonium chloride	0.7	0.2	1	0.6	0.0	0.0
AE3S	0.9	1	0.9	0.0	0.5	0.9
AE7	0.0	0.0	0.0	1	0.0	3
Sodium tripolyphosphate	5	0.0	4	9	2	0.0
Zeolite A	0.0	1	0.0	1	4	1
1.6R Silicate (SiO2:Na2O at rati 1.6:1)	7	5	2	3	3	5
Sodium carbonate	25	20	25	17	18	19
Polyacrylate MW 4500	1	0.6	1	1	1.5	1
Random graft copolymer[1]	0.1	0.2	0.0	0.0	0.0	0.0

Carboxymethyl cellulose	1	0.3	1	1	1	1
Protease (Savinase®, 32.89 m active/g)	0.1	0.1	0.1	0.1		0.1
[5]DNase as defined herein (mgs c active enzyme per 100g c detergent)	4.0	6.0	10.0	2.2	4.4	1.5
Lipase - Lipex® (18 mg active /g)	0.03	0.07	0.3	0.1	0.07	0.4
[4]Amylase Stainzyme® Plus (m active)	3.0	5.0	3.0	2.2	6.0	6.0
[6]Endo-ß-1,3-glucanase as define herein (mg active)	12.0	15.0	3.2	4.3	9.2	17.0
Fluorescent Brightener 1	0.06	0.0	0.06	0.18	0.06	0.06
Fluorescent Brightener 2	0.1	0.06	0.1	0.0	0.1	0.1
DTPA	0.6	0.8	0.6	0.25	0.6	0.6
MgSO4	1	1	1	0.5	1	1
Sodium Percarbonate	0.0	5.2	0.1	0.0	0.0	0.0
Sodium Perborate Monohydrate	4.4	0.0	3.85	2.09	0.78	3.63
NOBS	1.9	0.0	1.66	0.0	0.33	0.75
TAED	0.58	1.2	0.51	0.0	0.015	0.28
Sulphonated zinc phthalocyanine	0.0030	0.0	0.0012	0.0030	0.0021	0.0
S-ACMC	0.1	0.0	0.0	0.0	0.06	0.0

Direct Violet 9	0.0	0.0	0.0003	0.0005	0.0003	0.0
Acid Blue 29	0.0	0.0	0.0	0.0	0.0	0.0003
Sulfate/Moisture	Balance					

Examples 7-13. Granular laundry detergent compositions designed for front-loading automatic washing machines.

	7 (wt%)	8 (wt%)	9 (wt%)	10 (wt%)	11 (wt%)	12 (wt%)	13 (wt%)
Linear alkylbenzenesulfonate	8	7.1	7	6.5	7.5	7.5	11
AE3S	0	4.8	0	5.2	4	4	0
C12-14 Alkylsulfate	1	0	1	0	0	0	1
AE7	2.2	0	3.2	0	0	0	1
C10-12 Dimethyl hydroxyethylammonium chloride	0.75	0.94	0.98	0.98	0	0	0
Crystalline layered silicate (δ-Na2Si2O5)	4.1	0	4.8	0	0	0	7
Zeolite A	5	0	5	0	2	2	4
Citric Acid	3	5	3	4	2.5	3	0.5
Sodium Carbonate	15	20	14	20	23	23	14
Silicate 2R (SiO2:Na2O at ratio 2:1)	0.08	0	0.11	0	0	0	0.01
Soil release agent	0.75	0.72	0.71	0.72	0	0	0.1

