

(51) International Patent Classification:
C12N 9/24 (2006.01)(21) International Application Number:
PCT/US2013/075829(22) International Filing Date:
17 December 2013 (17.12.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/739,267 19 December 2012 (19.12.2012) US(71) Applicant: **DANISCO US INC.** [US/US]; 925 Page Mill Road, Palo Alto, California 94304 (US).(72) Inventors: **BARNETT, Christopher C.**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US). **DEMASO, Connie**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US). **DODGE, Eric**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US). **HARRIS, Kathleen A.**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US). **QI, Rachel B.**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US).(74) Agent: **KONG, Lawrence B.**; Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: NOVEL MANNANASE, COMPOSITIONS AND METHODS OF USE THEREOF

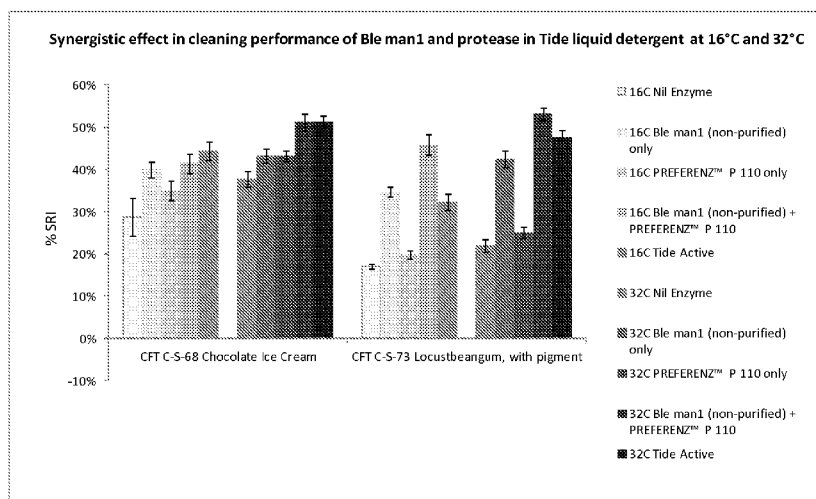


Figure 6-1. Cleaning performance of Bleman1 in the presence of protease.

(57) Abstract: The present compositions and methods relate to an endo-beta-mannanase cloned from *Bacillus lentus*, polynucleotides encoding the endo-beta-mannanase, and methods of use thereof. Formulations containing the endo-beta-mannanase are highly suitable for use as detergents.

NOVEL MANNANASE, COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 [001] This application claims benefit of priority from U.S. provisional patent application no. USSN 61/739,267, filed on 19 December 2012, and is incorporated herein by reference in its entirety.

BACKGROUND

10 [002] Current laundry detergent and fabric care compositions include a complex combination of active ingredients such as surfactants, enzymes (e.g., protease, amylase, lipase, mannanase, and/or cellulase), bleaching agents, a builder system, suds suppressors, soil-suspending agents, soil-release agents, optical brighteners, softening agents, dispersants, dye transfer inhibition compounds, abrasives, bactericides, and perfumes.

15 [003] Mannanase enzymes have been employed in detergent cleaning compositions for the removal of gum stains by hydrolyzing mannans. However, enzymes are often inhibited by surfactants and other components present in cleaning compositions, which interferes with their ability to remove stains. For instance, proteases in laundry detergents may degrade mannases before the removal of a gum stain. In addition, mannases may have a limited pH and/or temperature range at which they are active, which may make them unsuitable for certain formulations and washing conditions. Accordingly, the need exists
20 for mannases that retain activity in the harsh environment of cleaning compositions.

SUMMARY

- [004] The present compositions and methods relate to a mannanase cloned from *Bacillus lentus* (Bleman1). Formulations containing the endo- β -mannanase are highly suitable for use as detergents.
- 25 [005] In one embodiment, the invention is a recombinant polypeptide comprising a catalytic domain of an endo- β -mannanase, wherein the catalytic domain is at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention is a recombinant polypeptide comprising a mature form of an endo- β -mannanase, wherein the mature form is at least 60%,
30 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:2. In each embodiment, the polypeptide of the invention has mannanase activity. In some embodiments, the polypeptide of the invention has mannanase activity on an AZO-Carob Galactomannan or Locust Bean Gum substrate.
- [006] In some embodiments, the polypeptide of the invention has mannanase activity at pH values
35 of between 5 and 11, or between 7 and 11, or between 7 and 9. In some embodiments, the polypeptide of the invention retains greater than 50% mannanase activity at pH values of between 5 and 11, or between

7 and 11, or between 7 and 9, or between 7 and 8. In some embodiments, the polypeptide of the invention retains greater than 75% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8. In some embodiments, the polypeptide of the invention has mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C.

[007] In some embodiments, the polypeptide of the invention has measurable mannanase activity in the presence of detergent. In some embodiments, the polypeptide has measurable mannanase activity in the presence of a protease. In some embodiments, the polypeptide retains greater than 50% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8 in the presence of protease. In some embodiments, the polypeptide of the invention retains greater than 75% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8 in the presence of protease. In some embodiments, the polypeptide has mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C in the presence of protease. In some embodiments, the polypeptide retains at least 50% mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C in the presence of protease. In some embodiments, the polypeptide is capable of hydrolyzing a substrate selected from the group consisting of chocolate ice cream, chocolate pudding, guar gum, locust bean gum, and combinations thereof. In some embodiments, the polypeptide further comprises a native or non-native signal peptide. In some embodiments, the polypeptide further comprises at least one carbohydrate-binding module. In other embodiments, the polypeptide does not comprise a carbohydrate-binding module.

[008] In some embodiments, the invention is a detergent composition having at least one recombinant polypeptide as described above. In some embodiments, the invention is a detergent composition having a polypeptide as described above, wherein the polypeptide is active at a temperature between 10°C and 60°C, or 20°C and 60°C, or 30°C and 60°C, or 40°C and 60°C, or 50°C and 60°C. In some embodiment of the invention, the mannanase polypeptide is active at 16°C or 32°C. In some embodiment of the invention, the mannanase polypeptide has mannanase activity in the presence of a protease. In some embodiments, the mannanase polypeptide retains activity in the presence of the bleaching agent. In some embodiments of the invention, the detergent composition having a mannanase polypeptide further comprises a bleaching agent. In some embodiment of the invention, the mannanase polypeptide has mannanase activity in the presence of a protease and an amylase. In some embodiments of the invention, the detergent composition having a mannanase polypeptide further comprises a protease and an amylase.

[009] In some embodiments, the composition further comprises a surfactant. In some embodiments, the surfactant is selected from the group consisting of sodium dodecylbenzene sulfonate, sodium hydrogenated cocoate, sodium laureth sulfate, C12-14 pareth-7, C12-15 pareth-7, sodium C12-15 pareth sulfate, C14-15 pareth-4, and combinations thereof. In some embodiments, the surfactant is an

ionic surfactant. In some embodiments, the ionic surfactant is selected from the group consisting of an anionic surfactant, a cationic surfactant, a zwitterionic surfactant, and a combination thereof.

[0010] In some embodiments, the composition further comprises an enzyme selected from the group consisting of proteases, proteases, peroxidases, cellulases, beta-glucanases, hemicellulases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo- β -mannanases, exo- β -mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof. In some embodiments, the combination comprises a protease and an amylase. In some embodiments, the detergent is selected from the group consisting of a laundry detergent, a fabric softening detergent, a dishwashing detergent, and a hard-surface cleaning detergent. In some embodiments, the detergent is in a form selected from the group consisting of a liquid, a powder, a granulated solid, and a tablet. In addition the present disclosure provides methods for hydrolyzing a mannan substrate present in a soil or stain on a surface, comprising: contacting the surface with the detergent composition to produce a clean surface. Also provided are methods of textile cleaning comprising: contacting a soiled textile with the detergent composition to produce a clean textile.

[0011] Moreover, the present disclosure provides isolated nucleic acids encoding the recombinant polypeptide of the preceding paragraphs. Also provided are expression vectors having the isolated nucleic acid in operable combination to a regulatory sequence. Additionally, host cells comprising the expression vector are provided. In some embodiments, the host cell is a bacterial cell or a fungal cell. The present disclosure further provides methods of producing an endo- β -mannanase, comprising: culturing the host cell in a culture medium, under suitable conditions to produce a culture comprising the endo- β -mannanase. In some embodiments, the methods further comprise removing the host cells from the culture by centrifugation, and removing debris of less than 10 kDa by filtration to produce an endo- β -mannanase-enriched supernatant. The present disclosure further provides methods for hydrolyzing a polysaccharide, comprising: contacting a polysaccharide comprising mannose with the supernatant to produce oligosaccharides comprising mannose. In some embodiments, the polysaccharide is selected from the group consisting of mannan, glucomannan, galactomannan, galactoglucomannan, and combinations thereof.

[0012] These and other aspects of Bleman1 compositions and methods will be apparent from the following description.

DESCRIPTION OF THE DRAWINGS

[0013] Figure 1-1 shows mannanase activity of Bleman1 on the substrate AZO-Carob Galactomannan.

[0014] Figure 1-2 shows mannanase activity of Bleman1 on the substrate Locust Bean Gum.

[0015] Figure 2-1 provides a pH profile of Bleman1.

[0016] Figure 3-1 provides a temperature profile of Bleman1.

[0017] Figure 4-1 shows the cleaning performance of Bleman1 in liquid laundry detergent on Locust Bean Gum and ASDA Chocolate Ice Cream stains in a dose response curve. The dose response for the cleaning performance of Bleman1 is based on the mannanase activity measured in Tide® HE Active. The bars from left to right on the chart for each corresponding mannanase stain, the following conditions were tested: Nil Enzyme (heat inactivated) Tide® HE, Bleman1 dosed at 0.5X the activity measured in commercially available Tide® HE, Bleman1 dosed at 1X the activity measured in commercially available Tide® HE, Bleman1 dosed at 2X the activity measured in commercially available Tide® HE, and commercially available Tide® HE.

[0018] Figure 5-1 shows the cleaning performance of Bleman1 in liquid laundry detergent at different temperatures. For each stain, the first set of 3 bars from left to right on the chart show the following test conditions at 16°C: Nil Enzyme (heat inactivated) Tide® HE, Bleman1 dosed at 1X the activity measured in commercially available Tide® HE, and commercially available Tide® HE. The second set of 3 bars from left to right show the same conditions at 32°C.

[0019] Figure 6-1 shows the cleaning performance of Bleman1 in liquid laundry detergent at different temperatures while in the presence of a protease. For each stain, the first set of 5 bars from left to right on the chart show the following test conditions at 16°C: Nil Enzyme (heat inactivated) Tide® HE, Bleman1 dosed at 1X the activity measured in commercially available Tide® HE, PREFERENZ® P 110 dosed at 1X the activity measured in commercially available Tide® HE, both Bleman1 and PREFERENZ® P 110 dosed at 1X the activity measured in commercially available Tide® HE, and commercially available Tide® HE. The second set of 5 bars from left to right show the same conditions at 32°C.

[0020] Figure 7-1 shows the cleaning performance of purified Bleman1 in liquid laundry detergent at different temperatures. The bar chart shows the benefit of Bleman1 wash performance in heat inactivated Tide liquid detergent at 16C and 32C. For each stain, the first set of 3 bars from left to right on the chart show the following test conditions at 16C: Nil Enzyme (heat inactivated) Tide(R) HE, Bleman1 dosed at 1X the activity measured in commercially available Tide(R) HE, and commercially available Tide(R) HE. The second set of 3 bars from left to right show the same conditions at 32C. The mannanase was dosed based on equal active levels of the activity measured in commercially available Tide(R) HE liquid detergent. The mannanase was dosed at 0.11 wt% of the 0.72 g/L dose of heat inactivated Tide(R) HE. At both 16C and 32C, the addition of Bleman1 alone provides a cleaning benefit over nil enzyme liquid detergent and at 16C provides roughly the same amount of cleaning on mannanase stains as the Tide(R) HE active benchmark.

[0021] Figure 8-1 shows the cleaning performance of purified Bleman1 in liquid laundry detergent at different temperatures while in the presence of a protease. The bar chart shows complex technical stains that show better wash performance of purified Bleman1 in the presence of protease. For each stain, the first set of 5 bars from left to right on the chart show the following test conditions at 16C: Nil Enzyme (heat inactivated) Tide(R) HE, purified Bleman1 dosed at 1X the activity measured in commercially available Tide(R) HE, PREFERENZ® P 110 dosed at 1X the activity measured in commercially available Tide(R) HE, both purified Bleman1 and PREFERENZ® P 110 dosed at 1X the activity measured in commercially available Tide(R) HE, and commercially available Tide(R) HE. The second set of 5 bars from left to right show the same conditions at 32C. The mannanase and protease were dosed based on equal active levels of the activity measured in commercially available Tide(R) HE liquid detergent. The mannanase was dosed at 0.2 wt% and the protease was dosed at 0.4 wt% of the 0.72 g/L dose of heat inactivated Tide(R) HE. The synergistic effects of the mannanase/protease combination are seen on the various stains. Overall, purified mannanase provides the same cleaning benefit as the non-purified Bleman1 sample.

[0022] Figure 9-1 shows the cleaning performance of purified Bleman1 in powder laundry detergent at different temperatures alone or in the presence of a protease and amylase and/or a bleaching agent. The first set of 4 bars for each stain were the following conditions run with bleach: Nil enzyme powder detergent, with 0.2wt% dose of Bleman1 only, with 2.0 wt% dose of SMARTENZ™ 1050 only, and 0.2 wt% Bleman1 + 2.0 wt% SMARTENZ™ 1050. The second set of 4 bars for each stain is the same as prior conditions but without bleach.

DETAILED DESCRIPTION

[0023] The present invention provides improved mannanase enzymes, especially mannanase enzymes useful for detergent compositions. Described are compositions and methods relating to endo- β -mannanase1 cloned from a *Bacillus circulans* or *Bacillus lentus* strain CMG1240 (Bleman1; see U.S. Patent No. 5,476,775). Both *Bacillus circulans* and *Bacillus lentus* strains are known to be highly related to each other. The compositions and methods are based, in part, on the observation that recombinant Bleman1 has glycosyl hydrolase activity in the presence of detergent compositions. This feature of Bleman1 makes it well suited for use in a variety of cleaning applications, where the enzyme can hydrolyze mannans in the presence of surfactants and other components found in detergent compositions. The invention includes compositions comprising a mannanase enzyme as set forth herein. Some such compositions comprise detergent compositions. The mannanase enzyme of the present invention can be combined with other enzymes useful in detergent compositions, such as protease and/or amylase enzymes. The mannanase enzyme of the present invention can be combined with bleaching agents useful in detergent compositions. The invention also provides enzyme compositions having comparable or

improved wash performance, as compared to known mannanase enzymes, such as, known endo- β -mannanase enzymes. The invention also provides methods of cleaning using a mannanase enzyme of the present invention.

[0024] Prior to describing the present compositions and methods in detail, the following terms are defined for clarity. Terms and abbreviations not defined should be accorded their ordinary meaning as used in the art:

[0025] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although many methods and materials similar or equivalent to those described herein find use in the practice of the present invention, some methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the specification as a whole. All patents, patent applications, articles and publications mentioned herein, both *supra* and *infra*, are hereby expressly incorporated herein by reference.

[0026] Also, as used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context in which they are used by those of skill in the art.

[0027] It is intended that every maximum numerical limitation given throughout this specification include every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0028] As used herein, a "mannan endo-1,4- β -mannosidase," "endo-1,4- β -mannanase," "endo- β -1,4-mannase," " β -mannanase B," " β -1, 4-mannan 4-mannanohydrolase," "endo- β -mannanase," " β -D-mannanase," "1,4- β -D-mannan mannanohydrolase," or "endo- β -mannanase" (EC 3.2.1.78) refers to an enzyme capable of the random hydrolysis of 1,4- β -D-mannosidic linkages in mannans, galactomannans and glucomannans. Endo-1,4- β -mannanases are members of several families of glycosyl hydrolases, including GH26 and GH5. In particular, endo- β -mannanases constitute a group of polysaccharases that degrade mannans and denote enzymes that are capable of cleaving polyose chains containing mannose units (*i.e.*, are capable of cleaving glycosidic bonds in mannans, glucomannans, galactomannans and galactogluco-mannans). The "endo- β -mannanases" of the present disclosure may possess additional enzymatic activities (*e.g.*, endo-1,4- β -glucanase, 1,4- β -mannosidase, cellodextrinase activities, etc.).

[0029] As used herein, a “mannanase,” “mannosidic enzyme,” “mannolytic enzyme,” “mannanase enzyme,” “mannanase polypeptides,” or “mannanase proteins” refers to an enzyme, polypeptide, or protein exhibiting a mannan degrading capability. The mannanase enzyme may be, for example, an endo- β -mannanase, an exo- β -mannanase, or a glycosyl hydrolase. As used herein, mannanase activity may be determined according to any procedure known in the art (*See, e.g.,* Lever, *Anal. Biochem.*, 47:248, 1972; U.S. Pat. No. 6, 602, 842; and International Publication No. WO 95/35362A1).

[0030] As used herein, “mannans” are polysaccharides having a backbone composed of β -1,4-linked mannose; “glucomannans” are polysaccharides having a backbone of more or less regularly alternating β -1,4 linked mannose and glucose; “galactomannans” and “galactoglucomannans” are mannans and glucomannans with α -1,6 linked galactose sidebranches. These compounds may be acetylated. The degradation of galactomannans and galactoglucomannans is facilitated by full or partial removal of the galactose sidebranches. Further the degradation of the acetylated mannans, glucomannans, galactomannans and galactoglucomannans is facilitated by full or partial deacetylation. Acetyl groups can be removed by alkali or by mannan acetyl esterases. The oligomers that are released from the mannans or by a combination of mannanases and α -galactosidase and/or mannan acetyl esterases can be further degraded to release free maltose by β -mannosidase and/or β -glucosidase

[0031] As used herein, “catalytic activity” or “activity” describes quantitatively the conversion of a given substrate under defined reaction conditions. The term “residual activity” is defined as the ratio of the catalytic activity of the enzyme under a certain set of conditions to the catalytic activity under a different set of conditions. The term “specific activity” describes quantitatively the catalytic activity per amount of enzyme under defined reaction conditions.

[0032] As used herein, “pH-stability” describes the property of a protein to withstand a limited exposure to pH-values significantly deviating from the pH where its stability is optimal (*e.g.,* more than one pH-unit above or below the pH-optimum, without losing its activity under conditions where its activity is measurable).

[0033] As used herein, the phrase “detergent stability” refers to the stability of a specified detergent composition component (such as a hydrolytic enzyme) in a detergent composition mixture.

[0034] As used herein, a “perhydrolase” is an enzyme capable of catalyzing a reaction that results in the formation of a peracid suitable for applications such as cleaning, bleaching, and disinfecting.

[0035] As used herein, the term “aqueous,” as used in the phrases “aqueous composition” and “aqueous environment,” refers to a composition that is made up of at least 50% water. An aqueous composition may contain at least 50% water, at least 60% water, at least 70% water, at least 80% water, at least 90% water, at least 95% water, at least 97% water, at least 99% water, or even at least 99% water.

[0036] As used herein, the term “surfactant” refers to any compound generally recognized in the art as having surface active qualities. Surfactants generally include anionic, cationic, nonionic, and zwitterionic compounds, which are further described, herein.

[0037] As used herein, “surface property” is used in reference to electrostatic charge, as well as properties such as the hydrophobicity and hydrophilicity exhibited by the surface of a protein.

[0038] The term “oxidation stability” refers to endo- β -mannanases of the present disclosure that retain a specified amount of enzymatic activity over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example while exposed to or contacted with bleaching agents or oxidizing agents. In some embodiments, the endo- β -mannanases retain at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- β -mannanase activity after contact with a bleaching or oxidizing agent over a given time period, for example, at least about 1 minute, about 3 minutes, about 5 minutes, about 8 minutes, about 12 minutes, about 16 minutes, about 20 minutes, etc.

[0039] The term “chelator stability” refers to endo- β -mannanases of the present disclosure that retain a specified amount of enzymatic activity over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example while exposed to or contacted with chelating agents. In some embodiments, the endo- β -mannanases retain at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- β -mannanase activity after contact with a chelating agent over a given time period, for example, at least about 10 minutes, about 20 minutes, about 40 minutes, about 60 minutes, about 100 minutes, etc.

[0040] The terms “thermal stability” and “thermostable” refer to endo- β -mannanases of the present disclosure that retain a specified amount of enzymatic activity after exposure to identified temperatures over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example, while exposed to altered temperatures. Altered temperatures include increased or decreased temperatures. In some embodiments, the endo- β -mannanases retain at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- β -mannanase activity after exposure to altered temperatures over a given time period, for example, at least about 60 minutes, about 120 minutes, about 180 minutes, about 240 minutes, about 300 minutes, etc.

[0041] The term “cleaning activity” refers to the cleaning performance achieved by the endo- β -mannanase under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein. In some embodiments, cleaning performance is determined by the application of various cleaning assays concerning enzyme sensitive stains, for example ice cream, ketchup, BBQ sauce, mayonnaise, chocolate milk, body lotion, locust bean gum, or guar gum as determined by various chromatographic, spectrophotometric or other quantitative methodologies after subsection of the stains to standard wash conditions. Exemplary assays include, but are not limited to those described in WO 99/34011, U.S. Pat. No. 6,605,458, and U.S. Pat. No. 6,566,114 (all of which are herein incorporated by reference), as well as those methods included in the Examples.

[0042] As used herein, the terms “clean surface” and “clean textile” refer to a surface or textile respectively that has a percent stain removal of at least 10%, preferably at least 15%, 20%, 25%, 30%, 35%, or 40% of a soiled surface or textile.

[0043] The term “cleaning effective amount” of an endo- β -mannanase refers to the quantity of endo- β -mannanase described hereinbefore that achieves a desired level of enzymatic activity in a specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular endo- β -mannanase used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (*e.g.*, granular, bar) composition is required, etc.

[0044] The term “cleaning adjunct materials,” as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (*e.g.*, liquid, granule, powder, bar, paste, spray, tablet, gel, or foam composition), which materials are also preferably compatible with the endo- β -mannanase enzyme used in the composition. In some embodiments, granular compositions are in “compact” form, while in other embodiments, the liquid compositions are in a “concentrated” form.

[0045] As used herein, “cleaning compositions” and “cleaning formulations” refer to admixtures of chemical ingredients that find use in the removal of undesired compounds (*e.g.*, soil or stains) from items to be cleaned, such as fabric, dishes, contact lenses, other solid surfaces, hair, skin, teeth, and the like. The composition or formulations may be in the form of a liquid, gel, granule, powder, or spray, depending on the surface, item or fabric to be cleaned, and the desired form of the composition or formulation.

[0046] As used herein, the terms “detergent composition” and “detergent formulation” refer to mixtures of chemical ingredients intended for use in a wash medium for the cleaning of soiled objects. Detergent compositions/formulations generally include at least one surfactant, and may optionally include hydrolytic enzymes, oxido-reductases, builders, bleaching agents, bleach activators, bluing agents and fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and solubilizers.

[0047] As used herein, “dishwashing composition” refers to all forms of compositions for cleaning dishware, including cutlery, including but not limited to granular and liquid forms. In some embodiments, the dishwashing composition is an “automatic dishwashing” composition that finds use in automatic dish washing machines. It is not intended that the present disclosure be limited to any particular type or dishware composition. Indeed, the present disclosure finds use in cleaning dishware (*e.g.*, dishes including, but not limited to plates, cups, glasses, bowls, etc.) and cutlery (*e.g.*, utensils including, but not limited to spoons, knives, forks, serving utensils, etc.) of any material, including but not limited to ceramics, plastics, metals, china, glass, acrylics, etc. The term “dishware” is used herein in reference to both dishes and cutlery.