Acrylic Acid/Maleic Acid Copolymer	1.1	3.7	1.0	3.7	2.6	3.8	2
Carboxymethylcellulose	0.15	1.4	0.2	1.4	1	0.5	0.2
Protease - Purafect® (84 mg active/g)	0.2	0.2	0.3	0.15	0.12	0.13	0.18
Lipase - Lipex® (18.00 mg active/g)	0.05	0.15	0.1	0	0	0	0.1
Cellulase - CellucleanTM (15.6 mg active/g)	0	0	0	0	0.1	0.1	0
[4]Amylase Stainzyme® Plus (mg active)	4.0	5.0	10	2.2	4.4	1.5	1.5
Mannanase - Mannaway® (4mg active/g)	0.05	0.1	0	0.05	0.1	0	0.1
[5]DNase as defined herein (mgs of active enzyme per 100g of detergent)	4.0	5.0	10.0	2.2	8.0	1.5	0.0
[6]Endo-ß-1,3-glucanase as defined herein (mg active)	3.3	9.2	12.0	4.7	3.7	13.2	3.3
TAED	3.6	4.0	3.6	4.0	2.2	1.4	1
Percarbonate	13	13.2	13	13.2	16	14	10
Na salt of Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer (EDDS)	0.2	0.2	0.001	0.2	0.2	0.2	0.001
Hydroxyethane di phosphonate (HEDP)	0.2	0.2	0.5	0.2	0.2	0.2	0.5

MgSO4	0.42	0.42	0.42	0.42	0.4	0.4	0
Perfume	0.5	0.6	0.5	0.6	0.6	0.6	0.8
Suds suppressor agglomerate	0.05	0.1	0.05	0.1	0.06	0.05	0.05
Soap	0.45	0.45	0.45	0.45	0	0	0
Sulphonated zinc phthalocyanine (active)	0.0007	0.0012	0.0007	0	0	0	0
S-ACMC	0.01	0.01	0	0.01	0	0	0
Direct Violet 9 (active)	0	0	0.0001	0.0001	0	0	0.001
Sulfate/ Water & Miscellaneous	Balance						

*DNase is shown as mgs of active enzyme per 100g of detergent.

Examples 14-21. Heavy Duty Liquid laundry detergent compositions

	14 (wt%)	15 (wt%)	16 (wt%)	17 (wt%)	18 (wt%)	19 (wt%)	20 (wt%)	21 (wt%)
C12-15 Alkylethoxy(1.8)sulfate	14.7	11.6	0.0	16.3	0.0	17.3	20	12
C11.8 Alkylbenzene sulfonate	4.3	11.6	8.3	7.8	11.7	7.8	7	0
C16-17 Branched alkyl sulfate	1.7	1.29	0.0	3.09	0.0	3.3	0	0
C12-14 Alkyl -9-ethoxylate	0.9	1.07	0.0	1.31	0.0	1.31	5	0
C12 dimethylamine oxide	0.6	0.64	0.0	1.03	0.0	1.03	2	3
Citric acid	3.5	0.65	3	0.66	2.27	0.67	1	0

C12-18 fatty acid	1.5	2.32	3.6	1.52	0.82	1.52	1	0
Sodium Borate (Borax)	2.5	2.46	1.2	2.53	0.0	2.53	0	1
Sodium C12-14 alkyl ethoxy 3 sulfate	0.0	0.0	2.9	0.0	3.9	0.0	0	14
C14-15 alkyl 7-ethoxylate	0.0	0.0	4.2	0.0	1.9	0.0	0	4.2
C12-14 Alkyl -7-ethoxylate	0.0	0.0	1.7	0.0	0.5	0.0	0	1.7
Ca chloride dihydrate	0.0	0.0	0.0	0.0	0.045	0.0	0	0
Ca formate	0.09	0.09	0.0	0.09	0.0	0.09	0.09	0
A compound: bis((C2H5O)(C2H4O)n)(CH3)-N+-CxH2x-N+-(CH3)-bis((C2H5O)(C2H4O)n); n is 20 to 30; x is 3 to 8, optionally sulphated or sulphonated	0.0	0.0	1.2	0.0	0.66	0.0	0.0	1.2
Random graft co-polymer[1]	0.0	1.46	0.5	0.0	0.83	0.0	0.0	0.5
Ethoxylated Polyethylenimine [2]	1.5	1.29	0.0	1.44	0.0	1.44	1.44	0.0
Diethylene triamine pentaacetic acid	0.34	0.64	0.0	0.34	0.0	0.34	0.34	0.0
Diethylene triamine penta (methylene phosphonic acid)	0.0	0.0	0.3	0.0	0.3	0.0	0.0	0.3
1-hydroxyethyidene-1,1-diphosphonic acid	0.0	0.0	0.0	0.0	0.18	0.0	0.0	0.0
Dihydroxybenzene-3,5-disulfonic acid disodium salt hydrate	0.0	0.0	0.0	0.0	0.0	0.19	0.19	0.0