[0048] As used herein, the term “bleaching” refers to the treatment of a material (*e.g.*, fabric, laundry, pulp, etc.) or surface for a sufficient length of time and under appropriate pH and temperature conditions to effect a brightening (*i.e.*, whitening) and/or cleaning of the material. Examples of chemicals suitable for bleaching include but are not limited to ClO₂, H₂O₂, peracids, NO₂, etc.

5 [0049] As used herein, “wash performance” of a variant endo-β-mannanase refers to the contribution of a variant endo-β-mannanase to washing that provides additional cleaning performance to the detergent without the addition of the variant endo-β-mannanase to the composition. Wash performance is compared under relevant washing conditions.

10 [0050] The term “relevant washing conditions” is used herein to indicate the conditions, particularly washing temperature, time, washing mechanics, sud concentration, type of detergent, and water hardness, actually used in households in a dish or laundry detergent market segment.

[0051] As used herein, the term “disinfecting” refers to the removal of contaminants from the surfaces, as well as the inhibition or killing of microbes on the surfaces of items. It is not intended that the present disclosure be limited to any particular surface, item, or contaminant(s) or microbes to be removed.

15 [0052] The “compact” form of the cleaning compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt. Inorganic filler salts are conventional ingredients of detergent compositions in powder form. In conventional detergent compositions, the filler salts are present in substantial amounts, typically about 17 to about 35% by weight of the total composition. In contrast, in compact compositions, the filler salt is present in amounts not exceeding about 15% of the total composition. In some embodiments, the filler salt is present in amounts that do not exceed about 10%, or more preferably, about 5%, by weight of the composition. In some embodiments, the inorganic filler salts are selected from the alkali and alkaline-earth-metal salts of sulfates and chlorides. In some embodiments, a preferred filler salt is sodium sulfate.

25 [0053] As used herein, the terms “textile” or “textile material” refer to woven fabrics, as well as staple fibers and filaments suitable for conversion to or use as yarns, woven, knit, and non-woven fabrics. The term encompasses yarns made from natural, as well as synthetic (*e.g.*, manufactured) fibers.

[0054] As used herein, the terms “purified” and “isolated” refer to the physical separation of a subject molecule, such as Bleman1, from its native source (*e.g.*, *Bacillus agaradhaerens*) or other molecules, such as proteins, nucleic acids, lipids, media components, and the like. Once purified or isolated, a subject molecule may represent at least 50%, and even at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or more, of the total amount of material in a sample (wt/wt).

30 [0055] As used herein, a “polypeptide” refers to a molecule comprising a plurality of amino acids linked through peptide bonds. The terms “polypeptide,” “peptide,” and “protein” are used interchangeably. Proteins may optionally be modified (*e.g.*, glycosylated, phosphorylated, acylated, farnesylated, prenylated, sulfonated, and the like) to add functionality. Where such amino acid sequences exhibit activity, they may be referred to as an “enzyme.” The conventional one-letter or three-letter

codes for amino acid residues are used, with amino acid sequences being presented in the standard amino-to-carboxy terminal orientation (*i.e.*, N→C).

[0056] The terms “polynucleotide” encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a polypeptide. Nucleic acids may be single-stranded or double-stranded, and may have chemical modifications. The terms “nucleic acid” and “polynucleotide” are used interchangeably. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present compositions and methods encompass nucleotide sequences which encode a particular amino acid sequence. Unless otherwise indicated, nucleic acid sequences are presented in a 5' -to-3' orientation.

[0057] As used herein, the terms “wild-type” and “native” refer to polypeptides or polynucleotides that are found in nature.

[0058] The terms, “wild-type,” “parental,” or “reference,” with respect to a polypeptide, refer to a naturally-occurring polypeptide that does not include a man-made substitution, insertion, or deletion at one or more amino acid positions. Similarly, the terms “wild-type,” “parental,” or “reference,” with respect to a polynucleotide, refer to a naturally-occurring polynucleotide that does not include a man-made nucleoside change. However, note that a polynucleotide encoding a wild-type, parental, or reference polypeptide is not limited to a naturally-occurring polynucleotide, and encompasses any polynucleotide encoding the wild-type, parental, or reference polypeptide.

[0059] As used herein, a “variant polypeptide” refers to a polypeptide that is derived from a parent (or reference) polypeptide by the substitution, addition, or deletion, of one or more amino acids, typically by recombinant DNA techniques. Variant polypeptides may differ from a parent polypeptide by a small number of amino acid residues and may be defined by their level of primary amino acid sequence homology/identity with a parent polypeptide. Preferably, variant polypeptides have at least 70%, at least 75%, at least 80%, at least 85%, 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 even at least 99% amino acid sequence identity with a parent polypeptide.

[0060] Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (*See, e.g.*, Altschul *et al.* [1990] *J. Mol. Biol.* 215:403-410; Henikoff *et al.* [1989] *Proc. Natl. Acad. Sci. USA* 89:10915; Karin *et al.* [1993] *Proc. Natl. Acad. Sci. USA* 90:5873; and Higgins *et al.* [1988] *Gene* 73:237-244). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Databases may also be searched using FASTA (Pearson *et al.* [1988] *Proc. Natl. Acad. Sci. USA* 85:2444-2448). One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

[0061] As used herein, a “variant polynucleotide” encodes a variant polypeptide, has a specified degree of homology/identity with a parent polynucleotide, or hybridized under stringent conditions to a parent polynucleotide or the complement, thereof. Preferably, a variant polynucleotide has at least 70%, at least 75%, at least 80%, at least 85%, 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 even at least 99% nucleotide sequence identity with a parent polynucleotide. Methods for determining percent identity are known in the art and described immediately above.

[0062] The term “derived from” encompasses the terms “originated from,” “obtained from,” “obtainable from,” “isolated from,” and “created from,” and generally indicates that one specified material find its origin in another specified material or has features that can be described with reference to the another specified material.

[0063] As used herein, the term “hybridization” refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing, as known in the art.

[0064] As used herein, the phrase “hybridization conditions” refers to the conditions under which hybridization reactions are conducted. These conditions are typically classified by degree of “stringency” of the conditions under which hybridization is measured. The degree of stringency can be based, for example, on the melting temperature (T_m) of the nucleic acid binding complex or probe. For example, “maximum stringency” typically occurs at about $T_m-5^\circ\text{C}$ (5° below the T_m of the probe); “high stringency” at about $5-10^\circ$ below the T_m ; “intermediate stringency” at about $10-20^\circ$ below the T_m of the probe; and “low stringency” at about $20-25^\circ$ below the T_m . Alternatively, or in addition, hybridization conditions can be based upon the salt or ionic strength conditions of hybridization and/or one or more stringency washes, *e.g.*, 6X SSC = very low stringency; 3X SSC = low to medium stringency; 1X SSC = medium stringency; and 0.5X SSC = high stringency. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe. For applications requiring high selectivity, it is typically desirable to use relatively stringent conditions to form the hybrids (*e.g.*, relatively low salt and/or high temperature conditions are used). As used herein, stringent conditions are defined as 50°C and 0.2X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0).

[0065] The phrases “substantially similar” and “substantially identical” in the context of at least two nucleic acids or polypeptides means that a polynucleotide or polypeptide comprises a sequence that has at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identical to a parent or reference sequence, or does not include amino acid substitutions, insertions, deletions, or modifications made only to circumvent the present description without adding functionality.

[0066] As used herein, an “expression vector” refers to a DNA construct containing a DNA sequence that encodes a specified polypeptide and is operably linked to a suitable control sequence

capable of effecting the expression of the polypeptides in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself.

[0067] The term “recombinant,” refers to genetic material (*i.e.*, nucleic acids, the polypeptides they encode, and vectors and cells comprising such polynucleotides) that has been modified to alter its sequence or expression characteristics, such as by mutating the coding sequence to produce an altered polypeptide, fusing the coding sequence to that of another gene, placing a gene under the control of a different promoter, expressing a gene in a heterologous organism, expressing a gene at a decreased or elevated levels, expressing a gene conditionally or constitutively in manner different from its natural expression profile, and the like. Generally recombinant nucleic acids, polypeptides, and cells based thereon, have been manipulated by man such that they are not identical to related nucleic acids, polypeptides, and cells found in nature.

[0068] A “signal sequence” refers to a sequence of amino acids bound to the N-terminal portion of a polypeptide, and which facilitates the secretion of the mature form of the protein from the cell. The mature form of the extracellular protein lacks the signal sequence which is cleaved off during the secretion process.

[0069] The term “selective marker” or “selectable marker” refers to a gene capable of expression in a host cell that allows for ease of selection of those hosts containing an introduced nucleic acid or vector. Examples of selectable markers include but are not limited to antimicrobial substances (*e.g.*, hygromycin, bleomycin, or chloramphenicol) and/or genes that confer a metabolic advantage, such as a nutritional advantage, on the host cell.

[0070] The term “regulatory element” as used herein refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Additional regulatory elements include splicing signals, polyadenylation signals and termination signals.

[0071] As used herein, “host cells” are generally prokaryotic or eukaryotic hosts which are transformed or transfected with vectors constructed using recombinant DNA techniques known in the art. Transformed host cells are capable of either replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or pro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

[0072] The term “introduced” in the context of inserting a nucleic acid sequence into a cell, means transformation, transduction or transfection. Means of transformation include protoplast transformation, calcium chloride precipitation, electroporation, naked DNA, and the like as known in the art. (*See, Chang*

and Cohen [1979] *Mol. Gen. Genet.* 168:111-115; Smith *et al.* [1986] *Appl. Env. Microbiol.* 51:634; and the review article by Ferrari *et al.*, in Harwood, Bacillus, Plenum Publishing Corporation, pp. 57-72, 1989).

[0073] The terms “selectable marker” or “selectable gene product” as used herein refer to the use of a gene, which encodes an enzymatic activity that confers resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed.

[0074] Other technical and scientific terms have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains (*See, e.g.*, Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology*, 2d Ed., John Wiley and Sons, NY 1994; and Hale and Marham, *The Harper Collins Dictionary of Biology*, Harper Perennial, NY 1991).

[0075] As used herein in connection with a numerical value, the term “about” refers to a range of -10% to +10% of the numerical value. For instance, the phrase a “pH value of about 6” refers to pH values of from 5.4 to 6.6.

[0076] Headings are provided for convenience and should not be construed as limitations. The description included under one heading may apply to the specification as a whole.

Mannanase enzymes of the invention

[0077] As used herein, a mannanase enzyme of the invention includes an enzyme, polypeptide, or protein exhibiting a mannanase activity. In one aspect, the present compositions and methods provide a recombinant Bleman1 endo- β -mannanase polypeptide, fragments thereof, or variants thereof. An exemplary Bleman1 polypeptide was recombinantly expressed from a polynucleotide obtained from *Bacillus lentus*. The mature Bleman1 polypeptide has the amino acid sequence set forth as SEQ ID NO:2, as shown below. Similar, substantially identical Bleman1 polypeptides may occur in nature, *e.g.*, in other strains or isolates of *B. lentus*. These and other isolated Bleman1 polypeptides are encompassed by the present compositions and methods.

[0078] Polynucleotide sequence of Bleman1:

[0079] GCTTCAGGTTTTATGTAAGCGGTACCATCCTGTGTGATTCGACAGGTAATCCGT
TAAAATTCGTGGGATCAATCATGCTCACTCCTGGTTAAAAATGACTCAGCAACCGCAATG
GAGGCCATTGCGGCAACAGGTGCCAATACGGTGCGAATTGTTCTGTCTAATGGACAGCAGT
ATGCGAAGGACGATGCTAACACGGTGAGTAATTTGTTATCACTAGCAAATCAACATAAGCT
AATCGCTATTCTTGAAGTTCATGACGCTACAGGCAGTGATTGAGTCTCTGCGCTAGATCATG
CCGTGGATTATTGGATTGAAATGAAGAATGTGCTTGTAGGAAAAGAGGATCGCGTATTGAT
TAATATTGCCAATGAGTGGTACGGAACGTGGGATAGCAACGGGTGGGCTGATGGTTACAAA
AGCGCAATTCCGAAATTGAGAAATGCTGGAATCAACCATACGTTAATCGTGGATGCGGCGG
GCTGGGGACAATATCCGCAGTCGATCGTTGATAAAGGGAATGAAGTGTTTAAACAGTGATCC
GCTGCGGAATACGATATTTCCATTCATATGTATGAATATGCAGGCGGAAATGCCGATATGG
TGAGAGCGAATATTGATCAGGTCTTAAATAAGGGGTTGGCCGTTATCATTGGGGAATTTGGC

CATTATCATACGGGTGGCGATGTGGATGAGACGGCTATAATGAGTTATACGCAGCAGAAGG
GGGTTGGATGGCTCGCTTGGTCATGGAAAGGAAATGGTGCAGAATGGTTGTATCTGGACTT
ATCTTATGACTGGGCGGGCAACCATCTGACCGAATGGGGCGAGACGATCGTCAACGGTGCA
AACGGGCTGAAAGCAACGAGTACGCGAGCCCCCTATTTTTGGGAAT (SEQ ID NO:1)

5 [0080] Polypeptide sequence of Bleman1:

[0081] ASGFYVSGTILCDSTGNPFKIRGINHAHSWFKNDSATAMEAIAATGANTVRIVLSNG
QQYAKDDANTVSNLLSLANQHKLIAILEVHDATGSDSVSALDHAVDYWIEMKNVLVGKEDRV
LINIANEWYGTWDSNGWADGYKSAIPKLNRNAGINHTLIVDAAGWGQYPQSIVDKGNEVFNSDP
LRNTIFSIHMYEYAGGNADMVRANIDQVLNKGGLAVIIGFEFGHYHTGGDVDETAIMS YTQQKGV
10 GWLAWSWKNGAEWLYLDLSYDWAGNHLTEWGETIVNGANGLKATSTRAPIFGN (SEQ ID
NO:2)

[0082] In some embodiments, a polypeptide of the present invention is a variant Bleman1
polypeptide having a specified degree of amino acid sequence identity to the exemplified Bleman1
polypeptide, *e.g.*, at least 60%, at least 70%, at least 80%, at least 85%, at least 86%, at least 87%, at least
15 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%, at least 99%, or even at least 100% sequence identity to the amino
acid sequence of SEQ ID NO:2. Sequence identity can be determined by amino acid sequence
alignment, *e.g.*, using a program such as BLAST, ALIGN, or CLUSTAL, as described herein.

[0083] Polypeptides of the present invention have mannanase activity. In some embodiments, the
20 polypeptide of the present invention has mannanase activity on an AZO-Carob Galactomannan or Locust
Bean Gum substrate. In some embodiments, the polypeptide of the present invention has mannanase
activity on a substrate such as chocolate ice cream or chocolate pudding.

[0084] In certain embodiments, the Bleman1 polypeptides are produced recombinantly, while in
others the Bleman1 polypeptides are produced synthetically, or are purified from a native source (*B.*
25 *lentus*).

[0085] In certain other embodiments, the isolated Bleman1 polypeptide includes substitutions that
do not substantially affect the structure and/or function of the polypeptide. Exemplary substitutions are
conservative mutations, as summarized in Table I.

30 **Table I. Amino Acid Substitutions**

Original Residue	Code	Acceptable Substitutions
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln

Original Residue	Code	Acceptable Substitutions
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-1-thioazolidine-4- carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0086] Substitutions involving naturally occurring amino acids are generally made by mutating a nucleic acid encoding a recombinant Bleman1 polypeptide, and then expressing the variant polypeptide in an organism. Substitutions involving non-naturally occurring amino acids or chemical modifications to amino acids are generally made by chemically modifying a recombinant Bleman1 polypeptide after it has been synthesized by an organism.

[0087] In some embodiments, variant isolated Bleman1 polypeptides are substantially identical to SEQ ID NO:2, meaning that they do not include amino acid substitutions, insertions, or deletions that do not significantly affect the structure, function, or expression of the polypeptide. Such variant isolated Bleman1 polypeptides include those designed only to circumvent the present description.

[0088] In some embodiments, the isolated Bleman1 polypeptide (including a variant thereof) has 1,4- β -D-mannosidic hydrolase activity, which includes mannanase, endo-1,4- β -D-mannanase, exo-1,4- β -D-mannanasegalactomannanase, and/or glucomannanase activity. 1,4- β -D-mannosidic hydrolase activity can be determined and measured using the assays described herein, or by other assays known in the art.

In some embodiments, the isolated Bleman1 polypeptide has activity in the presence of a detergent composition.

[0089] In some embodiments, the polypeptide of the invention has mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9. In some embodiments, the polypeptide of the invention retains greater than 50% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8. Retained mannanase activity is measured compared to peak levels for the mannanase. In some embodiments, the mannanase activity is measured by mannanase activity on an AZO-Carob Galactomannan or Locust Bean Gum substrate, as further described in Example 2. In some embodiments, the polypeptide of the invention retains greater than 75% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8.

[0090] In some embodiments, the polypeptide of the invention has mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C. Mannanase activity can be measured, for example, by hydrolysis of a substrate, such as AZO-Carob Galactomannan and release of Remazolbrilliant Blue R dye.

[0091] In some embodiments, the polypeptide of the invention has measurable mannanase activity in the presence of detergent. In some embodiments, the polypeptide has measurable mannanase activity in the presence of a protease. In some embodiments, the polypeptide and the protease are both present at from about 0.1 to about 10.0 ppm. In some embodiments, the polypeptide retains greater than 50% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8 in the presence of protease. In some embodiments, the polypeptide of the invention retains greater than 75% mannanase activity at pH values of between 5 and 11, or between 7 and 11, or between 7 and 9, or between 7 and 8 in the presence of protease. In some embodiments, the polypeptide has mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C in the presence of protease. In some embodiments, the polypeptide retains at least 50% mannanase activity at temperature values of between 10°C and 60°C, or between 20°C and 60°C, or between 30°C and 60°C, or between 40°C and 60°C in the presence of protease. In some embodiments, the polypeptide is capable of hydrolyzing a substrate selected from the group consisting of chocolate ice cream, chocolate pudding, guar gum, locust bean gum, and combinations thereof. In some embodiments, the polypeptide further comprises a native or non-native signal peptide. In some embodiments, the polypeptide further comprises at least one carbohydrate-binding module. In other embodiments, the polypeptide does not comprise a carbohydrate-binding module.

[0092] Bleman1 polypeptides include fragments of “full-length” Bleman1 polypeptides that retain 1,4-β-D-mannosidic hydrolase activity. Such fragments preferably retain the active site of the full-length polypeptides but may have deletions of non-critical amino acid residues. The activity of fragments can readily be determined using the assays described, herein, or by other assays known in the art. In some embodiments, the fragments of Bleman1 polypeptides retain 1,4-β-D-mannosidic hydrolase activity in the presence of a detergent composition.

[0093] In some embodiments, the Bleman1 amino acid sequences and derivatives are produced as a N- and/or C-terminal fusion protein, for example to aid in extraction, detection and/or purification and/or to add functional properties to the Bleman1 polypeptides. Examples of fusion protein partners include, but are not limited to, glutathione-S-transferase (GST), 6XHis, GAL4 (DNA binding and/or transcriptional activation domains), FLAG, MYC, BCE103 (WO 2010/044786), or other tags well known to anyone skilled in the art. In some embodiments, a proteolytic cleavage site is provided between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably, the fusion protein does not hinder the activity of the isolated Bleman1 polypeptide.

[0094] In some embodiments, the isolated Bleman1 polypeptide is fused to a functional domain including a leader peptide, propeptide, one or more binding domain (modules) and/or catalytic domain. Suitable binding domains include, but are not limited to, carbohydrate-binding modules (*e.g.*, CBM) of various specificities, providing increased affinity to carbohydrate components present during the application of the isolated Bleman1 polypeptide. As described herein, the CBM and catalytic domain of the Bleman1 polypeptide are operably linked.

[0095] A carbohydrate-binding module (CBM) is defined as a contiguous amino acid sequence within a carbohydrate-active enzyme with a discreet fold having carbohydrate-binding activity. A few exceptions are CBMs in cellosomal scaffoldin proteins and rare instances of independent putative CBMs. The requirement of CBMs existing as modules within larger enzymes sets this class of carbohydrate-binding protein apart from other non-catalytic sugar binding proteins such as lectins and sugar transport proteins. CBMs were previously classified as cellulose-binding domains (CBDs) based on the initial discovery of several modules that bound cellulose (Tomme et al., *Eur J Biochem*, 170:575-581, 1988; and Gilkes et al., *J Biol Chem*, 263:10401-10407, 1988). However, additional modules in carbohydrate-active enzymes are continually being found that bind carbohydrates other than cellulose yet otherwise meet the CBM criteria, hence the need to reclassify these polypeptides using more inclusive terminology. Previous classification of cellulose-binding domains was based on amino acid similarity. Groupings of CBDs were called "Types" and numbered with roman numerals (*e.g.* Type I or Type II CBDs). In keeping with the glycoside hydrolase classification, these groupings are now called families and numbered with Arabic numerals. Families 1 to 13 are the same as Types I to XIII (Tomme et al., in Enzymatic Degradation of Insoluble Polysaccharides (Saddler, J.N. & Penner, M., eds.), Cellulose-binding domains: classification and properties. pp. 142-163, American Chemical Society, Washington, 1995). A detailed review on the structure and binding modes of CBMs can be found in (Boraston et al., *Biochem J*, 382:769-81, 2004). The family classification of CBMs is expected to: aid in the identification of CBMs, in some cases, predict binding specificity, aid in identifying functional residues, reveal evolutionary relationships and possibly be predictive of polypeptide folds. Because the fold of proteins is better conserved than their sequences, some of the CBM families can be grouped into superfamilies or clans. The current CBM families are 1-63. CBMs/CBDs have also been found in algae,

e.g., the red alga *Porphyra purpurea* as a non-hydrolytic polysaccharide-binding protein. However, most of the CBDs are from cellulases and xylanases. CBDs are found at the N-and C-termini of proteins or are internal. Enzyme hybrids are known in the art (See *e.g.*, WO 90/00609 and WO 95/16782) and may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding a disclosed Bleman1 polypeptide and growing the host cell to express the fused gene. Enzyme hybrids may be described by the following formula:

CBM-MR-X or X-MR-CBM

[0096] In the above formula, the CBM is the N-terminal or the C-terminal region of an amino acid sequence corresponding to at least the carbohydrate-binding module; MR is the middle region (the linker), and may be a bond, or a short linking group preferably of from about 2 to about 100 carbon atoms, more preferably of from 2 to 40 carbon atoms; or is preferably from about 2 to about 100 amino acids, more preferably from 2 to 40 amino acids; and X is an N-terminal or C-terminal region of a disclosed Bleman1 polypeptide having mannanase catalytic activity. In addition, a mannanase may contain more than one CBM or other module(s)/domain(s) of non-glycolytic function. The terms “module” and “domain” are used interchangeably in the present disclosure.

[0097] Suitable enzymatically active domains possess an activity that supports the action of the isolated Bleman1 polypeptide in producing the desired product. Non-limiting examples of catalytic domains include: cellulases, hemicellulases such as xylanase, exo-mannanases, glucanases, arabinases, galactosidases, pectinases, and/or other activities such as proteases, lipases, acid phosphatases and/or others or functional fragments thereof. Fusion proteins are optionally linked to the isolated Bleman1 polypeptide through a linker sequence that simply joins the Bleman1 polypeptide and the fusion domain without significantly affecting the properties of either component, or the linker optionally has a functional importance for the intended application.

[0098] Alternatively, the isolated Bleman1 polypeptides described herein are used in conjunction with one or more additional proteins of interest. Non-limiting examples of proteins of interest include: proteases, peroxidases, cellulases, beta-glucanases, hemicellulases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo- β -mannanases, exo- β -mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof and/or other enzymes.

[0099] In other embodiments, the isolated Bleman1 polypeptide is fused to a signal peptide for directing the extracellular secretion of the isolated Bleman1 polypeptide. For example, in certain embodiments, the signal peptide is the native Bleman1 signal peptide. In other embodiments, the signal peptide is a non-native signal peptide such as the *B. subtilis* AprE signal peptide. In some embodiments,

the isolated Bleman1 polypeptide has an N-terminal extension of Ala-Gly-Lys between the mature form and the signal peptide.

Polypeptides of the Invention

- 5 **[00100]** The present invention provides novel polypeptides, which may be collectively referred to as “polypeptides of the invention.” Polypeptides of the invention include isolated, recombinant, substantially pure, or non-naturally occurring variant mannanase enzyme polypeptides, including for example, variant mannanase enzyme polypeptides, having enzymatic activity (*e.g.*, mannanase activity). In some embodiments, polypeptides of the invention are useful in cleaning applications and can be
- 10 incorporated into cleaning compositions that are useful in methods of cleaning an item or a surface (*e.g.*, of surface of an item) in need of cleaning.
- [00101]** In some embodiments, the mannanase enzyme variant can be a variant of a parent mannanase enzyme from the Genus *Bacillus*. All of the mannanase enzyme variants described in the section above can be a variant of a parent mannanase enzyme from the Genus *Bacillus*, and more specifically a variant
- 15 of the mannanase enzyme from *Bacillus lentus* as shown in SEQ ID NO:2.
- [00102]** In some embodiments, the mannanase enzyme variant can be a variant having 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99 or 100% identity to a mannanase enzyme from the genus *Bacillus*. In various embodiments, the mannanase enzyme variant can be a variant having 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99 or 100% identity to the mannanase enzyme from *Bacillus lentus* as shown in SEQ ID NO:2.
- 20 **[00103]** Described are compositions and methods relating to mannanase cloned from *Bacillus lentus*. The compositions and methods are based, in part, on the observation that cloned and expressed mannanase from *Bacillus lentus* has mannan hydrolase activity in the presence of a detergent composition. These features of polypeptides of the present invention makes them well suited for use in a variety of cleaning applications, where the enzyme can hydrolyze mannans in the presence of surfactants
- 25 and other components found in detergent compositions.
- [00104]** In some embodiments, the invention includes an isolated, recombinant, substantially pure, or non-naturally occurring variant mannanase enzyme having mannanase activity, which polypeptide comprises a polypeptide sequence having at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least
- 30 about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% sequence identity to a parent mannanase enzyme as provided herein.
- [00105]** In some embodiments, the variant polypeptide is a variant having a specified degree of amino acid sequence homology to the exemplified mannanase polypeptide, *e.g.*, at least 70%, at least
- 35 75%, at least 80%, at least 85%, 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 even at least 99% sequence homology to the amino

acid sequence of SEQ ID NO: 2. Homology can be determined by amino acid sequence alignment, *e.g.*, using a program such as BLAST, ALIGN, or CLUSTAL, as described herein.

[00106] Also provided is an isolated, recombinant, substantially pure, or non-naturally occurring sequence which encodes a variant mannanase enzyme having mannanase activity, said variant mannanase enzyme comprising an amino acid sequence which differs from the amino acid sequence of SEQ ID NO:2 by no more than 50, no more than 40, no more than 30, no more than 35, no more than 25, no more than 20, no more than 19, no more than 18, no more than 17, no more than 16, no more than 15, no more than 14, no more than 13, no more than 12, no more than 11, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 amino acid residue(s), wherein amino acid positions of the variant mannanase are numbered according to the numbering of corresponding amino acid positions in the amino acid sequence of *Bacillus lentus* mannanase *Bleman1* shown in SEQ ID NO:2 as determined by alignment of the variant mannanase enzyme amino acid sequence with the *Bacillus lentus* mannanase *Bleman1* amino acid sequence.

[00107] As noted above, the variant mannanase enzyme polypeptides of the invention have enzymatic activities (*e.g.*, mannanase activities) and thus are useful in cleaning applications, including but not limited to, methods for cleaning dishware items, tableware items, fabrics, and items having hard surfaces (*e.g.*, the hard surface of a table, table top, wall, furniture item, floor, ceiling, etc.). Exemplary cleaning compositions comprising one or more variant mannanase enzyme polypeptides of the invention are described *infra*. The enzymatic activity (*e.g.*, mannanase enzyme activity) of a variant mannanase enzyme polypeptide of the invention can be determined readily using procedures well known to those of ordinary skill in the art. The Examples presented *infra* describe methods for evaluating the enzymatic activity, cleaning performance, detergent stability and/or thermostability. The performance of variant mannanase enzymes of the invention in removing stains (*e.g.*, a lipid stain), cleaning hard surfaces, or cleaning laundry, dishware or tableware item(s) can be readily determined using procedures well known in the art and/or by using procedures set forth in the Examples.

[00108] A polypeptide of the invention can be subject to various changes, such as one or more amino acid insertions, deletions, and/or substitutions, either conservative or non-conservative, including where such changes do not substantially alter the enzymatic activity of the polypeptide. Similarly, a nucleic acid of the invention can also be subject to various changes, such as one or more substitutions of one or more nucleic acids in one or more codons such that a particular codon encodes the same or a different amino acid, resulting in either a silent variation (*e.g.*, mutation in a nucleotide sequence results in a silent mutation in the amino acid sequence, for example when the encoded amino acid is not altered by the nucleic acid mutation) or non-silent variation, one or more deletions of one or more nucleic acids (or codons) in the sequence, one or more additions or insertions of one or more nucleic acids (or codons) in the sequence, and/or cleavage of or one or more truncations of one or more nucleic acids (or codons) in the sequence. Many such changes in the nucleic acid sequence may not substantially alter the enzymatic

activity of the resulting encoded variant mannanase enzyme compared to the variant mannanase enzyme encoded by the original nucleic acid sequence. A nucleic acid of the invention can also be modified to include one or more codons that provide for optimum expression in an expression system (*e.g.*, bacterial expression system), while, if desired, said one or more codons still encode the same amino acid(s).

5 **[00109]** In some embodiments, the present invention provides a genus of polypeptides comprising variant mannanase enzyme polypeptides having the desired enzymatic activity (*e.g.*, mannanase enzyme activity or cleaning performance activity) which comprise sequences having the amino acid substitutions described herein and also which comprise one or more additional amino acid substitutions, such as conservative and non-conservative substitutions, wherein the polypeptide exhibits, maintains, or
10 approximately maintains the desired enzymatic activity (*e.g.*, mannanase enzyme activity, as reflected in the cleaning activity or performance of the variant mannanase enzyme). Amino acid substitutions in accordance with the invention may include, but are not limited to, one or more non-conservative substitutions and/or one or more conservative amino acid substitutions. A conservative amino acid residue substitution typically involves exchanging a member within one functional class of amino acid
15 residues for a residue that belongs to the same functional class (identical amino acid residues are considered functionally homologous or conserved in calculating percent functional homology). A conservative amino acid substitution typically involves the substitution of an amino acid in an amino acid sequence with a functionally similar amino acid. For example, alanine, glycine, serine, and threonine are functionally similar and thus may serve as conservative amino acid substitutions for one another.
20 Aspartic acid and glutamic acid may serve as conservative substitutions for one another. Asparagine and glutamine may serve as conservative substitutions for one another. Arginine, lysine, and histidine may serve as conservative substitutions for one another. Isoleucine, leucine, methionine, and valine may serve as conservative substitutions for one another. Phenylalanine, tyrosine, and tryptophan may serve as conservative substitutions for one another.

25 **[00110]** Other conservative amino acid substitution groups can be envisioned. For example, amino acids can be grouped by similar function or chemical structure or composition (*e.g.*, acidic, basic, aliphatic, aromatic, sulfur-containing). For instance, an aliphatic grouping may comprise: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I). Other groups containing amino acids that are considered conservative substitutions for one another include: aromatic: Phenylalanine (F), Tyrosine (Y),
30 Tryptophan (W); sulfur-containing: Methionine (M), Cysteine (C); Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E); non-polar uncharged residues, Cysteine (C), Methionine (M), and Proline (P); hydrophilic uncharged residues: Serine (S), Threonine (T), Asparagine (N), and Glutamine (Q). Additional groupings of amino acids are well-known to those of skill in the art and described in various standard textbooks. Listing of a polypeptide sequence herein, in conjunction
35 with the above substitution groups, provides an express listing of all conservatively substituted polypeptide sequences.

[00111] More conservative substitutions exist within the amino acid residue classes described above, which also or alternatively can be suitable. Conservation groups for substitutions that are more conservative include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Thus, for example, in some embodiments, the invention provides an isolated or recombinant variant mannanase enzyme polypeptide having mannanase activity, said variant mannanase enzyme polypeptide comprising an amino acid sequence having at least about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% sequence identity to the amino acid sequence of SEQ ID NO:2. A conservative substitution of one amino acid for another in a variant mannanase enzyme of the invention is not expected to alter significantly the enzymatic activity or cleaning performance activity of the variant mannanase enzyme. Enzymatic activity or cleaning performance activity of the resultant mannanase enzyme can be readily determined using the standard assays and the assays described herein.

[00112] Conservatively substituted variations of a polypeptide sequence of the invention (*e.g.*, variant mannanase enzymes of the invention) include substitutions of a small percentage, sometimes less than about 25%, about 20%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, or about 6% of the amino acids of the polypeptide sequence, or less than about 5%, about 4%, about 3%, about 2%, or about 1%, of the amino acids of the polypeptide sequence, with a conservatively selected amino acid of the same conservative substitution group.

Activities of Bleman1

[00113] The isolated Bleman1 polypeptides disclosed herein may have enzymatic activity over a broad range of pH conditions. In certain embodiments the disclosed Bleman1 polypeptides have enzymatic activity from about pH 4.5 to about pH 11.0. In preferred embodiments, the Bleman1 polypeptides have substantial enzymatic activity from about pH 5.0 to about pH 11.0, or from about pH 7.0 to about pH 11.0, or from about pH 7.0 to about pH 9.0. It should be noted that the pH values described herein may vary by ± 0.2 . For example a pH value of about 8.0 could vary from pH 7.8 to pH 8.2.

[00114] The isolated Bleman1 polypeptides disclosed herein may have enzymatic activity over a wide range of temperatures, *e.g.*, from 10°C or lower to about 80°C or higher. In certain embodiments, the Bleman1 polypeptides have substantial enzymatic activity at a temperature range of about 10°C to about 60°C, or about 20°C to about 60°C, or about 20°C to about 60°C, or about 30°C to about 60°C, or about 40°C to about 60°C. It should be noted that the temperature values described herein may vary by $\pm 0.2^\circ\text{C}$. For example a temperature of about 50°C could vary from 49.8°C to 50.2°C.

[00115] Polypeptides of the present invention have endo- β -mannanase activity (for example, against locust bean gum, guar gum, chocolate ice cream and/or chocolate pudding) in the presence of one or more other enzymes, such as, for example, one or more protease, amylase, cellulase, lipase, pectate lyase, peroxidase, beta-glucanase, hemicellulase, acyl transferase, phospholipase, esterase, laccase,

catalase, aryl esterase, alpha-amylase, glucoamylase, cutinase, pectinase, keratinase, reductase, oxidase, phenoloxidase, lipxygenase, ligninase, carrageenase, pullulanase, tannase, arabinosidase, hyaluronidase, chondroitinase, xyloglucanase, xylanase, pectin acetyl esterase, polygalacturonase, rhamnogalacturonase, other endo- β -mannanase, exo- β -mannanase, pectin methylesterase, cellobiohydrolase, and/or transglutaminase, or any combination thereof. As shown in Examples 6 and 8, the Bleman1 polypeptide had endo- β -mannanase activity against locust bean gum, chocolate ice cream, chocolate pudding and salad dressing in the presence of a protease. As shown in Example 9, the Bleman1 polypeptide had endo- β -mannanase activity against locust bean gum, chocolate pudding and salad dressing in the presence of protease and amylase, with or without a bleaching agent. The endo- β -mannanase activity of the Bleman1 polypeptide was at least as effective as, and in some cases more effective than, a commercial detergent containing endo- β -mannanase in hydrolyzing mannans such as locust bean gum and chocolate ice cream. In fact, Bleman1 showed hydrolysis activity against exemplary gum stained material, in the presence of both powder and liquid detergent. Accordingly, in certain embodiments, any of the isolated Bleman1 polypeptides described herein may hydrolyze mannan substrates that include, but are not limited to, locust bean gum, guar gum, chocolate ice cream, chocolate pudding, salad dressing, and combinations thereof.

Nucleic Acids of the Invention

[00116] The invention provides isolated, non-naturally occurring, or recombinant nucleic acids (also referred to herein as “polynucleotides”), which may be collectively referred to as “nucleic acids of the invention” or “polynucleotides of the invention”, which encode polypeptides of the invention. Nucleic acids of the invention, including all described below, are useful in recombinant production (*e.g.*, expression) of polypeptides of the invention, typically through expression of a plasmid expression vector comprising a sequence encoding the polypeptide of interest or fragment thereof. As discussed above, polypeptides include variant mannanase enzyme polypeptides, including variant mannanase polypeptides having enzymatic activity (*e.g.*, mannanase activity) which are useful in cleaning applications and cleaning compositions for cleaning an item or a surface (*e.g.*, surface of an item) in need of cleaning.

[00117] In some embodiments, the invention provides an isolated, recombinant, substantially pure, or non-naturally occurring nucleic acid comprising a nucleotide sequence encoding any polypeptide (including any fusion protein, etc.) of the invention described above in the section entitled "Polypeptides of the Invention" and elsewhere herein. The invention also provides an isolated, recombinant, substantially pure, or non-naturally-occurring nucleic acid comprising a nucleotide sequence encoding a combination of two or more of any polypeptides of the invention described above and elsewhere herein.

[00118] Another aspect of the compositions and methods is a polynucleotide that encodes an isolated Bleman1 polypeptide (including variants and fragments, thereof), provided in the context of an expression vector for directing the expression of a Bleman1 polypeptide in a heterologous organism, such as those identified, herein. The polynucleotide that encodes a Bleman1 polypeptide may be operably-

linked to regulatory elements (*e.g.*, a promoter, terminator, enhancer, and the like) to assist in expressing the encoded polypeptides.

[00119] An exemplary polynucleotide sequence encoding a Bleman1 polypeptide has the nucleotide sequence of SEQ ID NO: 1. Similar, including substantially identical, polynucleotides encoding

5 Bleman1 polypeptides and variants may occur in nature, *e.g.*, in other strains or isolates of *B. lentus*. In view of the degeneracy of the genetic code, it will be appreciated that polynucleotides having different nucleotide sequences may encode the same Bleman1 polypeptides, variants, or fragments.

[00120] In some embodiments, polynucleotides encoding Bleman1 polypeptides have a specified degree of amino acid sequence identity to the exemplified polypeptide encoding a Bleman1 polypeptide, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at
10 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 even at least 99% sequence identity to the amino acid sequence of SEQ ID NO:2. Homology can be determined by amino acid sequence alignment, *e.g.*, using a program such as BLAST, ALIGN, or CLUSTAL, as described herein.

[00121] Also provided is an isolated, recombinant, substantially pure, or non-naturally occurring nucleic acid comprising a polynucleotide sequence which encodes a variant mannanase enzyme having mannanase activity, said variant mannanase enzyme comprising an amino acid sequence which differs from the amino acid sequence of SEQ ID NO:2 by no more than 50, no more than 40, no more than 30,
15 no more than 35, no more than 25, no more than 20, no more than 19, no more than 18, no more than 17, no more than 16, no more than 15, no more than 14, no more than 13, no more than 12, no more than 11, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 amino acid residue(s), wherein amino acid positions of the variant mannanase are numbered according to the numbering of corresponding amino acid positions in the amino acid sequence of *Bacillus lentus* mannanase *Bleman1* shown in SEQ
20 ID NO:2 as determined by alignment of the variant mannanase enzyme amino acid sequence with the *Bacillus lentus* mannanase *Bleman1* amino acid sequence.

[00122] The present invention provides nucleic acids encoding a mannanase variant of *Bacillus* mannanase, as described previously, wherein the amino acid positions of the mannanase variant are numbered by correspondence with the amino acid sequence of *Bacillus lentus* mannanase Bleman1 set
25 forth as SEQ ID NO:2.

[00123] In some embodiments, the polynucleotide that encodes a Bleman1 polypeptide is fused in frame behind (*i.e.*, downstream of) a coding sequence for a signal peptide for directing the extracellular secretion of a Bleman1 polypeptide. Heterologous signal sequences include those from bacterial cellulase genes. Expression vectors may be provided in a heterologous host cell suitable for expressing a
30 Bleman1 polypeptide, or suitable for propagating the expression vector prior to introducing it into a suitable host cell.

[00124] In some embodiments, polynucleotides encoding Bleman1 polypeptides hybridize to the exemplary polynucleotide of SEQ ID NO:1 (or the complement thereof) under specified hybridization conditions. Exemplary conditions are stringent condition and highly stringent conditions, which are described, herein.

5 [00125] Bleman1 polynucleotides may be naturally occurring or synthetic (*i.e.*, man-made), and may be codon-optimized for expression in a different host, mutated to introduce cloning sites, or otherwise altered to add functionality.

[00126] Nucleic acids of the invention can be generated by using any suitable synthesis, manipulation, and/or isolation techniques, or combinations thereof. For example, a polynucleotide of the invention may be produced using standard nucleic acid synthesis techniques, such as solid-phase synthesis techniques that are well-known to those skilled in the art. The synthesis of the nucleic acids of the invention can be also facilitated (or alternatively accomplished) by any suitable method known in the art, including but not limited to chemical synthesis using the classical phosphoramidite method (*See e.g.*, Beaucage *et al.* Tetrahedron Letters 22:1859-69 (1981)); or the method described by Matthes *et al.* (*See*, 10 Matthes *et al.*, EMBO J. 3:801-805 (1984), as is typically practiced in automated synthetic methods. Nucleic acids of the invention also can be produced by using an automatic DNA synthesizer. Customized nucleic acids can be ordered from a variety of commercial sources (*e.g.*, The Midland Certified Reagent Company, the Great American Gene Company, Operon Technologies Inc., and DNA2.0). Other techniques for synthesizing nucleic acids and related principles are known in the art 15 (*See e.g.*, Itakura *et al.*, Ann. Rev. Biochem. 53:323 (1984); and Itakura *et al.*, Science 198:1056 (1984)).

Methods for Making Modified Variant Mannanase Enzymes of the Invention

[00127] In order to produce a disclosed Bleman1 polypeptide, the DNA encoding the polypeptide can be chemically synthesized from published sequences or obtained directly from host cells harboring the 25 gene (*e.g.*, by cDNA library screening or PCR amplification). In some embodiments, the Bleman1 polynucleotide is included in an expression cassette and/or cloned into a suitable expression vector by standard molecular cloning techniques. Such expression cassettes or vectors contain sequences that assist initiation and termination of transcription (*e.g.*, promoters and terminators), and generally contain a selectable marker.

30 [00128] A variety of methods are known in the art that are suitable for generating modified polynucleotides of the invention that encode variant mannanase enzymes of the invention, including, but not limited to, for example, site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, deletion mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches. Methods for making modified polynucleotides and proteins 35 (*e.g.*, variant mannanase enzymes) include DNA shuffling methodologies, methods based on non-homologous recombination of genes, such as ITCHY (*See*, Ostermeier *et al.*, 7:2139-44 (1999)), SCRACHY (*See*, Lutz *et al.* 98:11248-53 (2001)), SHIPREC (*See*, Sieber *et al.*, 19:456-60 (2001)), and

NRR (*See, Bittker et al.*, 20:1024-9 (2001); Bittker *et al.*, 101:7011-6 (2004)), and methods that rely on the use of oligonucleotides to insert random and targeted mutations, deletions and/or insertions (*See, Ness et al.*, 20:1251-5 (2002); Coco *et al.*, 20:1246-50 (2002); Zha *et al.*, 4:34-9 (2003); Glaser *et al.*, 149:3903-13 (1992)).

5

Vectors, Cells, and Methods for Producing Variant Mannanase Enzymes of the Invention

[00129] The present invention provides isolated or recombinant vectors comprising at least one polynucleotide of the invention described herein (*e.g.*, a polynucleotide encoding a variant mannanase enzyme of the invention described herein), isolated or recombinant expression vectors or expression
10 cassettes comprising at least one nucleic acid or polynucleotide of the invention, isolated, substantially pure, or recombinant DNA constructs comprising at least one nucleic acid or polynucleotide of the invention, isolated or recombinant cells comprising at least one polynucleotide of the invention, cell cultures comprising cells comprising at least one polynucleotide of the invention, cell cultures comprising at least one nucleic acid or polynucleotide of the invention, and compositions comprising one
15 or more such vectors, nucleic acids, expression vectors, expression cassettes, DNA constructs, cells, cell cultures, or any combination or mixtures thereof.

[00130] In some embodiments, the invention provides recombinant cells comprising at least one vector (*e.g.*, expression vector or DNA construct) of the invention which comprises at least one nucleic acid or polynucleotide of the invention. Some such recombinant cells are transformed or transfected with
20 such at least one vector. Such cells are typically referred to as host cells. Some such cells comprise bacterial cells, including, but are not limited to *Bacillus sp.* cells, such as *B. subtilis* cells, mammalian cells or fungal cells. The invention also provides recombinant cells (*e.g.*, recombinant host cells) comprising at least one variant mannanase enzyme of the invention.

[00131] In some embodiments, the invention provides a vector comprising a nucleic acid or
25 polynucleotide of the invention. In some embodiments, the vector is an expression vector or expression cassette in which a polynucleotide sequence of the invention which encodes a variant mannanase enzyme of the invention is operably linked to one or additional nucleic acid segments required for efficient gene expression (*e.g.*, a promoter operably linked to the polynucleotide of the invention which encodes a variant mannanase enzyme of the invention). A vector may include a transcription terminator and/or a
30 selection gene, such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media.

[00132] For expression and production of a protein of interest (*e.g.*, variant mannanase enzyme) in a cell, at least one expression vector comprising at least one copy of a polynucleotide encoding the modified mannanase enzyme, and preferably comprising multiple copies, is transformed into the cell
35 under conditions suitable for expression of the mannanase enzyme. In some embodiments of the present invention, a polynucleotide sequence encoding the variant mannanase enzyme (as well as other sequences included in the vector) is integrated into the genome of the host cell, while in other

embodiments, a plasmid vector comprising a polynucleotide sequence encoding the variant mannanase enzyme remains as autonomous extra-chromosomal element within the cell. The invention provides both extrachromosomal nucleic acid elements as well as incoming nucleotide sequences that are integrated into the host cell genome. The vectors described herein are useful for production of the variant mannanase enzymes of the invention. In some embodiments, a polynucleotide construct encoding the variant mannanase enzyme is present on an integrating vector that enables the integration and optionally the amplification of the polynucleotide encoding the variant mannanase enzyme into the bacterial chromosome. Examples of sites for integration are well known to those skilled in the art. In some embodiments, transcription of a polynucleotide encoding a variant mannanase enzyme of the invention is effectuated by a promoter that is the wild-type promoter for the selected precursor mannanase enzyme. In some other embodiments, the promoter is heterologous to the precursor mannanase enzyme, but is functional in the host cell. Specifically, examples of suitable promoters for use in bacterial host cells include, but are not limited to, for example, the amyE, amyQ, amyL, pstS, sacB, pSPAC, pAprE, pVeg, pHpaII promoters, the promoter of the *B. stearothermophilus* maltogenic amylase gene, the *B. amyloliquefaciens* (BAN) amylase gene, the *B. subtilis* alkaline mannanase enzyme gene, the *B. clausii* alkaline mannanase enzyme gene, the *B. pumilis* xylosidase gene, the *B. thuringiensis* cryIIIA, and the *B. licheniformis* alpha-amylase gene. Additional promoters include, but are not limited to the A4 promoter, as well as phage Lambda P_R or P_L promoters, and the *E. coli* lac, trp or tac promoters.