Tinopal AMS-GX	0.0	0.06	0.0	0.0	0.0	0.29	0.29	0.0
Tinopal CBS-X	0.2	0.17	0.0	0.29	0.0	0.0	0.0	0.0
Tinopal TAS-X B36	0.0	0.0	0.0	0.0	0.091	0.0	0.0	0.0
Amphiphilic alkoxylated grease cleaning polymer [3]	1.28	1	0.4	1.93	0.0	1.93	1.93	0.4
CHEC	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2
Ethanol	2	1.58	1.6	5.4	1.2	3.57	0	1.6
Propylene Glycol	3.9	3.59	1.3	4.3	0.0	3.8	3.8	1.3
Diethylene glycol	1.05	1.54	0.0	1.15	0.0	1.15	1.15	0.0
Polyethylene glycol	0.06	0.04	0.0	0.1	0.0	0.1	0.1	0.0
[4]Amylase Amplify® (mg active)	8.0	7.0	2.5	4.0	3.0	1.7	3	2.5
[5]DNase (mgs of active enzyme per 100g of detergent)	7.0	3.0	2.5	4.0	1.25	10.0	3	2.5
[6]Endo-ß-1,3-glucanase as defined herein (mg active)	3.2	4.1	7.9	12.4	3.7	5.0	17.3	2.1
Monoethanolamine	3.05	2.41	0.4	1.26	0.31	1.13	1.13	0.4
NaOH	2.44	1.8	0.0	3.01	3.84	0.24	0.24	0.0
Sodium Cumene Sulphonate	0.0	0.0	1	0.0	0.95	0.0	0.0	1
Sodium Formate	0.0	0.11	0.0	0.09	0.2	0.12	0.12	0.0
Polyethoxylated azo thiophene dye	0.001	0.001	0.001	0.05	0.0001	0.0001	0.0001	0.001

Water, Aesthetics (Dyes, perfumes) and Minors (Enzymes including lipase, protease, additional amylase each at 0.2% active protein, solvents, structurants)	balance

Examples 22-28. Unit Dose Laundry detergent compositions. Such unit dose formulations can comprise one or multiple compartments.

	22 (wt%)	23 (wt%)	24 (wt%)	25 (wt%)	26 (wt%)	27 (wt%)	28 (wt%)
Alkylbenzene sulfonic acid	14.5	14.5	14.5	14.5	14.5	23	23
C12-18 alkyl ethoxy 2.5 sulfate	7.5	7.5	7.5	7.5	7.5	16	16
C12-18 alkyl 7-ethoxylate	13.0	13.0	13.0	13.0	13.0	3.1	3.8
C14-15 alkyl 9-ethoxylate	0	0	0	0	0	1	0
Citric Acid	0.6	0.6	0.6	0.6	0.6	0.9	0.7
Fatty Acid	14.8	14.8	14.8	14.8	14.8	6.5	6
Amylase (mg active)	6	12	8	2	10	2	2
Ethoxylated Polyethylenimine[2]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Protease (Purafect Prime®, 40.6 mg active/g)	1.4	2.0	0.9	1.2	0	1	1
Cellulase (Celluclean, active protein)	0.1	0.2	0.0	0.0	0.1	0	0