[00133] Variant mannanase enzymes of the present invention can be produced in host cells of any suitable Gram-positive microorganism, including bacteria and fungi. For example, in some embodiments, the variant mannanase enzyme is produced in host cells of fungal and/or bacterial origin. In some embodiments, the host cells are *Bacillus sp.*, *Streptomyces sp.*, *Escherichia sp.* or *Aspergillus sp.* In some embodiments, the variant mannanase enzymes are produced by *Bacillus sp.* host cells. Examples of *Bacillus sp.* host cells that find use in the production of the variant mannanase enzymes of the invention include, but are not limited to *B. licheniformis*, *B. lentus*, *B. subtilis*, *T. lanuginosus*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilis*, *B. thuringiensis*, *B. clausii*, and *B. megaterium*, as well as other organisms within the genus *Bacillus*. In some embodiments, *B. subtilis* host cells are used for production of variant mannanase enzymes. U.S. Patents 5,264,366 and 4,760,025 (RE 34,606) describe various *Bacillus* host strains that can be used for producing variant mannanase enzymes of the invention, although other suitable strains can be used.

[00134] Several industrial bacterial strains that can be used to produce variant mannanase enzymes of the invention include non-recombinant (*i.e.*, wild-type) *Bacillus sp.* strains, as well as variants of naturally-occurring strains and/or recombinant strains. In some embodiments, the host strain is a recombinant strain, wherein a polynucleotide encoding a polypeptide of interest has been introduced into the host. In some embodiments, the host strain is a *B. subtilis* host strain and particularly a recombinant *Bacillus subtilis* host strain. Numerous *B. subtilis* strains are known, including, but not limited to for example, 1A6 (ATCC 39085), 168 (1A01), SB19, W23, Ts85, B637, PB1753 through PB1758, PB3360,

JH642, 1A243 (ATCC 39,087), ATCC 21332, ATCC 6051, MI113, DE100 (ATCC 39,094), GX4931, PBT 110, and PEP 211 strain (See e.g., Hoch *et al.*, Genetics 73:215–228 [1973]; See also, U.S. Patent Nos. 4,450,235 and 4,302,544, and EP 0134048, each of which is incorporated by reference in its entirety). The use of *B. subtilis* as an expression host cells is well known in the art (See e.g., Palva *et al.*, Gene 19:81–87 [1982]; Fahnestock and Fischer, J. Bacteriol., 165:796–804 [1986]; and Wang *et al.*, Gene 69:39–47 [1988]).

[00135] In some embodiments, the *Bacillus* host cell is a *Bacillus sp.* that includes a mutation or deletion in at least one of the following genes, *degU*, *degS*, *degR* and *degQ*. Preferably the mutation is in a *degU* gene, and more preferably the mutation is *degU(Hy)32* (See e.g., Msadek *et al.*, J. Bacteriol. 172:824–834 [1990]; and Olmos *et al.*, Mol. Gen. Genet. 253:562–567 [1997]). One suitable host strain is a *Bacillus subtilis* carrying a *degU32(Hy)* mutation. In some embodiments, the *Bacillus* host comprises a mutation or deletion in *scoC4* (See e.g., Caldwell *et al.*, J. Bacteriol. 183:7329–7340 [2001]); *spoIIE* (See e.g., Arigoni *et al.*, Mol. Microbiol. 31:1407–1415 [1999]); and/or *oppA* or other genes of the *opp* operon (See e.g., Perego *et al.*, Mol. Microbiol. 5:173–185 [1991]). Indeed, it is contemplated that any mutation in the *opp* operon that causes the same phenotype as a mutation in the *oppA* gene will find use in some embodiments of the altered *Bacillus* strain of the invention. In some embodiments, these mutations occur alone, while in other embodiments, combinations of mutations are present. In some embodiments, an altered *Bacillus* host cell strain that can be used to produce a variant mannanase enzyme of the invention is a *Bacillus* host strain that already includes a mutation in one or more of the above-mentioned genes. In addition, *Bacillus sp.* host cells that comprise mutation(s) and/or deletions of endogenous mannanase enzyme genes find use. In some embodiments, the *Bacillus* host cell comprises a deletion of the *aprE* and the *nprE* genes. In other embodiments, the *Bacillus sp.* host cell comprises a deletion of 5 mannanase enzyme genes, while in other embodiments, the *Bacillus sp.* host cell comprises a deletion of 9 mannanase enzyme genes (See e.g., U.S. Pat. Appln. Pub. No. 2005/0202535, incorporated herein by reference).

[00136] Host cells are transformed with at least one nucleic acid encoding at least one variant mannanase enzyme of the invention using any suitable method known in the art. Whether the nucleic acid is incorporated into a vector or is used without the presence of plasmid DNA, it is typically introduced into a microorganism, in some embodiments, preferably an *E. coli* cell or a competent *Bacillus* cell. Methods for introducing a nucleic acid (e.g., DNA) into *Bacillus* cells or *E. coli* cells utilizing plasmid DNA constructs or vectors and transforming such plasmid DNA constructs or vectors into such cells are well known. In some embodiments, the plasmids are subsequently isolated from *E. coli* cells and transformed into *Bacillus* cells. However, it is not essential to use intervening microorganisms such as *E. coli*, and in some embodiments, a DNA construct or vector is directly introduced into a *Bacillus* host.

[00137] Those of skill in the art are well aware of suitable methods for introducing nucleic acid or polynucleotide sequences of the invention into *Bacillus* cells (See e.g., Ferrari *et al.*, “Genetics,” *in*

Harwood *et al.* [eds.], *Bacillus*, Plenum Publishing Corp. [1989], pp. 57-72; Saunders *et al.*, *J. Bacteriol.* 157:718-726 [1984]; Hoch *et al.*, *J. Bacteriol.* 93:1925-1937 [1967]; Mann *et al.*, *Current Microbiol.* 13:131-135 [1986]; Holubova, *Folia Microbiol.* 30:97 [1985]; Chang *et al.*, *Mol. Gen. Genet.* 168:11-115 [1979]; Vorobjeva *et al.*, *FEMS Microbiol. Lett.* 7:261-263 [1980]; Smith *et al.*, *Appl. Env. Microbiol.* 51:634 [1986]; Fisher *et al.*, *Arch. Microbiol.* 139:213-217 [1981]; and McDonald, *J. Gen. Microbiol.* 130:203 [1984]). Indeed, such methods as transformation, including protoplast transformation and congression, transduction, and protoplast fusion are well known and suited for use in the present invention. Methods of transformation are used to introduce a DNA construct or vector comprising a nucleic acid encoding a variant mannanase enzyme of the present invention into a host cell. Methods known in the art to transform *Bacillus* cells include such methods as plasmid marker rescue transformation, which involves the uptake of a donor plasmid by competent cells carrying a partially homologous resident plasmid (*See*, Contente *et al.*, *Plasmid* 2:555-571 [1979]; Haima *et al.*, *Mol. Gen. Genet.* 223:185-191 [1990]; Weinrauch *et al.*, *J. Bacteriol.* 154:1077-1087 [1983]; and Weinrauch *et al.*, *J. Bacteriol.* 169:1205-1211 [1987]). In this method, the incoming donor plasmid recombines with the homologous region of the resident "helper" plasmid in a process that mimics chromosomal transformation.

[00138] The expression cassette or vector is introduced in a suitable expression host cell, which then expresses the corresponding Bleman1 polynucleotide. Particularly suitable expression hosts are bacterial expression host genera including *Escherichia* (*e.g.*, *Escherichia coli*), *Pseudomonas* (*e.g.*, *P. fluorescens* or *P. stutzeri*), *Proteus* (*e.g.*, *Proteus mirabilis*), *Ralstonia* (*e.g.*, *Ralstonia eutropha*), *Streptomyces*, *Staphylococcus* (*e.g.*, *S. carnosus*), *Lactococcus* (*e.g.*, *L. lactis*), or *Bacillus* (*subtilis*, *megaterium*, *licheniformis*, *etc.*). Also particularly suitable are yeast expression hosts such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Hansenula polymorpha*, *Kluyveromyces lactis* or *Pichia pastoris*. Especially suited are fungal expression hosts such as *Aspergillus niger*, *Chrysosporium lucknowense*, *Aspergillus* (*e.g.*, *A. oryzae*, *A. niger*, *A. nidulans*, *etc.*) or *Trichoderma reesei*. Also suited are mammalian expression hosts such as mouse (*e.g.*, NS0), Chinese Hamster Ovary (CHO) or Baby Hamster Kidney (BHK) cell lines. Other eukaryotic hosts such as insect cells or viral expression systems (*e.g.*, bacteriophages such as M13, T7 phage or Lambda, or viruses such as Baculovirus) are also suitable for producing the Bleman1 polypeptide.

[00139] In some embodiments, the isolated Bleman1 polypeptide is expressed in a heterologous organism, *i.e.*, an organism other than *Bacillus lentus*. Exemplary heterologous organisms are Gram(+) bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Geobacillus* (formerly *Bacillus*) *stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Streptomyces lividans*, or *Streptomyces murinus*; Gram(-) bacteria such as *Escherichia coli*; yeast such as *Saccharomyces* spp. or *Schizosaccharomyces* spp., *e.g.* *Saccharomyces cerevisiae*; and filamentous fungi such as *Aspergillus* spp., *e.g.*, *Aspergillus oryzae* or *Aspergillus niger*, and *Trichoderma reesei*. Methods

from transforming nucleic acids into these organisms are well known in the art. A suitable procedure for transformation of *Aspergillus* host cells is described in EP 238 023.

[00140] In particular embodiments, the isolated Bleman1 polypeptide is expressed in a heterologous organism as a secreted polypeptide, in which case, the compositions and method encompass a method for expressing a Bleman1 polypeptide as a secreted polypeptide in a heterologous organism.

[00141] An expression vector may be derived from plasmid or viral DNA, or in alternative embodiments, contains elements of both. Exemplary vectors include, but are not limited to pXX, pC194, pJH101, pE194, pHP13 (*See*, Harwood and Cutting [eds.], Chapter 3, Molecular Biological Methods for *Bacillus*, John Wiley & Sons [1990]; suitable replicating plasmids for *B. subtilis* include those listed on p. 92; *See also*, Perego, Integrational Vectors for Genetic Manipulations in *Bacillus subtilis*, in Sonenshein *et al.*, [eds.] *Bacillus subtilis* and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics, American Society for Microbiology, Washington, D.C. [1993], pp. 615-624).

[00142] Promoters and/or signal sequences associated with secreted proteins in a particular host of interest are candidates for use in the heterologous production and secretion of endo- β -mannanases in that host or in other hosts. As an example, in filamentous fungal systems, the promoters that drive the genes for cellobiohydrolase I (*cbh1*), glucoamylase A (*glaA*), TAKA-amylase (*amyA*), xylanase (*exlA*), the *gpd*-promoter *cbh1*, *cbhl1*, endoglucanase genes EGI-EGV, Cel61B, Cel74A, *egl1-egl5*, *gpd* promoter, *Pgk1*, *pki1*, EF-1 α , *tef1*, *cDNA1* and *hex1* are particularly suitable and can be derived from a number of different organisms (*e.g.*, *A. niger*, *T. reesei*, *A. oryzae*, *A. awamori* and *A. nidulans*). In some embodiments, the Bleman1 polynucleotide is recombinantly associated with a polynucleotide encoding a suitable homologous or heterologous signal sequence that leads to secretion of the Bleman1 polypeptide into the extracellular (or periplasmic) space, thereby allowing direct detection of enzyme activity in the cell supernatant (or periplasmic space or lysate). Particularly suitable signal sequences for *Escherichia coli*, other Gram negative bacteria and other organisms known in the art include those that drive expression of the HlyA, DsbA, Pbp, PhoA, PelB, OmpA, OmpT or M13 phage Gill genes. For *Bacillus subtilis*, Gram-positive organisms and other organisms known in the art, particularly suitable signal sequences further include those that drive expression of the AprE, NprB, Mpr, AmyA, AmyE, Blac, SacB, and for *S. cerevisiae* or other yeast, include the killer toxin, Bar1, Suc2, Mating factor alpha, Inu1A or Ggplp signal sequence. Signal sequences can be cleaved by a number of signal peptidases, thus removing them from the rest of the expressed protein. In some embodiments, the rest of the Bleman1 polypeptide is expressed alone or as a fusion with other peptides, tags or proteins located at the N- or C-terminus (*e.g.*, 6XHis, HA or FLAG tags). Suitable fusions include tags, peptides or proteins that facilitate affinity purification or detection (*e.g.*, BCE103, 6XHis, HA, chitin binding protein, thioredoxin or FLAG tags), as well as those that facilitate expression, secretion or processing of the target endo- β -mannanase. Suitable processing sites include enterokinase, STE13, Kex2 or other protease cleavage sites for cleavage in vivo or in vitro.

[00143] Bleman1 polynucleotides are introduced into expression host cells by a number of transformation methods including, but not limited to, electroporation, lipid-assisted transformation or transfection (“lipofection”), chemically mediated transfection (*e.g.*, CaCl and/or CaP), lithium acetate-mediated transformation (*e.g.*, of host-cell protoplasts), biolistic “gene gun” transformation, PEG-mediated transformation (*e.g.*, of host-cell protoplasts), protoplast fusion (*e.g.*, using bacterial or eukaryotic protoplasts), liposome-mediated transformation, *Agrobacterium tumefaciens*, adenovirus or other viral or phage transformation or transduction.

[00144] Alternatively, the Bleman1 polypeptides are expressed intracellularly. Optionally, after intracellular expression of the enzyme variants, or secretion into the periplasmic space using signal sequences such as those mentioned above, a permeabilisation or lysis step can be used to release the Bleman1 polypeptide into the supernatant. The disruption of the membrane barrier is effected by the use of mechanical means such as ultrasonic waves, pressure treatment (French press), cavitation or the use of membrane-digesting enzymes such as lysozyme or enzyme mixtures. As a further alternative, the polynucleotides encoding the Bleman1 polypeptide are expressed by use of a suitable cell-free expression system. In cell-free systems, the polynucleotide of interest is typically transcribed with the assistance of a promoter, but ligation to form a circular expression vector is optional. In other embodiments, RNA is exogenously added or generated without transcription and translated in cell free systems.

[00145] In addition to commonly used methods, in some embodiments, host cells are directly transformed with a DNA construct or vector comprising a nucleic acid encoding a variant mannanase enzyme of the invention (*i.e.*, an intermediate cell is not used to amplify, or otherwise process, the DNA construct or vector prior to introduction into the host cell). Introduction of the DNA construct or vector of the invention into the host cell includes those physical and chemical methods known in the art to introduce a nucleic acid sequence (*e.g.*, DNA sequence) into a host cell without insertion into a plasmid or vector. Such methods include, but are not limited to calcium chloride precipitation, electroporation, naked DNA, liposomes and the like. In additional embodiments, DNA constructs or vector are co-transformed with a plasmid, without being inserted into the plasmid. In further embodiments, a selective marker is deleted from the altered *Bacillus* strain by methods known in the art (*See*, Stahl *et al.*, J. Bacteriol. 158:411-418 [1984]; and Palmeros *et al.*, Gene 247:255 -264 [2000]).

[00146] In some embodiments, the transformed cells of the present invention are cultured in conventional nutrient media. The suitable specific culture conditions, such as temperature, pH and the like are known to those skilled in the art and are well described in the scientific literature. In some embodiments, the invention provides a culture (*e.g.*, cell culture) comprising at least one variant mannanase enzyme or at least one nucleic acid of the invention. Also provided are compositions comprising at least one nucleic acid, vector, or DNA construct of the invention.

[00147] In some embodiments, host cells transformed with at least one polynucleotide sequence encoding at least one variant mannanase enzyme of the invention are cultured in a suitable nutrient medium under conditions permitting the expression of the present mannanase enzyme, after which the

resulting mannanase enzyme is recovered from the culture. The medium used to culture the cells comprises any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*See e.g.*, the catalogues of the American Type Culture Collection). In some embodiments, the mannanase enzyme produced by the cells is recovered from the culture medium by conventional procedures, including, but not limited to for example, separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt (*e.g.*, ammonium sulfate), chromatographic purification (*e.g.*, ion exchange, gel filtration, affinity, etc.). Any method suitable for recovering or purifying a variant mannanase enzyme finds use in the present invention.

[00148] In some embodiments, a variant mannanase enzyme produced by a recombinant host cell is secreted into the culture medium. A nucleic acid sequence that encodes a purification facilitating domain may be used to facilitate purification of soluble proteins. A vector or DNA construct comprising a polynucleotide sequence encoding a variant mannanase enzyme may further comprise a nucleic acid sequence encoding a purification facilitating domain to facilitate purification of the variant mannanase enzyme (*See e.g.*, Kroll *et al.*, DNA Cell Biol. 12:441-53 [1993]). Such purification facilitating domains include, but are not limited to, for example, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (*See*, Porath, Protein Expr. Purif. 3:263-281 [1992]), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (*e.g.*, protein A domains available from Immunex Corp., Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (*e.g.*, sequences available from Invitrogen, San Diego, CA) between the purification domain and the heterologous protein also find use to facilitate purification.

[00149] Assays for detecting and measuring the enzymatic activity of an enzyme, such as a mannanase enzyme of the invention, are well known. A variety of methods can be used to determine the level of production of a mature mannanase enzyme (*e.g.*, mature variant mannanase enzymes of the present invention) in a host cell. Such methods include, but are not limited to, for example, methods that utilize either polyclonal or monoclonal antibodies specific for the mannanase enzyme. Exemplary methods include, but are not limited to enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), fluorescent immunoassays (FIA), and fluorescent activated cell sorting (FACS). These and other assays are well known in the art (*See e.g.*, Maddox *et al.*, J. Exp. Med. 158:1211 [1983]).

[00150] In some other embodiments, the invention provides methods for making or producing a mature variant mannanase enzyme of the invention. A mature variant mannanase enzyme does not include a signal peptide or a propeptide sequence. Some methods comprise making or producing a variant mannanase enzyme of the invention in a recombinant bacterial host cell, such as for example, a *Bacillus sp.* cell (*e.g.*, a *B. subtilis* cell). In some embodiments, the invention provides a method of

producing a variant mannanase enzyme of the invention, the method comprising cultivating a recombinant host cell comprising a recombinant expression vector comprising a nucleic acid encoding a variant mannanase enzyme of the invention under conditions conducive to the production of the variant mannanase enzyme. Some such methods further comprise recovering the variant mannanase enzyme from the culture.

[00151] In some embodiments the invention provides methods of producing a variant mannanase enzyme of the invention, the methods comprising: (a) introducing a recombinant expression vector comprising a nucleic acid encoding a variant mannanase enzyme of the invention into a population of cells (*e.g.*, bacterial cells, such as *B. subtilis* cells); and (b) culturing the cells in a culture medium under conditions conducive to produce the variant mannanase enzyme encoded by the expression vector. Some such methods further comprise: (c) isolating the variant mannanase enzyme from the cells or from the culture medium.

Fabric and Home Care Products

[00152] In some embodiments, the mannanase enzyme variants of the present invention can be used in compositions comprising an adjunct material and a mannanase enzyme variant, wherein the composition is a fabric and home care product. Examples of suitable compositions are described in Examples 10- 18.

[00153] In some embodiments, the fabric and home care product compositions comprising at least one mannanase enzyme variant comprise one or more of the following ingredients (based on total composition weight): from about 0.0005 wt% to about 0.5 wt%, from about 0.001 wt% to about 0.1 wt%, or even from about 0.002 wt% to about 0.05 wt% of said mannanase enzyme variant; and one or more of the following: from about 0.00003 wt% to about 0.1 wt% fabric hueing agent; from about 0.001 wt% to about 5 wt %, perfume capsules; from about 0.001 wt% to about 1 wt%, cold-water soluble brighteners; from about 0.00003 wt% to about 0.1 wt% bleach catalysts; from about 0.00003 wt% to about 0.1 wt% bacterial cleaning cellulases; and/or from about 0.05wt% to about 20 wt% Guerbet nonionic surfactants.

[00154] As used herein, “wash performance” of a mannanase enzyme (*e.g.*, a variant mannanase enzyme of the invention) refers to the contribution of the mannanase enzyme to washing that provides additional cleaning performance to the detergent as compared to the detergent without the addition of the variant mannanase enzyme to the composition. Wash performance is compared under relevant washing conditions. In some test systems, other relevant factors, such as detergent composition, sud concentration, water hardness, washing mechanics, time, pH, and/or temperature, can be controlled in such a way that condition(s) typical for household application in a certain market segment (*e.g.*, hand or manual dishwashing, automatic dishwashing, dishwashing machine cleaning, dishware cleaning, tableware cleaning, fabric cleaning, etc.) are imitated.

[00155] In some embodiments, the fabric and home care product composition is a granular or powder laundry detergent.

[00156] In some embodiments, the fabric and home care product composition is a liquid laundry detergent or a dish washing detergent.

5 [00157] It is intended that the fabric and home care product is provided in any suitable form, including a fluid or solid. The fabric and home care product can be in the form of a unit dose pouch, especially when in the form of a liquid, and typically the fabric and home care product is at least partially, or even completely, enclosed by a water-soluble pouch. In addition, in some embodiments of the fabric and home care products comprising at least one mannanase enzyme of the present invention,
10 the fabric and home care product may have any combination of parameters and/or characteristics detailed above.

Cleaning Compositions

[00158] Cleaning compositions and cleaning formulations include any composition that is suited for
15 cleaning, bleaching, disinfecting, and/or sterilizing any object, item, and/or surface. Such compositions and formulations include, but are not limited to for example, liquid and/or solid compositions, including cleaning or detergent compositions (*e.g.*, liquid, tablet, gel, bar, granule, and/or solid laundry cleaning or detergent compositions and fine fabric detergent compositions; hard surface cleaning compositions and formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners;
20 oven cleaners; fabric fresheners; fabric softeners; and textile, laundry booster cleaning or detergent compositions, laundry additive cleaning compositions, and laundry pre-spotter cleaning compositions; dishwashing compositions, including hand or manual dishwash compositions (*e.g.*, “hand” or “manual” dishwashing detergents) and automatic dishwashing compositions (*e.g.*, “automatic dishwashing detergents”).

25 [00159] Cleaning composition or cleaning formulations, as used herein, include, unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, granular, gel, solid, tablet, or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) detergent or heavy-duty powder detergent (HDD) types; liquid fine-fabric detergents; hand or manual dishwashing agents, including those of the high-foaming type; hand or
30 manual dishwashing, automatic dishwashing, or dishware or tableware washing agents, including the various tablet, powder, solid, granular, liquid, gel, and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, mouthwashes, denture cleaners, car shampoos, carpet shampoos, bathroom cleaners; hair shampoos and/or hair-rinses for humans and other animals; shower gels and foam baths and metal cleaners; as well
35 as cleaning auxiliaries, such as bleach additives and “stain-stick” or pre-treat types. In some embodiments, granular compositions are in “compact” form; in some embodiments, liquid compositions are in a “concentrated” form.

[00160] As used herein, the term “detergent composition” or “detergent formulation” is used in reference to a composition intended for use in a wash medium for the cleaning of soiled or dirty objects, including particular fabric and/or non-fabric objects or items. Such compositions of the present invention are not limited to any particular detergent composition or formulation. Indeed, in some embodiments, the detergents of the invention comprise at least one variant mannanase enzyme of the invention and, in addition, one or more surfactants, transferase(s), hydrolytic enzymes, oxido reductases, builders (*e.g.*, a builder salt), bleaching agents, bleach activators, bluing agents, fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and/or solubilizers. In some instances, a builder salt is a mixture of a silicate salt and a phosphate salt, preferably with more silicate (*e.g.*, sodium metasilicate) than phosphate (*e.g.*, sodium tripolyphosphate). Some compositions of the invention, such as, but not limited to, cleaning compositions or detergent compositions, do not contain any phosphate (*e.g.*, phosphate salt or phosphate builder).