[5]DNase described herein (mgs of active enzyme per 100g of detergent	3.0	2.0	1.0	4.0	2.0	1	1
[4]Amylase Amplify® (active protein)	0.0	0.0	0.1	0.2	0.1	0.5	0.5
[6]Endo-ß-1,3-glucanase as defined herein (mg active)	2.2	3.1	2.3	5.2	5.3	12.2	5.4
Hydroxyethane diphosphonic acid	1.2	1.2	1.2	1.2	1.2	0	2.3
Brightener	0.3	0.3	0.3	0.3	0.3	0.2	0.2
P-diol	15.8	13.8	13.8	13.8	13.8	12.2	12.2
Glycerol	6.1	6.1	6.1	6.1	6.1	4.0	3.8
MEA	8.0	8.0	8.0	8.0	8.0	8.6	10.2
TIPA	0.0	0.0	2.0	0.0	0.0	0.0	0.0
TEA	0.0	2.0	0.0	0.0	0.0	0.0	0.0
Cumene sulphonate	0.0	0.0	0.0	0.0	2.0	0.0	0.0
Cyclohexyl dimethanol	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Water	10	10	10	10	10	10	10
Structurant	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Perfume	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Buffers (monoethanolamine)	To pH 8.0						

Solvents (1,2 propanediol, ethanol) & minors	To 100%

Example 29. Multiple Compartment Unit Dose Composition

Multiple compartment unit dose laundry detergent formulations of the present invention are provided below. In these examples the unit dose has three compartments, but similar compositions can be made with two, four or five compartments. The film used to encapsulate the compartments is polyvinyl alcohol.

Base composition 1	24 (wt%)
Glycerol (min 99)	5.3
1,2-propanediol	10.0
Citric Acid	0.5
Monoethanolamine	10.0
Caustic soda	-
Dequest 2010	1.1
Potassium sulfite	0.2
[5]DNase as defined herein (mg active)	8.0
[6]Endo-ß-1,3-glucanase as defined herein (mg active)	12.2
Nonionic Marlipal C24EO7	20.1

HLAS	24.6
Optical brightener FWA49	0.2
C12-15 Fatty acid	16.4
Polymer Lutensit Z96	2.9
Polyethyleneimine ethoxylate PEI600 E20	1.1
MgCl2	0.2
Solvents (1,2 propanediol, ethanol)	To 100%

Multi-compartment formulations

Composition	1			2		
Compartment	A	B	C	A	B	C
Volume of each compartment	40 ml	5 ml	5 ml	40 ml	5 ml	5 ml
Active material in Wt.%						
Perfume	1.6	1.6	1.6	1.6	1.6	1.6
Dyes	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
TiO2	0.1	-	-	-	0.1	-
Sodium Sulfite	0.4	0.4	0.4	0.3	0.3	0.3
Acusol 305	1.2			2	-	-
Hydrogenated castor oil	0.14	0.14	0.14	0.14	0.14	0.14

Base Composition 1	Add to 100%	Add to 100%	Add to 100%	Add to 100%	Add to 100%	Add to 100%

Example 30-33. Fabric softener compositions of the present invention

	Weight %			
	Ex. 30	Ex. 31	Ex. 32	Ex. 33
NaHEDP	0.007	0.007	0.007	0.007
Formic acid	0.044	0.044	0.044	-
HCl	-	0.009	0.009	0.009
Preservative[a]	0.022	0.01	0.01	0.01
FSA[b]	7.6	7.6	7.6	7.6
Antifoam[c]	0.1	0.1	0.1	0.1
coconut oil	0.3	0.3	0.3	0.3
sopropanol	0.78	0.78	0.77	0.77
Encapsulated perfume[d]	0.15	0.15	0.15	0.15
dye	0.015	0.015	0.015	0.015
Cationic polymeric thickener[e]	0.15	0.20	0.28	0.35
[5]DNase as defined herein (mgs of active enzyme per 100g of detergent)	6.0	2.0	1.0	0.5
[6]Endo-ß-1,3-glucanase as defined herein (mg active)	3.2	5.2	2.2	9.4

50:50 Blend of alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl ethylbenzyl ammonium chloride[f]	-	-	0.4	-
Succinic acid	-	-	-	5
Perfume	1.0	1.0	1.0	1.0
deionized water	Balance	Balance	Balance	Balance

Raw Materials and Notes For Composition Examples 1-33

Linear alkylbenzenesulfonate having an average aliphatic carbon chain length C11-C18

C12-18 Dimethylhydroxyethyl ammonium chloride

AE3S is C12-15 alkyl ethoxy (3) sulfate

AE7 is C12-15 alcohol ethoxylate, with an average degree of ethoxylation of 7

AE9 is C12-16 alcohol ethoxylate, with an average degree of ethoxylation of 9

HSAS is a mid-branched primary alkyl sulfate with carbon chain length of about 16-17 as disclosed in US 6,020,303 and US 6,060,443

Polyacrylate MW 4500 is supplied by BASF

Carboxymethyl cellulose is Finnfix® V supplied by CP Kelco, Arnhem, Netherlands

CHEC is a cationically modified hydroxyethyl cellulose polymer.

Phosphonate chelants are, for example, diethylenetetraamine pentaacetic acid (DTPA) Hydroxyethane di phosphonate (HEDP)

Savinase®, Natalase®, Stainzyme®, Lipex®, CellucleanTM, Mannaway® and Whitezyme® are all products of Novozymes, Bagsvaerd, Denmark.

Purafect®, Purafect Prime® are products of Genencor International, Palo Alto, California, USA

Fluorescent Brightener 1 is Tinopal® AMS, Fluorescent Brightener 2 is Tinopal® CBS-X, Direct Violet 9 is Pergasol® Violet BN-Z NOBS is sodium nonanoyloxybenzenesulfonate

TAED is tetraacetylethylenediamine

S-ACMC is carboxymethylcellulose conjugated with C.I. Reactive Blue 19product name AZO-CM-CELLULOSE

Soil release agent is Repel-o-tex® PF

Acrylic Acid/Maleic Acid Copolymer is molecular weight 70,000 and acrylate:maleate ratio 70:30

EDDS is a sodium salt of ethylenediamine-N,N'-disuccinic acid, (S,S) isomer Suds suppressor agglomerate is supplied by Dow Corning, Midland, Michigan, USA

HSAS is mid-branched alkyl sulfate

Liquitint® Violet CT polymeric hueing dye, supplied by Milliken, Spartanburg, South Carolina, USA

Polyethoxylated azo thiophene dye is Violet DD™ polymeric hueing dye, supplied by Milliken, Spartanburg, South Carolina, USA.

[1] Random graft copolymer is a polyvinyl acetate grafted polyethylene oxide copolymer having a polyethylene oxide backbone and multiple polyvinyl acetate side chains. The molecular weight of the polyethylene oxide backbone is about 6000 and the weight ratio of the polyethylene oxide to polyvinyl acetate is about 40 to 60 and no more than 1 grafting point per 50 ethylene oxide units.

[2] Polyethyleneimine (MW = 600) with 20 ethoxylate groups per -NH.

[3] Amphiphilic alkoxylated polymer is a polyethylenimine (MW 600), prepared from a polymer that is derivatised to contain 24 ethoxylate groups per –NH and 16 Propoxylate groups per –NH.

[4]Amylase is shown as mgs of active enzyme per 100g of detergent.

[5]DNase in all of these examples is shown as mgs of active enzyme per 100g of detergent optionally in admixture with hexosaminidase enzyme. DNase may contain minor amounts of super oxide dismutase.

[66]Endo-ß-1,3-glucanase in all of these examples is shown as mgs of active enzyme per 100g of detergent

[a] Proxel GXL, 20% aqueous dipropylene glycol solution of 1,2-benzisothiazolin-3-one, supplied by Lonza.

[b] N,N-bis(hydroxyethyl)-N,N-dimethyl ammonium chloride fatty acid ester. The iodine value of the parent fatty acid of this material is between 18 and 22. The material as obtained from Evonik contains impurities in the form of free fatty acid, the monoester form of N,N-bis(hydroxyethyl)-N,N-dimethyl ammonium chloride fatty acid ester, and fatty acid esters of N,N-bis(hydroxyethyl)-N-methylamine.