[00161] Unless otherwise noted, all component or composition levels provided herein are made in reference to the active level of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources. Enzyme components weights are based on total active protein. All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated. In the exemplified detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions.

[00162] As indicated herein, in some embodiments, the cleaning compositions of the present invention further comprise adjunct materials including, but not limited to, surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids, pigments, and pH control agents (*See e.g.*, U.S. Pat. Nos. 6,610,642, 6,605,458, 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101, all of which are incorporated herein by reference). Embodiments of specific cleaning composition materials are exemplified in detail below. In embodiments in which the cleaning adjunct materials are not compatible with the variant mannanase enzymes of the present invention in the cleaning compositions, then suitable methods of keeping the cleaning adjunct materials and the mannanase enzyme(s) separated (*i.e.*, not in contact with each other) until combination of the two components is appropriate are used. Such separation methods include any suitable method known in the art (*e.g.*, gelscaps, encapsulation, tablets, physical separation, etc.).

Mannanase containing detergent compositions

[00163] The detergent compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

[00164] An aspect of the compositions and methods disclosed herein is a detergent composition comprising an isolated Bleman1 polypeptide (including variants or fragments, thereof) and methods for using such compositions in cleaning applications. Cleaning applications include, but are not limited to, laundry or textile cleaning, laundry or textile softening, dishwashing (manual and automatic), stain pre-treatment, and the like. Particular applications are those where mannans (*e.g.*, locust bean gum, guar gum, etc.) are a component of the soils or stains to be removed. Detergent compositions typically include an effective amount of any of the Bleman1 polypeptides described herein, *e.g.*, at least 0.0001 weight percent, from about 0.0001 to about 1, from about 0.001 to about 0.5, from about 0.01 to about 0.1 weight percent, or even from about 0.1 to about 1 weight percent, or more. An effective amount of a Bleman1 polypeptide in the detergent composition results in the Bleman1 polypeptide having enzymatic activity sufficient to hydrolyze a mannan-containing substrate, such as locust bean gum, guar gum, or combinations thereof.

[00165] Additionally, detergent compositions having a concentration from about 0.4 g/L to about 2.2 g/L, from about 0.4 g/L to about 2.0 g/L, from about 0.4 g/L to about 1.7 g/L, from about 0.4 g/L to about 1.5 g/L, from about 0.4 g/L to about 1 g/L, from about 0.4 g/L to about 0.8 g/L, or from about 0.4 g/L to about 0.5 g/L may be mixed with an effective amount of an isolated Bleman1 polypeptide. The detergent composition may also be present at a concentration of about 0.4 ml/L to about 2.6 ml/L, from about 0.4 ml/L to about 2.0 ml/L, from about 0.4 ml/L to about 1.5 ml/L, from about 0.4 ml/L to about 1 ml/L, from about 0.4 ml/L to about 0.8 ml/L, or from about 0.4 ml/L to about 0.5 ml/L.

[00166] Unless otherwise noted, all component or composition levels provided herein are made in reference to the active level of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources. Enzyme components weights are based on total active protein. All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated. In the exemplified detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions.

[00167] The detergent composition according to the invention can be in liquid, paste, gels, bars or granular forms. The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, *e.g.* in the range of 5-11, or 7-11, or 9-11. Granular compositions according to the present invention can also be in "compact form", *i.e.* they may have a relatively higher density than conventional granular detergents, *i.e.* from 550 to 950 g/l.

[00168] The present compositions can include one or more adjuvants (for example, surfactants that are efficient in removal of fatty acids from the fabric) and one or more mannanase enzymes. In some embodiments, the adjuvant and mannanase enzyme are present in a single composition. In other embodiments, the adjuvant and mannanase enzyme are present in separate compositions that are combined before contacting an oil stain on fabric, or combined on the oil stain.

[00169] The present cleaning compositions can include one or more adjuvants (surfactants) for use in combination with a mannanase enzyme. Suitable adjuvants can have a relatively small hydrophilic portion with no net charge and hydrophobic portion that is linear or saturated. In some embodiments, the hydrophobic portion includes at least, six, seven, eight, or nine adjacent aliphatic carbons. In some embodiments, the hydrophobic portion is cyclic. In some embodiments, the hydrophobic portion is not branched. Suitable surfactants include sugar-based compounds and zwitterionic compounds. Suitable adjuvants are disclosed, and hereby incorporated by reference in its entirety, in WO2011078949.

[00170] In some embodiments, the detergent composition comprises one or more surfactants, which may be non-ionic, semi-polar, anionic, cationic, zwitterionic, or combinations and mixtures thereof. The surfactants are typically present at a level of from about 0.1% to 60% by weight. Exemplary surfactants include but are not limited to sodium dodecylbenzene sulfonate, C12-14 pareth-7, C12-15 pareth-7, sodium C12-15 pareth sulfate, C14-15 pareth-4, sodium laureth sulfate (*e.g.*, Steol CS-370), sodium hydrogenated cocoate, C12 ethoxylates (Alfonic 1012-6, Hetoxol LA7, Hetoxol LA4), sodium alkyl benzene sulfonates (*e.g.*, Nacconol 90G), and combinations and mixtures thereof.

[00171] Sugar-based surfactants include maltopyranosides, thiomaltopyranosides, glucopyranosides, and their derivatives. Maltose-based surfactants were generally more effective than glucose-based surfactants. In some embodiments, a preferred sugar-based surfactant has a hydrophobic tail chain length of at least 4, at least 5, at least 6, and even at least 7 carbons. The tail can be aliphatic or cyclic. The tail can be unbranched, although branching is acceptable with sufficient chain length.

[00172] Particular examples of sugar-based surfactants include nonyl- β -D-maltopyranoside, decyl- β -D-maltopyranoside, undecyl- β -D-maltopyranoside, dodecyl- β -D-maltopyranoside, tridecyl- β -D-maltopyranoside, tetradecyl- β -D-maltopyranoside, hexadecyl- β -D-maltopyranoside, n-dodecyl- β -D-maltopyranoside and the like, 2,6-dimethyl-4-heptyl- β -D-maltopyranoside, 2-propyl-1-pentyl- β -D-maltopyranoside, nonyl- β -D-glucopyranoside, nonyl- β -D-glucopyranoside, decyl- β -D-glucopyranoside, dodecyl- β -D-glucopyranoside, sucrose monododecanoate, certain cyclohexylalkyl- β -D-maltosides (*e.g.*, the CYMAL[®]s and CYGLAs), and the MEGA[™] surfactants.

[00173] The adjuvant can be a non-sugar, non-ionic surfactant. Exemplary surfactants include Tritons with an ethoxylate repeat of nine or less. Particular Tritons are ANAPOE[®]-X-100 and ANAPOE[®]-X-114. In some embodiments, the adjuvant is a non-ionic phosphine oxide surfactant, having a hydrophobic tail of at least about 9 carbons. Exemplary surfactants include dimethyldodecylphosphine oxide and dimethyldodecylphosphine oxide.

[00174] The adjuvant can be a zwitterionic surfactant, such as a FOS-choline. In some embodiments, the FOS-choline has a hydrophobic tail with a chain length of 12 or greater. The hydrophobic tail can be saturated and unsaturated and can be cyclic. Exemplary FOS-choline surfactants include FOS-CHOLINE[®]-12, FOS-CHOLINE[®]-13, FOS-CHOLINE[®]-14, LYSO FOS-CHOLINE[®]-14, FOS-CHOLINE[®]-15, FOS-CHOLINE[®]-16, FOS-MEA[®]-12, DODECAFOS, ISO unsat 11-10, ISO 11-6, CYOFO, NOPOL-FOS, CYCLOFOS[®] (CYMAL[®])-5, -6, -7, -8, etc., and the like.

[00175] In some cases, the adjuvant can be a sulfobetaine zwitterionic surfactant. Preferred sulfobetaine surfactants have a hydrophobic tail having at least 12 carbons, *e.g.*, ANZERGENT[®] 3-12 and ANZERGENT[®] 3-14. The zwitterionic oxides and CHAPS-based surfactants (*e.g.* CHAPS and CHAPSO) are also effective, typically at higher doses than the sulfobetaines.

[00176] Anionic surfactants that may be used with the detergent compositions described herein include but are not limited to linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyl dimethylamine oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide (*e.g.*, as described in WO 92/06154), and combinations and mixtures thereof.

[00177] Nonionic surfactants that may be used with the detergent compositions described herein include but are not limited to polyoxyethylene esters of fatty acids, polyoxyethylene sorbitan esters (*e.g.*, TWEENS), polyoxyethylene alcohols, polyoxyethylene isoalcohols, polyoxyethylene ethers (*e.g.*, TRITONs and BRIJ), polyoxyethylene esters, polyoxyethylene-*p*-tert-octylphenols or octylphenyl-ethylene oxide condensates (*e.g.*, NONIDET P40), ethylene oxide condensates with fatty alcohols (*e.g.*, LUBROL), polyoxyethylene nonylphenols, polyalkylene glycols (SYNPERONIC F108), sugar-based surfactants (*e.g.*, glycopyranosides, thioglycopyranosides), and combinations and mixtures thereof.

[00178] The detergent compositions disclosed herein may have mixtures that include, but are not limited to 5-15% anionic surfactants, < 5% nonionic surfactants, cationic surfactants, phosphonates, soap, enzymes, perfume, butylphenyl methylptopionate, geraniol, zeolite, polycarboxylates, hexyl cinnamal, limonene, cationic surfactants, citronellol, and benzisothiazolinone.

[00179] In some cases, the adjuvant can be an anionic detergent, for example, a sarcosine. Preferred sarcosines have a hydrophobic tail having at least 10 carbons. In some cases, the adjuvant can also be deoxycholate.

[00180] The adjuvant can be present in a composition in an amount of at least .001%, at least 0.005%, at least 0.01%, at least 0.05%, at least 0.1%, or more, or at least 0.01 ppm, at least 0.05 ppm, at least 0.1 ppm, at least 0.5 ppm, at least 1 ppm, at least 5 ppm, at least 10 ppm, or more. In some cases, the adjuvant may be present in a preselected range, *e.g.*, about 0.001-0.01%, about 0.01-0.1%, about 0.1-

1%, or about 0.01-1 ppm, about 0.1-1 ppm, or about 1-10 ppm. In some cases, optimum activity is observed over a range, above and below which activity is reduced.

[00181] The surfactant system of the detergent can comprise nonionic, anionic, cationic, ampholytic, and/or zwitterionic surfactants. The surfactant is typically present at a level from 0.1% to 60% by weight, e.g. 1% to 40%, particularly 10-40% preferably from about 3% to about 20% by weight. The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefin sulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkane sulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid or soap.

[00182] The detergent can comprise 0-40% of nonionic surfactant polyalkylene oxide (e.g. polyethylene oxide) condensates of alkyl phenols. Preferred nonionic surfactants are alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyl dimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, alkyl(N-methyl)-glucoseamide or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92106154).

[00183] Semi-polar nonionic surfactants are another category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; water soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms. The amine oxide surfactants in particular include C₁₀-C₁₈ alkyl dimethyl amine oxides and C₈-C₁₂ alkoxy ethyl dihydroxy ethyl amine oxides.

[00184] The detergent composition can further comprise cationic surfactants. Cationic detergents used are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyl trimethyl ammonium halogenides. Highly preferred cationic surfactants are the water soluble quaternary ammonium compounds. Examples of suitable quaternary ammonium compounds include coconut trimethyl ammonium chloride or bromide; coconut methyl dihydroxy ethyl ammonium chloride or bromide; decyl triethyl ammonium chloride; decyl dimethyl hydroxyl ethyl ammonium chloride or bromide; C12-15 dimethyl hydroxyl ethyl ammonium chloride or bromide; coconut dimethyl hydroxyl ethyl ammonium chloride or bromide; myristyl trimethyl ammonium methyl sulphate; lauryl dimethyl benzyl ammonium chloride or bromide; lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide; choline esters, dialkyl imidazolines.

[00185] The detergent composition can further comprise ampholytic surfactants. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives

of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight-, or branched-chain. One of the aliphatic substituent contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. Examples of compounds falling within this definition are sodium 3-(dodecylamino) propionate, sodium 3-(dodecylamino)- propane-1-sulfonate, sodium 2-(dodecylamino)ethyl sulfate, sodium 2-(dimethylamino)octadecanoate, di-sodium 3-(N -carboxymethyldodecylamino)propane-I-sulfonate, disodium octadecyl-iminodiacetate, sodium l-carboxymethyl-2-undecylimidazole, and sodium N ,N-bis(2-hydroxyethyl)-2-sulfato-3-dodecoxy-propylamine. Sodium 3-(dodecylamino)propane-I-sulfonate is preferred.

[00186] Zwitterionic surfactants are also used in detergent compositions especially within laundry. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. The cationic atom in the quaternary compound can be part of a heterocyclic ring. In all of these compounds, there is at least one aliphatic group, straight chain or branched, containing from about 3 to 18 carbon atoms and at least one aliphatic substituent containing an anionic water solubilizing group, e.g. carboxy, sulfonate, sulfate, phosphate or phosphonate. Ethoxylated zwitterionic compounds in combination with zwitterionic surfactants have been particularly used for clay soil removal in laundry applications.

[00187] In some embodiments incorporating at least one builder, the detergent compositions comprise at least about 1%, from about 3% to about 60% or even from about 5% to about 40% builder by weight of the cleaning composition. Builders may include, but are not limited to, the alkali metals, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicates, polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metals, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. Indeed, it is contemplated that any suitable builder will find use in various embodiments of the present disclosure.

[00188] Detergent compositions may additionally include one or more detergent builders or builder systems, a complexing agent, a polymer, a bleaching system, a stabilizer, a foam booster, a suds suppressor, an anti-corrosion agent, a soil-suspending agent, an anti-soil redeposition agent, a dye, a bactericide, a hydrotrope, a tarnish inhibitor, an optical brightener, a fabric conditioner, and a perfume.

The detergent compositions may also include enzymes, including but not limited to proteases, amylases, cellulases, lipases, pectin degrading enzymes, xyloglucanases, or additional carboxylic ester hydrolases. The pH of the detergent compositions should be neutral to basic, as described herein.

[00189] The detergent may contain 1-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt i.e. essentially free of detergent builder.

[00190] The detergent builders may be subdivided into phosphorus-containing and non-phosphorous-containing types. Examples of phosphorus-containing inorganic alkaline detergent builders include the water-soluble salts, especially alkali metal pyrophosphates, orthophosphates, polyphosphates and phosphonates. Examples of non-phosphorus-containing inorganic builders include water soluble alkali metal carbonates, borates and silicates as well as layered disilicates and the various types of water insoluble crystalline or amorphous alumino silicates of which zeolites is the best known representative. Examples of suitable organic builders include alkali metal, ammonium or substituted ammonium salts of succinates, malonates, fatty acid malonates, fatty acid sulphonates, carboxymethoxy succinates, polyacetates, carboxylates, polycarboxylates, aminopolycarboxylates and polyacetyl carboxylates.

[00191] A suitable chelant for inclusion in the detergent compositions is ethylenediamine- N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include Na2EDDS and Na4EDDS. Examples of such preferred magnesium salts of EDDS include MgEDDS and Mg2EDDS. The magnesium salts are the most preferred for inclusion in compositions.

[00192] The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly (vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly (vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

[00193] In some embodiments, the builders form water-soluble hardness ion complexes (e.g., sequestering builders), such as citrates and polyphosphates (e.g., sodium tripolyphosphate and sodium tripolyphosphate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the present disclosure, including those known in the art (See, e.g., EP 2 100 949).

[00194] The detergent composition may contain bleaching agents of the chlorine/bromine-type or the oxygen-type. The bleaching agents may be coated or encapsulated. Examples of inorganic chlorine/bromine-type bleaches are lithium, sodium or calcium hypochlorite or hypobromite as well as chlorinated trisodium phosphate. The bleaching system may also comprise a hydrogen peroxide source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetyl-ethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Examples of organic chlorine/bromine-type bleaches are heterocyclic N-bromo and N-chloro imides such as trichloroisocyanuric, tribromoisocyanuric, dibromoisocyanuric and dichloroisocyanuric acids, and salts

thereof with water solubilizing cations such as potassium and sodium. Hydantoin compounds are also suitable. The bleaching system may also comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

[00195] In dishwashing detergents the oxygen bleaches are preferred, for example in the form of an inorganic persalt, preferably with a bleach precursor or as a peroxy acid compound. Typical examples of suitable peroxy bleach compounds are alkali metal perborates, both tetrahydrates and monohydrates, alkali metal percarbonates, persulfates and perphosphates. Preferred activator materials are tetraacetylenediamine (TAED), nonanoyloxybenzenesulfonate (NOBS), 3,5-trimethylhexanoyloxybenzenesulfonate (ISONOBS) or pentaacetylglucose (PAG).

[00196] The mannanase of the invention, or optionally another enzyme incorporated in the detergent composition, is normally incorporated in the detergent composition at a level from 0.00001 % to 3% of enzyme protein by weight of the composition, preferably at a level from 0.0001 % to 1% of enzyme protein by weight of the composition, more preferably at a level from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level from 0.01 % to 0.2% of enzyme protein by weight of the composition. The amount of mannanase protein may be 0.001-30 mg per gram of detergent or 0.001-100 mg per liter of wash liquor. The mannanase variants of the invention are particularly suited for detergents comprising of a combination of anionic and nonionic surfactant with 70-100% by weight of anionic surfactant and 0-30% by weight of nonionic, particularly 80-100% of anionic surfactant, and 0-20% nonionic surfactant. As further described, some preferred mannanases of the invention are also suited for detergents comprising 40-70% anionic and 30-60% non-ionic surfactant. The detergent composition may, in addition to the mannanase of the invention, comprise other enzyme(s) providing cleaning performance and/or fabric care benefits, e.g. proteases, peroxidases, cellulases, beta-glucanases, hemicellulases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo- β -mannanases, exo- β -mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof.

[00197] The enzymes of the detergent composition may be stabilized using conventional stabilizing agents (e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative as e.g. an aromatic borate ester). Boronic acid or borinic acid derivatives as enzyme stabilizers include Boric acid, Thiophene-3- boronic acid, Thiophene-2- boronic acid, 4-Methylthiophene- 2-boronic acid, 5-Ethylthiophene-2-boronic acid, 5-Methylthiophene-2-boronic acid, 5-Bromothiophene-2-boronic acid, 5-Chlorothiophene-2-boronic acid, Dibenzothiophene-1-boronic acid, Dibenzofuran-1-boronic acid, Dibenzofuran-4-boronic acid, Picoline-2-boronic acid, Diphenylborinic acid (ethanolamine complex), 5-Methoxythio-phen-2-boronic acid, Thionaphthrene-1- boronic acid,

Furan-2-boronic acid, Furan-3-boronic acid, 2,5-dimethyl- thiophene-3-boronic acid, Benzofuran-1-boronic acid, 3-Methoxythio-phene-2-boronic acid, 5-n-Propyl-thiophene-2-boronic acid, 5-Methoxyfuran-2-boronic acid, 3-Bromothiophene-2-boronic acid, 5-Ethylfuran-2-boronic acid, 4-Carbazole ethyl boronic acid.

[00198] An optional ingredient is a suds suppressor (e.g. exemplified by silicones-alkylated polysiloxane materials, and silica-silicone mixtures, where the silica is in the form of silica aerogels and xerogels and hydrophobic silicas of various types. The suds suppressor can be incorporated as particulates, in which the suds suppressor is advantageously releasable incorporated in a water-soluble or water dispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

[00199] The detergent may also contain inorganic or organic softening agents. Inorganic softening agents are exemplified by the smectite clays (5% to 15%). Organic fabric softening agents (0.5% to 5%) include the water insoluble tertiary amines and their combination with mono C₁₂ -C₁₄ quaternary ammonium salts and di-long-chain amides, or high molecular weight polyethylene oxide materials.

[00200] The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, deflocculant material, foam boosters/foam depressors (in dishwashing detergents foam depressors), anti-corrosion agents, soil-suspending or dispersing agents (0 to 10%), anti-soil-redeposition agents, dyes, dehydrating agents, bactericides, optical brighteners, abrasives, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes.

[00201]

Liquid detergent formulation	
Nonionic (Neodol 25-7) AE	25%
Anionic (Vista C-S50) LAS	5%
Triethanolamine	5%
Ethanol	10%
Stabilizer	0.5, 2.5, 5%
Protease	1%
Amylase	0.3%
Water	up to 100%
Adjust to pH = 9.0	
Mannanase insert after amylase	0.001- 1%

[00202]

Detergent formulations						
Ingredients	I (%) powder	II (%) powder	III (%) powder	IV (%) powder	V (%) liquid	VI (%) liquid
Linear alkylbenzenesulfonate (calculated as acid) or alkyl sulfate, alpha olefin sulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap	7-12	6-11	5-9	8-12	15-21	15-21
Alcohol ethoxysulfate (e.g. C ₁₂₋₁₈ alcohol 1-2 EO) or alkyl sulfate (e.g. C ₁₆₋₁₈)	1-4	1-3	--			

soap as fatty acid (e.g. C ₁₆₋₂₂ or oleic acid)	--	--	1-3		3-13	3-10
Alcohol ethoxylate (e.g. C ₁₄₋₁₅ or C ₁₂₋₁₅ 7EO or 5EO)	5-9	5-9	7-14	10-25	12-18	3-9
Alkenylsuccinic acid (C ₁₂₋₁₄)					0-13	
Aminoethanol					8-18	
sodium carbonate (Na ₂ CO ₃)	14-20	15-21	10-17	14-22		
soluble silicate (as Na ₂ O, 2SiO ₂)	2-6	1-4	3-9	1-5		
zeolite (as NaAlSiO ₄)	15-22	24-34	23-33	25-35		14-22
sodium sulfate (as Na ₂ SO ₄)	0-6	4-10	0-4	0-10		
sodium citrate/citric acid (C ₆ H ₅ Na ₃ O ₇ /C ₆ H ₈ O ₇) or potassium citrate	0-15	0-15	--		2-8	9-18
sodium perborate (as NaBO ₃ ·H ₂ O) or borate (as B ₄ O ₇)	11-18	--	8-16		0-2	0-2
TAED	2-6	--	2-8			
Phosphonate (e.g. EDTMPA)	--	--	0-1		0-3	
Ethanol					0-3	
carboxymethylcellulose	0-2	0-2	0-2	0-2		0-2
Polymers (PEG, PVP)					0-3	0-3
Anchoring polymers (e.g. maleic/acrylic acid copolymer PVP, PEG)	0-3	1-6	1-3	1-3		0-3
Propylene glycol					8-14	
Glycerol						0-5
Enzymes (mannanase)	0-5	0-5	0-5	0-5	0-5	0-5
minor ingredients (e.g. suds, suppressors, perfume, optical brightener, photobleach)	0-5	0-5	0-5	0-5	0-5	0-5

[00203]

Detergent formulations						
Ingredients	VII (%) powder	VIII (%) powder	IX (%) powder	X (%) liquid	XI (%) liquid	XII (%) powder
Linear alkylbenzenesulfonate (calculated as acid) or alkyl sulfate, alpha olefin sulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap		8-14	6-12	15-23	20-32	25-40
Fatty alcohol sulfate	5-10					
Ethoxylated fatty acid monoethanolamide	3-9	5-11				
Alcohol ethoxysulfate (e.g. C ₁₂₋₁₈ alcohol 1-2 EO, or C ₁₂₋₁₅ 2-3 EO) or alkyl sulfate (e.g. C ₁₆₋₁₈)				8-15		
soap as fatty acid (e.g. C ₁₆₋₂₂ or oleic acid or lauric acid)	0-3	0-3	2-6	0-3		
Alcohol ethoxylate (e.g. C ₁₄₋₁₅ or C ₁₂₋₁₅ 7EO or 5EO)			1-4	3-9	6-12	1-10
Alkenylsuccinic acid (C ₁₂₋₁₄)						
Aminoethanol				1-5	2-6	

sodium carbonate (Na_2CO_3)	5-10	4-10	14-22			8-25
soluble silicate (as Na_2O , 2SiO_2)	1-4	1-4				5-15
zeolite (as NaAlSiO_4)	20-40	30-50	18-32			15-28
sodium sulfate (as Na_2SO_4)	2-8	3-11	5-20			0-5
sodium citrate/citric acid ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$) or potassium citrate		5-12	3-8	5-10	8-14	
Hydrotrope (eg sodium toluenesulfonate)				2-6		
sodium perborate (as $\text{NaBO}_3\cdot\text{H}_2\text{O}$, or $\text{NaBO}_3\cdot 4\text{H}_2\text{O}$) or borate (as B_4O_7)	12-18		4-9	0-2	1-3	0-20
TAED (or NOBS)	2-7		1-5			0-5
Phosphonate (e.g. EDTMPA)						
Ethanol				1-3		
carboxymethylcellulose			0-2	0-1		
Polymers (PEG, PVP)						
Anchoring polymers (e.g. maleic/acrylic acid copolymer PVP, PEG)	1-5	1-5	1-5		0-3	
Propylene glycol				2-5		
Glycerol					3-8	
Enzymes (mannanase)	0-5	0-5	0-5	0-5	0-5	0-5
minor ingredients (e.g. suds, suppressors, perfume, optical brightener, photobleach)	0-5	0-5	0-5	0-5	0-5	0-3

Anionic Model Detergent A

[00204] A model granular detergent (90% anionic out of total surfactants, pH in solution 10.2) is made by mixing the following ingredients (% by weight):

- 5 8.7% anionic surfactant: LAS ($\text{C}_{10}\text{-C}_{13}$)
 7.4% anionic surfactant: AS (C_{12})
 1.8% Nonionic surfactant: alcohol ethoxylate ($\text{C}_{12}\text{-C}_{15}$ 7EO)
 30% Zeolite P (Wessalite P)
 18% Sodium Carbonate
- 10 5% Sodium Citrate
 17% Sodium sulfate
 0.3% Carboxy-Methyl-Cellulose
 6.5% Sodium-percarbonate monohydrate
 2.1% NOBS

15

Anionic model detergent B

[00205] A second model granular detergent (79% anionic out of total surfactants, pH in solution 10.2) is made by mixing the following ingredients (% by weight):

27% anionic surfactant: AS (C_{12})

7% Nonionic surfactant (C₁₂₋₁₅, 7EO)

60% Zeolite P (Wessalite P)

5% Sodium Carbonate

0.6% Sokalan CP5

5 1.5% Carboxy-Methyl-Cellulose

Anionic/non-ionic Model Detergent

[00206] A model detergent solution (32% anionic out of total surfactant, pH 10.2) is made by adding the following ingredients to 3.2 mM Ca²⁺/Mg²⁺(5:1) in pure water:

- 10 0.300 g/l of alkyl sulphate (AS; C₁₄₋₁₆);
 0.650 g/l of alcohol ethoxylate (AEO; C₁₂₋₁₄, 6EO);
 1.750 g/l of Zeolite P
 0.145 g/l of Na₂CO₃
 0.020 g/l of Sokalan CP5
 15 0.050 g/l of CMC (carboxy-methyl cellulose)

Low detergent compositions

European laundry powder detergent

[00207] 15% of surfactant of which 6% was LAS, 3% was AES and 6% was non ionic surfactants. It further contained 47% builder comprising fatty acid, zeolite A, carbonate and silicate.

[00208] 15% of surfactant of which 3% was AES, 6% was LAS and 6% was non ionic surfactants. It further comprised 47% builder comprising fatty acid, zeolite A, carbonate, silicate, and it comprised 5% polycarboxylate polymers.

[00209] 15% of surfactant of which 3% was AES, 6% was LAS and 6% was non ionic surfactants. It further contained 47% builder comprising fatty acid, zeolite A, carbonate, silicate, and it comprised 5 % polycarboxylate polymers.

[00210] 15% of surfactant of which 6% was LAS, 3% was AES and 6% was nonionic surfactants. It further contained 47% builder consisting of fatty acid, zeolite A, carbonate & silicate, 5% polycarboxylate dispersing polymers, 15% sodium perborate, and 4% tetraacetyl-ethylene-diamine (TAEO).

[00211] 15% of surfactant of which 6% was LAS, 3 % was AES and 6% was non ionic surfactants. It further contained 47% builder consisting of fatty acid, 22% zeolite A, carbonate and silicate, and 5% polycarboxylate dispersing polymers.

[00212] 15% of surfactant of which 6% was LAS, 3 % was AES and 6% was non ionic surfactants. It further contained 47% builder consisting of fatty acid, 22% zeolite A, carbonate and silicate, and 5% polycarboxylate dispersing polymers.

[00213] 15% of surfactant of which 6% was LAS, 3 % was AES and 6% was nonionic surfactants. It

further contained 47% builder consisting of fatty acid, 22% zeolite A, carbonate and silicate, and 5% polycarboxylate dispersing polymers.

[00214] 21 % of surfactant of which 8.1 % was LAS, 6.5% was AS, 4.0% was non ionic surfactants, and 2.5% was cationic surfactants (DSDMAC). It further contained 64% builder consisting of fatty acid, carbonate, zeolite A, silicates, and citrate, and also contained 2.7% of dispersing polymers.

[00215] 16.9 % surfactants including soap of which 11 % was LAS and 5.9 % non-ionic and 4.1 % soap, and 63 % builders.

European Liquid Laundry Detergent

[00216] 27% of surfactant of which 16.9% was AS, 6.7% was nonionic surfactants, and 3.5% was cationic surfactants (DSDMAC). It further contained 18.7% builder consisting of fatty acid, carbonate, citrate, and boric acid.

North American laundry liquid detergent

[00217] 23 % of surfactant of which 16% was AES, 5% was LAS and 2% was non ionic surfactants. It further contained 6% builder comprising soap, citric acid, DTPA and calcium formate.

[00218] 23% of surfactant of which 16% was AES, 5% was LAS and 2% was non ionic surfactants. It further contained 6% builder consisting of soap, citric acid, DTPA and calcium formate, and 5% polycarboxylate dispersing polymers.

North American Laundry Powder detergent

[00219] 16.3% of surfactant of which 7.8% was LAS, 6.7% was AS and 1.8% was nonionic surfactants, and 60% builder comprising fatty acid, zeolite A, carbonate and silicate.

[00220] 14.9% of surfactant of which 11.5% was LAS and 3.4% was non ionic surfactants, and 55% builder comprising fatty acid, zeolite A, carbonate and silicate.

[00221] 19.5% of surfactant of which 4.5% was LAS, 13% was AS and 2% was non ionic surfactants, and 61% builder comprising fatty acid, zeolite A, carbonate and silicate.

Japanese laundry powder detergent

[00222] 24.3% of surfactant of which 11.1 % was LAS, 11.6% was ester sulfonate and 1.6% was nonionic surfactants, and 60% builder comprising fatty acid, zeolite A, carbonate and silicate.

[00223] 27.9% of surfactant of which 15 27.5% was LAS and 0.4% was nonionic surfactants, and 64% builder comprising zeolite A, carbonate, citrate, phosphates and silicate.

European color compact laundry powder

[00224] 21.1 % of a surfactant system, of which 8.1 % was LAS, 6.5% was AS, 2.5% was Arguat 2T-70, and 4% was non-ionic surfactants, and 64% builder comprising fatty acid, zeolite A, carbonate, citric

acid and silicate. The surfactant system was prepared separately from the builder. The surfactant system was prepared either Neodol25-7 or Lutensol ON60 as nonionic surfactant.

Detergent composition				
Ingredients	Ex. 1	Ex. 2	Ex.3	Ex.4
Material	Level (parts as is)	Level (parts as is)	Level (parts as is)	Level (parts as is)
Glycerol	3.17	3.17	3.17	3.17
MPG	5.7	5.7	5.7	5.7
NaOH	2.13	2.13	2.13	2.13
TEA	2.05	2.05	2.05	2.05
Neodol 25-7	12.74	12.74	12.74	12.74
F-Dye	0.18	0.18	0.18	0.18
Citric Acid	1.71	1.71	1.71	1.71
LAS (as LAS Acid)	8.49	8.49	8.49	8.49
Fatty acid	3.03	3.03	3.03	3.03
Empigen BB	1.5	1.5	1.5	1.5
SLES	4.24	4.24	4.24	4.24
Dequest 2066	0.875	0.875	0.875	0.875
Patent Blue	0.00036	0.00036	0.00036	0.00036
Acid Yellow	0.00005	0.00005	0.00005	0.00005
Opacifier	0.0512	0.0512	0.0512	0.0512
Perfume	0.734	0.734	0.734	0.734
Borax	10	10	10	10
Savinase	2.362	2.362	2.362	2.362
Stainzyme	0.945	0.945	0.945	0.945
Soap	3.03	3.03	3.03	3.03
EPEI 20E0 (ex Nippon Shokubai) polyethyleneimine having a weight average molecular weight of about 600, and wherein the polyethyleneimine has been modified by alkoxylation with an average 20 ethylene oxide moieties	5.5	5.5	5.5	9
Mannanase	3	3	3	3
Texcare SRN170 (ex Clariant) soil release polymer	0	7.5	0	0
Sokolan CP5 (ex BASF) Soil-release polymer	0	0	20	0

Enzymatic detergent and bleaching composition	
Ingredients	% by weight
Sodium dodecyl benzene sulphonate	6.5
C14-C15 primary alcohol, condensed with 11 moles of ethylene oxide	2
Sodium stearate	1
Sodium silicate	7
Sodium carboxymethyl cellulose	0.5
Na ₂ SO ₄	37
Pentasodium triphosphate	15
Trisodium orthophosphate	5
Fluorescer	0.2
Ethylene diamine tetraacetic acid	0.5
Water	6.2
Dyes	0.01
Mannanase	0.001-1
bleach systems	sodium perborate + SNOBS, sodium perborate + TAED, DPDA, MPS All generating 1.5 mmol peracid in solution

Enzymatic detergent and bleaching composition	
Ingredients	% by weight
Sodium dodecyl benzene sulphonate	8.5
C14-C15 primary alcohol, condensed with 11 moles of ethylene oxide	4
sodium hardened rapeseed oil soap	1.5

sodium triphosphate	33
sodium carbonate	5
sodium silicate	6
sodium sulphate	20
water	9
fluorescers, soil-suspending agents, dyes, perfumes	minor amount
anti-foam granules	1.2
Dequest R 2047 (34% pure)	0.3
Mannanase	0.001-1

Detergent compositions		
Ingredients	% wt	% wt
sodium alkylbenzenesulphonate	24	28
pentasodium tripolyphosphate	15	2.1
alkaline sodium silicate sodium	10	12
carboxymethylcellulose sodium	0.6	0.6
sulphate	32.5	15.4
fluorescer	0.4	0.4
sodium carbonate	10	35
miscellaneous + water	to 100%	to 100%
Mannanase	0.001-1	0.001-1

Enzymatic Detergent composition	
Ingredients	% by weight
sodium linear dodecylbenzenesulphonate	13.35
sodium C ₁₂ -C ₁₃ alcohol (6.5 E0) sulphate	6.67
sodium carbonate	54.2
sodium tripolyphosphate	9.01
sodium silicate	4.6
sodium hydroxide	1.66
sodium carboxymethylcellulose	0.5
Dequest 2006	1.9
perfume, dye, water	q.s.
Mannanase	0.001-1
Protease	20GU/mL

Liquid laundry detergent formulation	
Ingredients	Parts by weight
Sodium dodecyl benzene sulphonate	8.5
C12-C15 primary alcohol, condensed with 7 moles of ethylene oxide	4
Sodium-hardened rapeseed oil soap	1.5
Sodium triphosphate	33
Sodium carbonate	5
Sodium silicate	6
Sodium sulphate	20
Water	9
Fluorescers, soil-suspending agents, dyes, perfumes	minor amount
Sodium perborate	12
Tetraacetyl ethylene diamine (TAED) (granules)	2
Proteolytic enzyme (Savinase ex NOVO)	0.4
Mannanase	0.001-1
Protease	20GU/mL

Liquid detergent compositions				
	A	B	C	D
sodium dodecylbenzene sulphonate	9	9	9	9
C13-C15 linear primary alcohol, condensed with 7 moles of ethylene oxide (e.g. Synperonic A7)	1	4	4	1
C13-C15 linear primary alcohol, condensed with 3 moles of ethylene oxide (e.g. Synperonic A3)	3	0	0	3
sodium tripolyphosphate	23	23	0	0
zeolite type 4A	0	0	24	24
copolymer of acrylic acid with maleic anhydride	0	0	4	4
sodium polyacrylate	2	2	0	0
alkaline silicate	5	5		
fluorescer	0.25	0.25	0.16	0.16

EDTA	0.15	0.15	0.18	0.18
SCMC	0.5	0.5	0.55	0.55
salt	2	2	0	0
sodium sulphate	26.8	26.8	22.31	22.31
sodium carbonate	0	0	10.3	10.3
moisture	10	10	11	11
TAED	3	3	3.3	3.3
sodium perborate monohydrate	10	10	8	8
calcium Dequest ^{® 2047}	0.7	0.7	0.3	0.3
foam depressor	3	3	2.5	2.5
perfume	0.2	0.2	0	0
alkaline protease	0.4	0.4	0.4	0.4
Mannanase	0.001-1			

Dishwashing composition	
Ingredients	% by weight
sodium tripolyphosphate	24
soda ash	20
sodium disilicate	11
linear C10 alcohol, condensed with 6 moles of ethylene oxide and 24 moles of propylene oxide	2.5
sodium sulphate	44
water	to 100
Mannanase	0.001-1

[00225] The cleaning compositions of the present invention are advantageously employed for example, in laundry applications, hard surface cleaning, dishwashing applications, as well as cosmetic applications such as dentures, teeth, hair and skin. In addition, due to the unique advantages of increased effectiveness in lower temperature solutions, the enzymes of the present invention are ideally suited for laundry applications. Furthermore, the enzymes of the present invention find use in granular and liquid compositions.

[00226] The variant mannanase enzymes of the present invention also find use in cleaning additive products. In some embodiments, low temperature solution cleaning applications find use. In some embodiments, the present invention provides cleaning additive products including at least one enzyme of the present invention is ideally suited for inclusion in a wash process when additional bleaching effectiveness is desired. Such instances include, but are not limited to low temperature solution cleaning applications. In some embodiments, the additive product is in its simplest form, one or more mannanase enzymes. In some embodiments, the additive is packaged in dosage form for addition to a cleaning process. In some embodiments, the additive is packaged in dosage form for addition to a cleaning

process where a source of peroxygen is employed and increased bleaching effectiveness is desired. Any suitable single dosage unit form finds use with the present invention, including but not limited to pills, tablets, gelcaps, or other single dosage units such as pre-measured powders or liquids. In some embodiments, filler(s) or carrier material(s) are included to increase the volume of such compositions.

5 Suitable filler or carrier materials include, but are not limited to, various salts of sulfate, carbonate and silicate as well as talc, clay and the like. Suitable filler or carrier materials for liquid compositions include, but are not limited to water or low molecular weight primary and secondary alcohols including polyols and diols. Examples of such alcohols include, but are not limited to, methanol, ethanol, propanol and isopropanol. In some embodiments, the compositions contain from about 5% to about 90% of such materials. Acidic fillers find use to reduce pH. Alternatively, in some embodiments, the cleaning additive includes adjunct ingredients, as more fully described below.

10 **[00227]** The present cleaning compositions and cleaning additives require an effective amount of at least one of the mannanase enzyme variants provided herein, alone or in combination with other mannanase enzymes and/or additional enzymes. The required level of enzyme is achieved by the addition of one or more mannanase enzyme variants of the present invention. Typically the present cleaning compositions comprise at least about 0.0001 weight percent, from about 0.0001 to about 10, from about 0.001 to about 1, or even from about 0.01 to about 0.1 weight percent of at least one of the variant mannanase enzymes of the present invention.

15 **[00228]** The required level of enzyme is achieved by the addition of one or more disclosed Bleman1 polypeptide. Typically the present cleaning compositions will comprise at least about 0.0001 weight percent, from about 0.0001 to about 10, from about 0.001 to about 1, or even from about 0.01 to about 0.1 weight percent of at least one of the disclosed Bleman1 polypeptides.

20 **[00229]** The cleaning compositions herein are typically formulated such that, during use in aqueous cleaning operations, the wash water will have a pH of from about 5.0 to about 11.5, or about 6.0 to 8.0 or even from about 7.5 to about 10.5. Liquid product formulations are typically formulated to have a neat pH from about 3.0 to about 9.0 or even from about 3 to about 8. Granular laundry products are typically formulated to have a pH from about 6 to about 11, or even from about 8 to about 10. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

25 **[00230]** Suitable "low pH cleaning compositions" typically have a neat pH of from about 3 to about 8, and are typically free of surfactants that hydrolyze in such a pH environment. Such surfactants include sodium alkyl sulfate surfactants that comprise at least one ethylene oxide moiety or even from about 1 to about 16 moles of ethylene oxide. Such cleaning compositions typically comprise a sufficient amount of a pH modifier, such as sodium hydroxide, monoethanolamine or hydrochloric acid, to provide such cleaning composition with a neat pH of from about 3 to about 8. Such compositions typically comprise at least one acid stable enzyme. In some embodiments, the compositions are liquids, while in other embodiments, they are solids. The pH of such liquid compositions is typically measured as a neat pH.

The pH of such solid compositions is measured as a 10% solids solution of said composition wherein the solvent is distilled water. In these embodiments, all pH measurements are taken at 20°C, unless otherwise indicated.

[00231] The term “granular composition” refers to a conglomeration of discrete solid, macroscopic particles. Powders are a special class of granular material due to their small particle size, which makes them more cohesive and more easily suspended.

[00232] In some embodiments, when the variant mannanase enzyme(s) is/are employed in a granular composition or liquid, it is desirable for the variant mannanase enzyme to be in the form of an encapsulated particle to protect the variant mannanase enzyme from other components of the granular composition during storage. In addition, encapsulation is also a means of controlling the availability of the variant mannanase enzyme during the cleaning process. In some embodiments, encapsulation enhances the performance of the variant mannanase enzyme(s) and/or additional enzymes. In this regard, the variant mannanase enzymes of the present invention are encapsulated with any suitable encapsulating material known in the art. In some embodiments, the encapsulating material typically encapsulates at least part of the catalyst for the variant mannanase enzyme(s) of the present invention. Typically, the encapsulating material is water-soluble and/or water-dispersible. In some embodiments, the encapsulating material has a glass transition temperature (T_g) of 0°C or higher. Glass transition temperature is described in more detail in WO 97/11151. The encapsulating material is typically selected from consisting of carbohydrates, natural or synthetic gums, chitin, chitosan, cellulose and cellulose derivatives, silicates, phosphates, borates, polyvinyl alcohol, polyethylene glycol, paraffin waxes, and combinations thereof. When the encapsulating material is a carbohydrate, it is typically selected from monosaccharides, oligosaccharides, polysaccharides, and combinations thereof. In some typical embodiments, the encapsulating material is a starch (*See e.g.*, EP 0 922 499; US 4,977,252; US 5,354,559, and US 5,935,826). In some embodiments, the encapsulating material is a microsphere made from plastic such as thermoplastics, acrylonitrile, methacrylonitrile, polyacrylonitrile, polymethacrylonitrile and mixtures thereof; commercially available microspheres that find use include, but are not limited to those supplied by EXPANCEL® (Stockviksverken, Sweden), and PM 6545, PM 6550, PM 7220, PM 7228, EXTENDOSPHERES®, LUXSIL®, Q-CEL®, and SPHERICEL® (PQ Corp., Valley Forge, PA).

[00233] As described herein, the variant mannanase enzymes of the present invention find particular use in the cleaning industry, including, but not limited to laundry and dish detergents. These applications place enzymes under various environmental stresses. The variant mannanase enzymes of the present invention provide advantages over many currently used enzymes, due to their stability under various conditions.

[00234] In some embodiments, the Bleman1 polypeptides find particular use in the cleaning industry, including, but not limited to laundry and dish detergents. These applications place enzymes under

various environmental stresses. The Bleman1 polypeptides may provide advantages over many currently used enzymes, due to their stability under various conditions.

[00235] Indeed, there are a variety of wash conditions including varying detergent formulations, wash water volumes, wash water temperatures, and lengths of wash time, to which mannanase enzymes involved in washing are exposed. In addition, detergent formulations used in different geographical areas have different concentrations of their relevant components present in the wash water. For example, European detergents typically have about 2000-10000 ppm of detergent components in the wash water, while Asian detergents typically have approximately 300-2500 ppm of detergent components in the wash water. In North America, particularly the United States, detergents typically have about 300ppm-1500ppm of detergent components present in the wash water.

[00236] A low detergent concentration system includes detergents where less than about 800 ppm of the detergent components are present in the wash water. Japanese detergents are typically considered low detergent concentration system as they have approximately 667 ppm of detergent components present in the wash water.

[00237] A medium detergent concentration includes detergents where between about 800 ppm and about 2000 ppm of the detergent components are present in the wash water. North American detergents are generally considered to be medium detergent concentration systems as they have approximately 975 ppm of detergent components present in the wash water. Brazil typically has approximately 1500 ppm of detergent components present in the wash water.

[00238] A high detergent concentration system includes detergents where greater than about 2000 ppm of the detergent components are present in the wash water. European detergents are generally considered to be high detergent concentration systems as they have approximately 2000-10000 ppm of detergent components in the wash water.

[00239] Latin American detergents are generally high suds phosphate builder detergents and the range of detergents used in Latin America can fall in both the medium and high detergent concentrations as they range from 1500 ppm to 6000 ppm of detergent components in the wash water. As mentioned above, Brazil typically has approximately 1500 ppm of detergent components present in the wash water. However, other high suds phosphate builder detergent geographies, not limited to other Latin American countries, may have high detergent concentration systems up to about 6000 ppm of detergent components present in the wash water.

[00240] In light of the foregoing, it is evident that concentrations of detergent compositions in typical wash solutions throughout the world varies from less than about 800 ppm of detergent composition ("low detergent concentration geographies"), for example about 667 ppm in Japan, to between about 800 ppm to about 2000 ppm ("medium detergent concentration geographies"), for example about 975 ppm in U.S. and about 1500 ppm in Brazil, to greater than about 2000 ppm ("high detergent concentration geographies"), for example about 4500 ppm to about 5000 ppm in Europe and about 6000 ppm in high suds phosphate builder geographies.

[00241] The concentrations of the typical wash solutions are determined empirically. For example, in the U.S., a typical washing machine holds a volume of about 64.4 L of wash solution. Accordingly, in order to obtain a concentration of about 1000 ppm of detergent within the wash solution about 64.4 g of detergent composition must be added to the 64.4 L of wash solution. This amount is the typical amount measured into the wash water by the consumer using the measuring cup provided with the detergent.

[00242] As a further example, different geographies use different wash temperatures. The temperature of the wash water in Japan is typically less than that used in Europe. For example, the temperature of the wash water in North America and Japan is typically between about 10 and about 30°C (*e.g.*, about 20°C), whereas the temperature of wash water in Europe is typically between about 30 and about 60°C (*e.g.*, about 40°C). However, in the interest of saving energy, many consumers are switching to using cold water washing. In addition, in some further regions, cold water is typically used for laundry, as well as dish washing applications. In some embodiments, the “cold water washing” of the present invention utilizes “cold water detergent” suitable for washing at temperatures from about 10°C to about 40°C, or from about 20°C to about 30°C, or from about 15°C to about 25°C, as well as all other combinations within the range of about 15°C to about 35°C, and all ranges within 10°C to 40°C.