[c] MP10®, supplied by Dow Corning, 8% activity

[d] as described in US 8,765,659, expressed as 100% encapsulated perfume oil

[e] Rheovis® CDE, cationic polymeric thickener supplied by BASF

[f] N,N-dimethyl octanamide and N,N-dimethyl decanamide in about a 55:45 weight ratio, tradename Steposol® M-8-10 from the Stepan Company

The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “40 mm” is intended to mean “about 40 mm”.

CLAIMS

What is claimed is:

1. A method of treating cotton comprising: (i) contacting cotton with an aqueous wash liquor comprising water and an endo-β-1,3-glucanase enzyme having at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, sequence identity to one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7; (ii) optionally rinsing the cotton; and (iii) drying the cotton.
2. A method according to claim 1 wherein the cotton is in the form of a mixed fabric comprising cotton and one or more additional materials, preferably polyester.
3. A method according to any preceding claim wherein the cotton is contacted with the aqueous wash liquor at a temperature of 60°C or less, or more preferably at a temperature of 40°C or 35°C or less, most preferably at a temperature of 30°C or less.
4. A method according to any preceding claim wherein the endo-β-1,3-glucanase enzyme is from E.C. class 3.2.1.39.
5. A method according to any preceding claim wherein the endo-β-1,3-glucanase enzyme is of microbial origin, preferably bacterial.
6. A method according to ay preceding claim wherein the endo-β-1,3-glucanase enzyme is obtainable from *Paenibacillus sp, Zobellia galactanivorans, Thermotoga petrophila* micro-organism, preferably *Paenibacillus sp or Zobellia galactanivorans, Trichoderma sp.,* most preferably *Paenibacillus sp.*
7. A method according to any preceding claim wherein the endo-β-1,3-glucanase enzyme is from glycosyl hydrolase (GH) family 16 or 64, preferably GH family 16.
8. A method according to any preceding claim wherein the endo-β-1,3-glucanase enzyme has a carbohydrate binding module CBM 6 or CBM 56.
9. A method according to any preceding claim wherein the endo-β-1,3-glucanase enzyme has at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, sequence identity to one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.
10. A method according to any preceding claim wherein the aqueous wash liquor comprises a

surfactant, preferably in an amount from 0.0001g/l to 10g/l aqueous wash liquor.

11. A method according to any claim 11 wherein the surfactant comprises nonionic and/or anionic surfactant, preferably both anionic and nonionic surfactant in a weight ratio of anionic to nonionic of from 30:1 to 2:3, preferably from 20:1 to 1:1.
12. A method according to any preceding claim wherein the aqueous wash liquor comprises additional enzyme selected from pectate lyase, cellulase, mannanase, xanthan lyase, xanthanase and mixtures thereof.
13. A method according to any preceding claim wherein the aqueous wash liquor comprises fabric shading dye, optical brightener or mixtures thereof.
14. A method according to any preceding claim for removal of callose from cotton.
15. Use of an endo-β-1,3-glucanase enzyme having at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, sequence identity to one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7 for manufacture of a cotton-containing fabric having improved soil repellency, reduced malodour, anti-wrinkle benefits, and/or improved drying.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/022383

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/24 C11D1/00 C11D1/83 C11D3/386
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C11D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95/31533 A1 (NOVO NORDISK AS [DK]; KOFOD LENE VENKE [DK] ET AL.) 23 November 1995 (1995-11-23) page 17, line 18 - line 20; claims 1, 24, 25, 35	1-15
Y	WO 2005/003319 A2 (DIVERSA CORP [US]; STEER BRIAN [US] ET AL.) 13 January 2005 (2005-01-13) page 18, line 12 - line 21 page 191, line 4 - page 197, line 10; sequence 152	1-15
Y	WO 92/06210 A1 (GENENCOR INT [US]) 16 April 1992 (1992-04-16) page 2, line 22; claim 21; example 3	1-15

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 April 2020	13/05/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Offermann, Stefanie

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/022383

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International application No
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