[00243] As a further example, different geographies typically have different water hardness. Water hardness is usually described in terms of the grains per gallon mixed $\text{Ca}^{2+}/\text{Mg}^{2+}$. Hardness is a measure of the amount of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the water. Most water in the United States is hard, but the degree of hardness varies. Moderately hard (60-120 ppm) to hard (121-181 ppm) water has 60 to 181 parts per million (parts per million converted to grains per U.S. gallon is ppm # divided by 17.1 equals grains per gallon) of hardness minerals.

Water	Grains per gallon	Parts per million
Soft	less than 1.0	less than 17
Slightly hard	1.0 to 3.5	17 to 60
Moderately hard	3.5 to 7.0	60 to 120
Hard	7.0 to 10.5	120 to 180
Very hard	greater than 10.5	greater than 180

[00244] European water hardness is typically greater than about 10.5 (for example about 10.5 to about 20.0) grains per gallon mixed $\text{Ca}^{2+}/\text{Mg}^{2+}$ (*e.g.*, about 15 grains per gallon mixed $\text{Ca}^{2+}/\text{Mg}^{2+}$). North American water hardness is typically greater than Japanese water hardness, but less than European water hardness. For example, North American water hardness can be between about 3 to about 10 grains, about 3 to about 8 grains or about 6 grains. Japanese water hardness is typically lower than North American water hardness, usually less than about 4, for example about 3 grains per gallon mixed $\text{Ca}^{2+}/\text{Mg}^{2+}$.

[00245] Accordingly, in some embodiments, the present invention provides variant mannanase enzymes that show surprising wash performance in at least one set of wash conditions (*e.g.*, water

temperature, water hardness, and/or detergent concentration). In some embodiments, the variant mannanase enzymes of the present invention are comparable in wash performance to other mannanase enzymes. In some embodiments, the variant mannanase enzymes of the present invention exhibit enhanced wash performance as compared to mannanase enzymes currently commercially available.

Thus, in some embodiments of the present invention, the variant mannanase enzymes provided herein exhibit enhanced oxidative stability, enhanced thermostability, enhanced cleaning capabilities under various conditions, and/or enhanced chelator stability. In addition, the variant mannanase enzymes of the present invention find use in cleaning compositions that do not include detergents, again either alone or in combination with builders and stabilizers.

[00246] In some embodiments, the present disclosure provides Bleman1 polypeptides that show surprising wash performance in at least one set of wash conditions (*e.g.*, water temperature, water hardness, and/or detergent concentration). In some embodiments, the Bleman1 polypeptides are comparable in wash performance to other endo- β -mannanases. In some embodiments, the Bleman1 polypeptides exhibit enhanced wash performance as compared to endo- β -mannanases currently commercially available. Thus, in some preferred embodiments, the Bleman1 polypeptides provided herein exhibit enhanced oxidative stability, enhanced thermal stability, enhanced cleaning capabilities under various conditions, and/or enhanced chelator stability. In addition, the Bleman1 polypeptides may find use in cleaning compositions that do not include detergents, again either alone or in combination with builders and stabilizers.

[00247] In some embodiments of the present invention, the cleaning compositions comprise at least one variant mannanase enzyme of the present invention at a level from about 0.00001 % to about 10% by weight of the composition and the balance (*e.g.*, about 99.999% to about 90.0%) comprising cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention comprises at least one variant mannanase enzyme at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% by weight of the composition and the balance of the cleaning composition (*e.g.*, about 99.9999% to about 90.0%, about 99.999 % to about 98%, about 99.995% to about 99.5% by weight) comprising cleaning adjunct materials.

[00248] In some embodiments, the cleaning compositions of the present invention comprise one or more additional detergent enzymes, which provide cleaning performance and/or fabric care and/or dishwashing benefits. Examples of suitable enzymes include, but are not limited to, proteases, peroxidases, cellulases, beta-glucanases, hemicellulases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo- β -mannanases, exo- β -mannanases, pectin

methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof. In some embodiments, a combination of enzymes is used (*i.e.*, a “cocktail”) comprising conventional applicable enzymes like protease, amylase, lipase and/or cellulase in conjunction with the mannanase of the present invention is used.

5 [00249] For example, a mannanase enzyme variant of the invention can be combined with a protease. Suitable proteolytic enzymes include those of animal, vegetable or microbial origin. In some
embodiments, microbial proteolytic enzymes are used. In some embodiments, the proteolytic enzyme is
preferably an alkaline microbial proteolytic enzyme or a trypsin-like proteolytic enzyme. Examples of
alkaline protease enzymes, especially those derived from *Bacillus* (*e.g.*, *lentus*, *amyloliquefaciens*,
10 Carlsberg, 309, 147 and 168). Additional examples include those mutant proteolytic enzymes described
in U.S. Pat. Nos. RE 34,606, 5,955,340, 5,700,676, 6,312,936, and 6,482,628, all of which are
incorporated herein by reference. Additional protease examples include, but are not limited to trypsin
(*e.g.*, of porcine or bovine origin), and the *Fusarium* protease enzyme described in WO 89/06270. In
some embodiments, commercially available protease enzymes that find use in the present invention
15 include, but are not limited to MAXATASE®, MAXACAL™, MAXAPEM™, OPTICLEAN®,
OPTIMASE®, PROPERASE®, PURAFECT®, PURAFECT® OXP, PURAMAX™, EXCELLASE™,
and PURAFAST™ (Genencor); ALCALASE®, SAVINASE®, PRIMASE®, DURAZYM™,
POLARZYME®, OVOZYME®, KANNASE®, LIQUANASE®, NEUTRASE®, RELEASE® and
ESPERASE® (Novozymes); BLAP™ and BLAP™ variants (Henkel Kommanditgesellschaft auf
20 Aktien, Duesseldorf, Germany), and KAP (B. alkalophilus protease; Kao Corp., Tokyo, Japan). Various
proteolytic enzymes are described in WO95/23221, WO 92/21760, U.S. Pat. Publ. No. 2008/0090747,
and U.S. Pat. Nos. 5,801,039, 5,340,735, 5,500,364, 5,855,625, US RE 34,606, 5,955,340, 5,700,676,
6,312,936, and 6,482,628, and various other patents. In some further embodiments, metalloprotease
enzymes find use in the present invention, including but not limited to the neutral metalloprotease
25 enzyme described in WO 07/044993.

[00250] In some embodiments of the present invention, any suitable amylase finds use in the present
invention. In some embodiments, any amylase (*e.g.*, alpha and/or beta) suitable for use in alkaline
solutions also find use. Suitable amylases include, but are not limited to those of bacterial or fungal
origin. Chemically or genetically modified mutants are included in some embodiments. Amylases that
30 find use in the present invention, include, but are not limited to α -amylases obtained from *B.*
licheniformis (See *e.g.*, GB 1,296,839). Commercially available amylases that find use in the present
invention include, but are not limited to DURAMYL®, TERMAMYL®, FUNGAMYL®,
STAINZYME®, STAINZYME PLUS®, STAINZYME ULTRA®, and BAN™ (Novozymes), as well as
POWERASE™, RAPIDASE® and MAXAMYL® P (Genencor).

35 [00251] In some embodiments of the present invention, the cleaning compositions of the present
invention further comprise amylases at a level from about 0.00001 % to about 10% of additional amylase
by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In

some other embodiments of the present invention, the cleaning compositions of the present invention also comprise amylases at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% amylase by weight of the composition.

[00252] In some embodiments of the present disclosure, any suitable pectin degrading enzyme may be used. As used herein, "pectin degrading enzyme(s)" encompass arabinanase (EC 3.2.1.99), galactanases (EC 3.2.1.89), polygalacturonase (EC 3.2.1.15) exo-polygalacturonase (EC 3.2.1.67), exo-poly-alpha-galacturonidase (EC 3.2.1.82), pectin lyase (EC 4.2.2.10), pectin esterase (EC 3.2.1.11), pectate lyase (EC 4.2.2.2), exo-polygalacturonate lyase (EC 4.2.2.9) and hemicellulases such as endo-1,3- β -xylosidase (EC 3.2.1.32), xylan-1,4- β -xylosidase (EC 3.2.1.37) and α -L-arabinofuranosidase (EC 3.2.1.55). Pectin degrading enzymes are natural mixtures of the above mentioned enzymatic activities. Pectin enzymes therefore include the pectin methylesterases which hydrolyse the pectin methyl ester linkages, polygalacturonases which cleave the glycosidic bonds between galacturonic acid molecules, and the pectin transeliminases or lyases which act on the pectic acids to bring about non-hydrolytic cleavage of α -1,4 glycosidic linkages to form unsaturated derivatives of galacturonic acid.

[00253] Suitable pectin degrading enzymes include those of plant, fungal, or microbial origin. In some embodiments, chemically or genetically modified mutants are included. In some embodiments, the pectin degrading enzymes are alkaline pectin degrading enzymes, *i.e.*, enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH of from about 7.0 to about 12. In certain other embodiments, the pectin degrading enzymes are enzymes having their maximum activity at a pH of from about 7.0 to about 12. Alkaline pectin degrading enzymes are produced by alkalophilic microorganisms *e.g.*, bacterial, fungal, and yeast microorganisms such as *Bacillus* species. In some embodiments, the microorganisms are *Bacillus firmus*, *Bacillus circulans*, and *Bacillus subtilis* as described in JP 56131376 and JP 56068393. Alkaline pectin decomposing enzymes may include but are not limited to galacturon-1,4- α -galacturonase (EC 3.2.1.67), poly-galacturonase activities (EC 3.2.1.15, pectin esterase (EC 3.1.1.11), pectate lyase (EC 4.2.2.2) and their iso enzymes. Alkaline pectin decomposing enzymes can be produced by the *Erwinia* species. In some embodiments, the alkaline pectin decomposing enzymes are produced by *E. chrysanthemi*, *E. carotovora*, *E. amylovora*, *E. herbicola*, and *E. dissolvens* as described in JP 59066588, JP 63042988, and in *World J. Microbiol. Microbiotechnol.* (8, 2, 115-120) 1992. In certain other embodiments, the alkaline pectin enzymes are produced by *Bacillus* species as disclosed in JP 73006557 and *Agr. Biol. Chem.* (1972), 36 (2) 285-93.

[00254] In some embodiments of the present disclosure, the disclosed cleaning compositions further comprise pectin degrading enzymes at a level from about 0.00001% to about 10% of additional pectin degrading enzyme by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions also comprise pectin degrading enzymes at a level of about 0.0001% to about 10%, about 0.001% to about 5%, about

0.001% to about 2%, about 0.005% to about 0.5% pectin degrading enzyme by weight of the composition.

[00255] In some other embodiments, any suitable xyloglucanase finds used in the cleaning compositions of the present disclosure. Suitable xyloglucanases include, but are not limited to those of plant, fungal, or bacterial origin. Chemically or genetically modified mutants are included in some embodiments. As used herein, "xyloglucanase(s)" encompass the family of enzymes described by Vincken and Voragen at Wageningen University [Vincken et al (1994) *Plant Physiol.*, 104, 99-107] and are able to degrade xyloglucans as described in Hayashi et al (1989) *Plant. Physiol. Plant Mol. Biol.*, 40, 139-168. Vincken et al demonstrated the removal of xyloglucan coating from cellulose of the isolated apple cell wall by a xyloglucanase purified from *Trichoderma viride* (endo-IV-glucanase). This enzyme enhances the enzymatic degradation of cell wall-embedded cellulose and work in synergy with pectic enzymes. Rapidase LIQ+ from Gist-Brocades contains a xyloglucanase activity.

[00256] In some embodiments of the present disclosure, the disclosed cleaning compositions further comprise xyloglucanases at a level from about 0.00001% to about 10% of additional xyloglucanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions also comprise xyloglucanases at a level of about 0.0001% to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% xyloglucanase by weight of the composition. In certain other embodiments, xyloglucanases for specific applications are alkaline xyloglucanases, *i.e.*, enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. In certain other embodiments, the xyloglucanases are enzymes having their maximum activity at a pH of from about 7.0 to about 12.

[00257] In some further embodiments, any suitable cellulase finds used in the cleaning compositions of the present invention. Suitable cellulases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Suitable cellulases include, but are not limited to *Humicola insolens* cellulases (*See e.g.*, U.S. Pat. No. 4,435,307). Especially suitable cellulases are the cellulases having color care benefits (*See e.g.*, EP 0 495 257). Commercially available cellulases that find use in the present include, but are not limited to CELLUZYME®, CAREZYME® (Novozymes), and KAC-500(B)TM (Kao Corporation) PURADAX HA 1200E (Danisco), PURADAX EG 7000L (Danisco). In some embodiments, cellulases are incorporated as portions or fragments of mature wild-type or variant cellulases, wherein a portion of the N-terminus is deleted (*See e.g.*, U.S. Pat. No. 5,874,276). In some embodiments, the cleaning compositions of the present invention further comprise cellulases at a level from about 0.00001 % to about 10% of additional cellulase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise cellulases at a level of about 0.0001 % to about 10%, about 0.001% to

about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% cellulase by weight of the composition.

[00258] In still further embodiments, any lipase suitable for use in detergent compositions also finds use in the present disclosure. Suitable lipases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Examples of useful lipases include *Humicola lanuginosa* lipase (See, e.g., EP 258 068, and EP 305 216), *Rhizomucor miehei* lipase (See, e.g., EP 238 023), *Candida* lipase, such as *C. antarctica* lipase (e.g., the *C. antarctica* lipase A or B; see, e.g., EP 214 761), *Pseudomonas* lipases such as *P. alcaligenes* lipase and *P. pseudoalcaligenes* lipase (See, e.g., EP 218 272), *P. cepacia* lipase (See, e.g., EP 331 376), *P. stutzeri* lipase (See, e.g., GB 1,372,034), *P. fluorescens* lipase, *Bacillus* lipase (e.g., *B. subtilis* lipase [Dartois *et al.*, (1993) *Biochem. Biophys. Acta* 1131:253-260]; *B. stearothermophilus* lipase [See, e.g., JP 64/744992]; and *B. pumilus* lipase [See, e.g., WO 91/16422]). Furthermore, a number of cloned lipases find use in some embodiments of the present disclosure, including but not limited to *Penicillium camembertii* lipase (See, Yamaguchi *et al.*, [1991] *Gene* 103:61-67), *Geotricum candidum* lipase (See, Schimada *et al.*, [1989] *J. Biochem.* 106:383-388), and various *Rhizopus* lipases such as *R. delemar* lipase (See, Hass *et al.*, [1991] *Gene* 109:117-113), *R. niveus* lipase (Kugimiya *et al.*, [1992] *Biosci. Biotech. Biochem.* 56:716-719), and *R. oryzae* lipase. Other types of lipolytic enzymes such as cutinases also find use in some embodiments of the present disclosure, including but not limited to the cutinase derived from *Pseudomonas mendocina* (See, WO 88/09367), and the cutinase derived from *Fusarium solani pisi* (See, WO 90/09446). Additional suitable lipases include commercially available lipases such as M1 LIPASE™, LUMA FAST™, and LIPOMAX™ (Genencor, A Danisco Division, Palo Alto, CA); LIPEX®, LIPOCLEAN®, LIPOLASE® and LIPOLASE® ULTRA (Novozymes A/S, Denmark); and LIPASE P™ "Amano" (Amano Pharmaceutical Co. Ltd., Japan).

[00259] In some embodiments, the disclosed cleaning compositions further comprise lipases at a level from about 0.00001% to about 10% of additional lipase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions also comprise lipases at a level of about 0.0001% to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% lipase by weight of the composition.

[00260] Any mannanase suitable for use in detergent compositions can be used in combination with the mannanase of the present invention. Suitable mannanases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Various mannanases are known which find use in the present invention (See e.g., U.S. Pat. No. 6,566,114, U.S. Pat. No. 6,602,842, and US Patent No. 6,440,991, all of which are incorporated herein by reference). In some embodiments, the cleaning compositions of the present invention further comprise mannanases at a level from about 0.00001 % to about 10% of additional mannanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some

embodiments of the present invention, the cleaning compositions of the present invention also comprise mannanases at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% mannanase by weight of the composition.

[00261] In addition to the Bleman1 polypeptides provided herein, any other suitable endo- β -

mannanases find use in the compositions of the present disclosure. Suitable endo- β -mannanases include, but are not limited to, endo- β -mannanases of the GH26 family of glycosyl hydrolases, endo- β -mannanases of the GH5 family of glycosyl hydrolases, acidic endo- β -mannanases, neutral endo- β -mannanases, and alkaline endo- β -mannanases. Examples of alkaline endo- β -mannanases include those described in U.S. Pat. Nos. 6, 060,299, 6,566,114, and 6,602,842; WO 9535362A1, WO 9964573A1, and WO9964619A1. Additionally, suitable endo- β -mannanases include, but are not limited to those of animal, plant, fungal, or bacterial origin. Chemically or genetically modified mutants are encompassed by the present disclosure.

[00262] Examples of useful endo- β -mannanases include *Bacillus* endo- β -mannanases such as *B. subtilis* endo- β -mannanase (See, e.g., U.S. Pat. No. 6, 060,299, and WO 9964573A1), *B. sp. I633* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *Bacillus sp. AAI12* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. sp. AA349* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. agaradhaerens* NCIMB 40482 endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. halodurans* endo- β -mannanase, *B. clausii* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. licheniformis* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *Humicola* endo- β -mannanases such as *H. insolens* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), and *Caldocellulosiruptor* endo- β -mannanases such as *C. sp.* endo- β -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1).

[00263] Furthermore, a number of identified mannanases (i.e., endo- β -mannanases and exo- β -

mannanases) find use in some embodiments of the present disclosure, including but not limited to *Agaricus bisporus* mannanase (See, Tang *et al.*, [2001] *Appl. Environ. Microbiol.* 67: 2298–2303), *Aspergillus tamarii* mannanase (See, Civas *et al.*, [1984] *Biochem. J.* 219: 857–863), *Aspergillus aculeatus* mannanase (See, Christgau *et al.*, [1994] *Biochem. Mol. Biol. Int.* 33: 917–925), *Aspergillus awamori* mannanase (See, Setati *et al.*, [2001] *Protein Express Purif.* 21: 105–114), *Aspergillus fumigatus* mannanase (See, Puchart *et al.*, [2004] *Biochimica et biophysica Acta.* 1674: 239–250), *Aspergillus niger* mannanase (See, Ademark *et al.*, [1998] *J. Biotechnol.* 63: 199–210), *Aspergillus oryzae* NRRL mannanase (See, Regalado *et al.*, [2000] *J. Sci. Food Agric.* 80: 1343–1350), *Aspergillus sulphureus* mannanase (See, Chen *et al.*, [2007] *J. Biotechnol.* 128(3): 452–461), *Aspergillus terreus* mannanase (See, Huang *et al.*, [2007] *Wei Sheng Wu Xue Bao.* 47(2): 280–284), *Bacillus agaradhaerens* mannanase (See, U.S. Pat No. 6,376,445.), *Bacillus AM001* mannanase (See, Akino *et al.*, [1989] *Arch. Microbiol.* 152: 10–15), *Bacillus brevis* mannanase (See, Araujo and Ward, [1990] *J. Appl. Bacteriol.* 68: 253–261), *Bacillus circulans* K-1 mannanase (See, Yoshida *et al.*, [1998] *Biosci. Biotechnol. Biochem.*

- 62(3): 514–520), *Bacillus polymyxa* mannanase (See, Araujo and Ward, [1990] *J. Appl. Bacteriol.* 68: 253–261), *Bacillus* sp JAMB-750 mannanase (See, Hatada *et al.*, [2005] *Extremophiles*. 9: 497–500), *Bacillus* sp. M50 mannanase (See, Chen *et al.*, [2000] *Wei Sheng Wu Xue Bao*. 40: 62–68), *Bacillus* sp. N 16-5 mannanase (See, Yanhe *et al.*, [2004] *Extremophiles* 8: 447–454), *Bacillus stearothermophilu*
- 5 mannanase (See, Talbot and Sygusch, [1990] *Appl. Environ. Microbiol.* 56: 3505–3510), *Bacillus subtilis* mannanase (See, Mendoza *et al.*, [1994] *World J. Microbiol. Biotechnol.* 10: 51–54), *Bacillus subtilis* B36 mannanase (Li *et al.*, [2006] *Z. Naturforsch (C)*. 61: 840–846), *Bacillus subtilis* BM9602 mannanase (See, Cui *et al.*, [1999] *Wei Sheng Wu Xue Bao*. 39(1): 60–63), *Bacillus subtilis* SA–22 mannanase (See, Sun *et al.*, [2003] *Sheng Wu Gong Cheng Xue Bao*. 19(3): 327–330), *Bacillus subtilis* 168 mannanase
- 10 (See, Helow and Khattab, [1996] *Acta Microbiol. Immunol. Hung.* 43: 289–299), *Bacteroides ovatus* mannanase (See, Gherardini *et al.*, [1987] *J. Bacteriol.* 169: 2038–2043), *Bacteroides ruminicola* mannanase (See, Matsushita *et al.*, [1991] *J. Bacteriol.* 173: 6919–6926), *Caldibacillus cellulovorans* mannanase (See, Sunna *et al.*, [2000] *Appl. Environ. Microbiol.* 66: 664–670), *Caldocellulosiruptor saccharolyticus* mannanase (See, Morris *et al.*, [1995] *Appl. Environ. Microbiol.* 61: 2262–2269),
- 15 *Caldocellum saccharolyticum* mannanase (See, Bicho *et al.*, [1991] *Appl. Microbiol. Biotechnol.* 36: 337–343), *Cellulomonas fimi* mannanase (See, Stoll *et al.*, [1999] *Appl. Environ. Microbiol.* 65(6):2598–2605), *Clostridium butyricum/beijerinckii* mannanase (See, Nakajima and Matsuura, [1997] *Biosci. Biotechnol. Biochem.* 61: 1739–1742), *Clostridium cellulolyticum* mannanase (See, Perret *et al.*, [2004] *Biotechnol. Appl. Biochem.* 40: 255–259), *Clostridium tertium* mannanase (See, Kataoka and Tokiwa,
- 20 [1998] *J. Appl. Microbiol.* 84: 357–367), *Clostridium thermocellum* mannanase (See, Halstead *et al.*, [1999] *Microbiol.* 145: 3101–3108), *Dictyoglomus thermophilum* mannanase (See, Gibbs *et al.*, [1999] *Curr. Microbiol.* 39(6): 351–357), *Flavobacterium* sp mannanase (See, Zakaria *et al.*, [1998] *Biosci. Biotechnol. Biochem.* 62: 655–660), *Gastropoda pulmonata* mannanase (See, Charrier and Rouland, [2001] *J. Expt. Zool.* 290: 125–135), *Littorina brevicula* mannanase (See, Yamamura *et al.*, [1996]
- 25 *Biosci. Biotechnol. Biochem.* 60: 674–676), *Lycopersicon esculentum* mannanase (See, Filichkin *et al.*, [2000] *Plant Physiol.* 134:1080–1087), *Paenibacillus curdolanolyticus* mannanase (See, Pason and Ratanakhanokchai, [2006] *Appl. Environ. Microbiol.* 72: 2483–2490), *Paenibacillus polymyxa* mannanase (See, Han *et al.*, [2006] *Appl. Microbiol. Biotechnol.* 73(3): 618–630), *Phanerochaete chrysosporium* mannanase (See, Wymelenberg *et al.*, [2005] *J. Biotechnol.* 118: 17–34), *Piromyces* sp.
- 30 mannanase (See, Fanutti *et al.*, [1995] *J. Biol. Chem.* 270(49): 29314–29322), *Pomacea insularis* mannanase (See, Yamamura *et al.*, [1993] *Biosci. Biotechnol. Biochem.* 7: 1316–1319), *Pseudomonas fluorescens* subsp. Cellulose mannanase (See, Braithwaite *et al.*, [1995] *Biochem J.* 305: 1005–1010), *Rhodothermus marinus* mannanase (See, Politz *et al.*, [2000] *Appl. Microbiol. Biotechnol.* 53 (6): 715–721), *Sclerotium rolfsii* mannanase (See, Sachslehner *et al.*, [2000] *J. Biotechnol.* 80:127–134),
- 35 *Streptomyces galbus* mannanase (See, Kansoh and Nagieb, [2004] *Anton. van. Leeuwenhoek*. 85: 103–114), *Streptomyces lividans* mannanase (See, Arcand *et al.*, [1993] *J. Biochem.* 290: 857–863), *Thermoanaerobacterium Polysaccharolyticum* mannanase (See, Cann *et al.*, [1999] *J. Bacteriol.* 181:

1643–1651), *Thermomonospora fusca* mannanase (See, Hilge *et al.*, [1998] *Structure* 6: 1433–1444), *Thermotoga maritima* mannanase (See, Parker *et al.*, [2001] *Biotechnol. Bioeng.* 75(3): 322–333), *Thermotoga neapolitana* mannanase (See, Duffaud *et al.*, [1997] *Appl. Environ. Microbiol.* 63: 169–177), *Trichoderma harzanium* strain T4 mannanase (See, Franco *et al.*, [2004] *Biotechnol Appl. Biochem.* 40: 255–259), *Trichoderma reesei* mannanase (See, Stalbrand *et al.*, [1993] *J. Biotechnol.* 29: 229–242), and *Vibrio sp.* mannanase (See, Tamaru *et al.*, [1997] *J. Ferment. Bioeng.* 83: 201–205).

[00264] Additional suitable endo- β -mannanases include commercially available endo- β -mannanases such as HEMICELL[®] (Chemgen); GAMANASE[®] and MANNAWAY[®], (Novozymes A/S, Denmark); PURABRITE[™] and MANNASTAR[™] (Genencor, A Danisco Division, Palo Alto, CA); and PYROLASE[®] 160 and PYROLASE[®] 200 (Diversa).

[00265] In some embodiments of the present disclosure, the cleaning compositions of the present disclosure further comprise endo- β -mannanases at a level from about 0.00001% to about 10% of additional endo- β -mannanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions of the present disclosure also comprise endo- β -mannanases at a level of about 0.0001% to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% endo- β -mannanase by weight of the composition.

[00266] In some embodiments, peroxidases are used in combination with hydrogen peroxide or a source thereof (*e.g.*, a percarbonate, perborate or persulfate) in the compositions of the present invention.

In some alternative embodiments, oxidases are used in combination with oxygen. Both types of enzymes are used for "solution bleaching" (*i.e.*, to prevent transfer of a textile dye from a dyed fabric to another fabric when the fabrics are washed together in a wash liquor), preferably together with an enhancing agent (See *e.g.*, WO 94/12621 and WO 95/01426). Suitable peroxidases/oxidases include, but are not limited to those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. In some embodiments, the cleaning compositions of the present invention further comprise peroxidase and/or oxidase enzymes at a level from about 0.00001 % to about 10% of additional peroxidase and/or oxidase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise, peroxidase and/or oxidase enzymes at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% peroxidase and/or oxidase enzymes by weight of the composition.

[00267] In some embodiments, additional enzymes find use, including but not limited to perhydrolases (See *e.g.*, WO 05/056782). In addition, in some embodiments, mixtures of the above mentioned enzymes are encompassed herein, in particular one or more additional lipolytic enzyme, amylase, protease, mannanase, and/or at least one cellulase. Indeed, it is contemplated that various mixtures of these enzymes will find use in the present invention. It is also contemplated that the varying levels of the variant mannanase enzyme(s) and one or more additional enzymes may both independently

range to about 10%, the balance of the cleaning composition being cleaning adjunct materials. The specific selection of cleaning adjunct materials are readily made by considering the surface, item, or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use (*e.g.*, through the wash detergent use).

5 **[00268]** Examples of suitable cleaning adjunct materials include, but are not limited to, surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dye transfer inhibiting agents, catalytic materials, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal agents, structure elasticizing agents, dispersants, suds suppressors, 10 dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, fabric softeners, carriers, hydrotropes, processing aids, solvents, pigments, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids, pigments, and pH control agents (*See e.g.*, U.S. Pat. Nos. 6,610,642, 6,605,458, 15 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101, all of which are incorporated herein by reference). Embodiments of specific cleaning composition materials are exemplified in detail below. In embodiments in which the cleaning adjunct materials are not compatible with the mannanase enzymes of the present invention in the cleaning compositions, then suitable methods of keeping the cleaning adjunct materials and the mannanase enzyme(s) separated (*i.e.*, not in contact with each other) 20 until combination of the two components is appropriate are used. Such separation methods include any suitable method known in the art (*e.g.*, gels, capsules, encapsulation, tablets, physical separation, etc.).

[00269] In some embodiments, an effective amount of one or more mannanase enzyme(s) provided herein is included in compositions useful for cleaning a variety of surfaces in need of lipid stain removal. Such cleaning compositions include cleaning compositions for such applications as cleaning hard 25 surfaces, fabrics, and dishes. Indeed, in some embodiments, the present invention provides fabric cleaning compositions, while in other embodiments, the present invention provides non-fabric cleaning compositions. It is intended that the present invention encompass detergent compositions in any form (*i.e.*, liquid, granular, bar, semi-solid, gels, emulsions, tablets, capsules, etc.).

[00270] By way of example, several cleaning compositions wherein the mannanase enzymes of the present invention find use are described in greater detail below. In some embodiments in which the cleaning compositions of the present invention are formulated as compositions suitable for use in laundry machine washing method(s), the compositions of the present invention preferably contain at least one surfactant and at least one builder compound, as well as one or more cleaning adjunct materials preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds 35 suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. In some embodiments, laundry compositions also contain softening agents (*i.e.*, as additional cleaning adjunct materials). The compositions of the present invention also find use detergent

additive products in solid or liquid form. Such additive products are intended to supplement and/or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process. In some embodiments, the density of the laundry detergent compositions herein ranges from about 400 to about 1200 g/liter, while in other embodiments, it ranges from about 500 to about 950 g/liter of composition measured at 20°C.

[00271] In embodiments formulated as compositions for use in manual dishwashing methods, the compositions of the invention preferably contain at least one surfactant and preferably at least one additional cleaning adjunct material selected from organic polymeric compounds, suds enhancing agents, group II metal ions, solvents, hydrotropes and additional enzymes.

[00272] In some embodiments, various cleaning compositions such as those provided in U.S. Pat. No. 6,605,458, find use with the variant mannanase enzymes of the present invention. Thus, in some embodiments, the compositions comprising at least one variant mannanase enzyme of the present invention is a compact granular fabric cleaning composition, while in other embodiments, the composition is a granular fabric cleaning composition useful in the laundering of colored fabrics, in further embodiments, the composition is a granular fabric cleaning composition which provides softening through the wash capacity, in additional embodiments, the composition is a heavy duty liquid fabric cleaning composition. In some embodiments, the compositions comprising at least one variant mannanase enzyme of the present invention are fabric cleaning compositions such as those described in U.S. Pat. Nos. 6,610,642 and 6,376,450. In addition, the variant mannanase enzymes of the present invention find use in granular laundry detergent compositions of particular utility under European or Japanese washing conditions (*See e.g.*, U.S. Pat. No. 6,610,642).

[00273] In some alternative embodiments, the present invention provides hard surface cleaning compositions comprising at least one variant mannanase enzyme provided herein. Thus, in some embodiments, the compositions comprising at least one variant mannanase enzyme of the present invention is a hard surface cleaning composition such as those described in U.S. Pat. Nos. 6,610,642, 6,376,450, and 6,376,450.

[00274] In yet further embodiments, the present invention provides dishwashing compositions comprising at least one variant mannanase enzyme provided herein. Thus, in some embodiments, the compositions comprising at least one variant mannanase enzyme of the present invention is a hard surface cleaning composition such as those in U.S. Pat. Nos. 6,610,642 and 6,376,450. In some still further embodiments, the present invention provides dishwashing compositions comprising at least one variant mannanase enzyme provided herein. In some further embodiments, the compositions comprising at least one variant mannanase enzyme of the present invention comprise oral care compositions such as those in U.S. Pat. No. 6,376,450, and 6,376,450. The formulations and descriptions of the compounds and cleaning adjunct materials contained in the aforementioned US Pat. Nos. 6,376,450, 6,605,458, 6,605,458, and 6,610,642, find use with the variant mannanase enzymes provided herein.

[00275] In still further embodiments, the compositions comprising at least one Bleman1 polypeptide

of the present disclosure comprise fabric softening compositions such as those in GB-A1 400898, GB-A1 514 276, EP 0 011 340, EP 0 026 528, EP 0 242 919, EP 0 299 575, EP 0 313 146, and U.S. Pat. No. 5,019,292. The formulations and descriptions of the compounds and softening agents contained in the aforementioned GB-A1 400898, GB-A1 514 276, EP 0 011 340, EP 0 026 528, EP 0 242 919, EP 0 299 575, EP 0 313 146, and U.S. Pat. No. 5,019,292 find use with the Bleman1 polypeptides provided herein.

[00276] The cleaning compositions of the present invention are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,565,422, 5,516,448, 5,489,392, and 5,486,303, all of which are incorporated herein by reference. When a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of a material such as monoethanolamine or an acidic material such as HCl.

[00277] While not essential for the purposes of the present invention, the non-limiting list of adjuncts illustrated hereinafter are suitable for use in the instant cleaning compositions. In some embodiments, these adjuncts are incorporated for example, to assist or enhance cleaning performance, for treatment of the substrate to be cleaned, or to modify the aesthetics of the cleaning composition as is the case with perfumes, colorants, dyes or the like. It is understood that such adjuncts are in addition to the variant mannanase enzymes of the present invention. The precise nature of these additional components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the cleaning operation for which it is to be used. Suitable adjunct materials include, but are not limited to, surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, additional enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids and/or pigments. In addition to the disclosure below, suitable examples of such other adjuncts and levels of use are found in U.S. Patent Nos. 5,576,282, 6,306,812, and 6,326,348, incorporated by reference. The aforementioned adjunct ingredients may constitute the balance of the cleaning compositions of the present invention.

[00278] In some embodiments, the cleaning compositions according to the present invention comprise at least one surfactant and/or a surfactant system wherein the surfactant is selected from nonionic surfactants, anionic surfactants, cationic surfactants, ampholytic surfactants, zwitterionic surfactants, semi-polar nonionic surfactants and mixtures thereof. In some low pH cleaning composition embodiments (*e.g.*, compositions having a neat pH of from about 3 to about 5), the composition typically does not contain alkyl ethoxylated sulfate, as it is believed that such surfactant may be hydrolyzed by such compositions the acidic contents. In some embodiments, the surfactant is present at a level of from about 0.1% to about 60%, while in alternative embodiments the level is from about 1% to about 50%, while in still further embodiments the level is from about 5% to about 40%, by weight of the cleaning composition.

[00279] In some embodiments, the cleaning compositions of the present disclosure contain at least one chelating agent. Suitable chelating agents may include, but are not limited to copper, iron, and/or manganese chelating agents, and mixtures thereof. In embodiments in which at least one chelating agent is used, the cleaning compositions of the present disclosure comprise from about 0.1% to about 15% or even from about 3.0% to about 10% chelating agent by weight of the subject cleaning composition.

[00280] In some still further embodiments, the cleaning compositions provided herein contain at least one deposition aid. Suitable deposition aids include, but are not limited to, polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

[00281] As indicated herein, in some embodiments, anti-redeposition agents find use in some embodiments of the present disclosure. In some preferred embodiments, non-ionic surfactants find use. For example, in automatic dishwashing embodiments, non-ionic surfactants find use for surface modification purposes, in particular for sheeting, to avoid filming and spotting and to improve shine. These non-ionic surfactants also find use in preventing the re-deposition of soils. In some preferred embodiments, the anti-redeposition agent is a non-ionic surfactant as known in the art (*See, e.g.*, EP 2 100 949).

[00282] In some embodiments, the cleaning compositions of the present disclosure include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylloxazolidones, and polyvinylimidazoles, or mixtures thereof. In embodiments in which at least one dye transfer inhibiting agent is used, the cleaning compositions of the present disclosure comprise from about 0.0001% to about 10%, from about 0.01% to about 5%, or even from about 0.1% to about 3% by weight of the cleaning composition.

[00283] In some embodiments, silicates are included within the compositions of the present disclosure. In some such embodiments, sodium silicates (*e.g.*, sodium disilicate, sodium metasilicate, and crystalline phyllosilicates) find use. In some embodiments, silicates are present at a level of from about 1% to about 20%. In some preferred embodiments, silicates are present at a level of from about 5% to about 15% by weight of the composition.

[00284] In some still additional embodiments, the cleaning compositions of the present disclosure also contain dispersants. Suitable water-soluble organic materials include, but are not limited to the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

[00285] In some further embodiments, the enzymes used in the cleaning compositions are stabilized by any suitable technique. In some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions that provide such ions to the enzymes. In some embodiments, the enzyme stabilizers include oligosaccharides, polysaccharides, and inorganic divalent metal salts, including alkaline earth metals,

such as calcium salts. It is contemplated that various techniques for enzyme stabilization will find use in the present disclosure. For example, 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)). Chlorides and sulfates also find use in some embodiments of the present disclosure. Examples of suitable oligosaccharides and polysaccharides (*e.g.*, dextrans) are known in the art (*See, e.g.*, WO 07/145964). In some embodiments, reversible protease inhibitors also find use, such as boron-containing compounds (*e.g.*, borate, 4-formyl phenyl boronic acid) and/or a tripeptide aldehyde find use to further improve stability, as desired.

[00286] In some embodiments, the cleaning compositions of the present invention comprise one or more detergent builders or builder systems. In some embodiments incorporating at least one builder, the cleaning compositions comprise at least about 1%, from about 3% to about 60% or even from about 5% to about 40% builder by weight of the cleaning composition. Builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicates, polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. Indeed, it is contemplated that any suitable builder will find use in various embodiments of the present invention.

[00287] In some embodiments, the builders form water-soluble hardness ion complexes (*e.g.*, sequestering builders), such as citrates and polyphosphates (*e.g.*, sodium tripolyphosphate and sodium tripolyphosphate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the present invention, including those known in the art (*See e.g.*, EP 2 100 949).

[00288] In some embodiments, the cleaning compositions of the present invention contain at least one chelating agent. Suitable chelating agents include, but are not limited to copper, iron and/or manganese chelating agents and mixtures thereof. In embodiments in which at least one chelating agent is used, the cleaning compositions of the present invention comprise from about 0.1% to about 15% or even from about 3.0% to about 10% chelating agent by weight of the subject cleaning composition.

[00289] In some still further embodiments, the cleaning compositions provided herein contain at least one deposition aid. Suitable deposition aids include, but are not limited to, polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

[00290] As indicated herein, in some embodiments, anti-redeposition agents find use in some embodiments of the present invention. In some embodiments, non-ionic surfactants find use. For example, in automatic dishwashing embodiments, non-ionic surfactants find use for surface modification purposes, in particular for sheeting, to avoid filming and spotting and to improve shine. These non-ionic surfactants also find use in preventing the re-deposition of soils. In some embodiments, the anti-redeposition agent is a non-ionic surfactant as known in the art (*See e.g.*, EP 2 100 949).

[00291] In some embodiments, the cleaning compositions of the present invention include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof. In embodiments in which at least one dye transfer inhibiting agent is used, the cleaning compositions of the present invention comprise from about 0.0001% to about 10%, from about 0.01% to about 5%, or even from about 0.1% to about 3% by weight of the cleaning composition.

[00292] In some embodiments, silicates are included within the compositions of the present invention. In some such embodiments, sodium silicates (*e.g.*, sodium disilicate, sodium metasilicate, and crystalline phyllosilicates) find use. In some embodiments, silicates are present at a level of from about 1% to about 20%. In some embodiments, silicates are present at a level of from about 5% to about 15% by weight of the composition.

[00293] In some still additional embodiments, the cleaning compositions of the present invention also contain dispersants. Suitable water-soluble organic materials include, but are not limited to the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

[00294] In some further embodiments, the enzymes used in the cleaning compositions are stabilized by any suitable technique. In some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions that provide such ions to the enzymes. In some embodiments, the enzyme stabilizers include oligosaccharides, polysaccharides, and inorganic divalent metal salts, including alkaline earth metals, such as calcium salts. It is contemplated that various techniques for enzyme stabilization will find use in the present invention. For example, 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)). Chlorides and sulfates also find use in some embodiments of the present invention. Examples of suitable oligosaccharides and polysaccharides (*e.g.*, dextrans) are known in the art (*See e.g.*, WO 07/145964). In some embodiments, reversible enzyme inhibitors also find use, such as boron-containing compounds (*e.g.*, borate, 4-formyl phenyl boronic acid) and/or a tripeptide aldehyde find use to further improve stability, as desired.

[00295] In some embodiments, bleaches, bleach activators and/or bleach catalysts are present in the compositions of the present invention. In some embodiments, the cleaning compositions of the present invention comprise inorganic and/or organic bleaching compound(s). Inorganic bleaches include, but are not limited to perhydrate salts (*e.g.*, perborate, percarbonate, perphosphate, persulfate, and persilicate salts). In some embodiments, inorganic perhydrate salts are alkali metal salts. In some embodiments, inorganic perhydrate salts are included as the crystalline solid, without additional protection, although in some other embodiments, the salt is coated. Any suitable salt known in the art finds use in the present invention (*See e.g.*, EP 2 100 949).

[00296] In some embodiments, bleach activators are used in the compositions of the present invention. Bleach activators are typically organic peracid precursors that enhance the bleaching action in the course of cleaning at temperatures of 60°C and below. Bleach activators suitable for use herein include compounds which, under perhydrolysis conditions, give aliphatic peroxycarboxylic acids having preferably from about 1 to about 10 carbon atoms, in particular from about 2 to about 4 carbon atoms, and/or optionally substituted perbenzoic acid. Additional bleach activators are known in the art and find use in the present invention (*See e.g.*, EP 2 100 949).

[00297] In addition, in some embodiments and as further described herein, the cleaning compositions of the present invention further comprise at least one bleach catalyst. In some embodiments, the manganese triazacyclononane and related complexes find use, as well as cobalt, copper, manganese, and iron complexes. Additional bleach catalysts find use in the present invention (*See e.g.*, US 4,246,612, 5,227,084, 4,810,410, WO 99/06521, and EP 2 100 949).

[00298] In some embodiments, the cleaning compositions of the present invention contain one or more catalytic metal complexes. In some embodiments, a metal-containing bleach catalyst finds use. In some embodiments, the metal bleach catalyst comprises a catalyst system comprising a transition metal cation of defined bleach catalytic activity, (*e.g.*, copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations), an auxiliary metal cation having little or no bleach catalytic activity (*e.g.*, zinc or aluminum cations), and a sequester having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra(methylenephosphonic acid) and water-soluble salts thereof are used (*See e.g.*, US Patent No. 4,430,243). In some embodiments, the cleaning compositions of the present invention are catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art (*See e.g.*, US Patent No. 5,576,282). In additional embodiments, cobalt bleach catalysts find use in the cleaning compositions of the present invention. Various cobalt bleach catalysts are known in the art (*See e.g.*, US Patent Nos. 5,597,936 and 5,595,967) and are readily prepared by known procedures.

[00299] In some additional embodiments, the cleaning compositions of the present invention include a transition metal complex of a macropolycyclic rigid ligand (MRL). As a practical matter, and not by way of limitation, in some embodiments, the compositions and cleaning processes provided by the present invention are adjusted to provide on the order of at least one part per hundred million of the

active MRL species in the aqueous washing medium, and in some embodiments, provide from about 0.005 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the MRL in the wash liquor.

[00300] In some embodiments, transition-metals in the instant transition-metal bleach catalyst include, but are not limited to manganese, iron and chromium. MRLs also include, but are not limited to special ultra-rigid ligands that are cross-bridged (*e.g.*, 5,12-diethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane). Suitable transition metal MRLs are readily prepared by known procedures (*See e.g.*, WO 2000/32601, and US Patent No. 6,225,464).

[00301] In some embodiments, the cleaning compositions of the present invention comprise metal care agents. Metal care agents find use in preventing and/or reducing the tarnishing, corrosion, and/or oxidation of metals, including aluminum, stainless steel, and non-ferrous metals (*e.g.*, silver and copper). Suitable metal care agents include those described in EP 2 100 949, WO 9426860 and WO 94/26859). In some embodiments, the metal care agent is a zinc salt. In some further embodiments, the cleaning compositions of the present invention comprise from about 0.1% to about 5% by weight of one or more metal care agent.

[00302] As indicated above, the cleaning compositions of the present invention are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,516,448, 5,489,392, and 5,486,303, all of which are incorporated herein by reference. In some embodiments in which a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of an acidic material such as HCl.

[00303] The cleaning compositions disclosed herein of find use in cleaning a *situs* (*e.g.*, a surface, item, dishware, or fabric). Typically, at least a portion of the situs is contacted with an embodiment of the present cleaning composition, in neat form or diluted in a wash liquor, and then the situs is optionally washed and/or rinsed. For purposes of the present invention, "washing" includes but is not limited to, scrubbing, and mechanical agitation. In some embodiments, the cleaning compositions are typically employed at concentrations of from about 300 ppm to about 15,000 ppm in solution. When the wash solvent is water, the water temperature typically ranges from about 5°C to about 90°C and, when the situs comprises a fabric, the water to fabric mass ratio is typically from about 1:1 to about 30:1.

Processes of Making and Using Cleaning Compositions

[00304] The cleaning compositions of the present invention are formulated into any suitable form and prepared by any suitable process chosen by the formulator, (*See e.g.*, US Patent Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,565,422, 5,516,448, 5,489,392, 5,486,303, 4,515,705, 4,537,706, 4,515,707, 4,550,862, 4,561,998, 4,597,898, 4,968,451, 5,565,145, 5,929,022, 6,294,514 and 6,376,445).

[00305] In some embodiments, the cleaning compositions of the present invention are provided in unit dose form, including tablets, capsules, sachets, pouches, and multi-compartment pouches. In some

embodiments, the unit dose format is designed to provide controlled release of the ingredients within a multi-compartment pouch (or other unit dose format). Suitable unit dose and controlled release formats are known in the art (*See e.g.*, EP 2 100 949, WO 02/102955, US Pat. Nos. 4,765,916 and 4,972,017, and WO 04/111178 for materials suitable for use in unit dose and controlled release formats). In some
5 embodiments, the unit dose form is provided by tablets wrapped with a water-soluble film or water-soluble pouches. Various formats for unit doses are provided in EP 2 100 947, and are known in the art.

Methods of Use

[00306] In some embodiments, the cleaning compositions of the present invention find use in
10 cleaning surfaces (*e.g.*, dishware), laundry, hard surfaces, contact lenses, etc. In some embodiments, at least a portion of the surface is contacted with at least one embodiment of the cleaning compositions of the present invention, in neat form or diluted in a wash liquor, and then the surface is optionally washed and/or rinsed. For purposes of the present invention, “washing” includes, but is not limited to, scrubbing, and mechanical washing. In some embodiments, the cleaning compositions of the present invention are
15 used at concentrations of from about 500 ppm to about 15,000 ppm in solution. In some embodiments in which the wash solvent is water, the water temperature typically ranges from about 5°C to about 90°C.

[00307] The present invention provides methods for cleaning or washing an item or surface (*e.g.*, hard surface) in need of cleaning, including, but not limited to methods for cleaning or washing a dishware item, a tableware item, a fabric item, a laundry item, personal care item, etc., or the like, and
20 methods for cleaning or washing a hard or soft surface (*e.g.*, a hard surface of an item).

[00308] In some embodiments, the present invention provides a method for cleaning an item, object, or surface in need of cleaning, the method comprising contacting the item or surface (or a portion of the item or surface desired to be cleaned) with at least one mannanase enzyme of the present invention or a composition of the present invention for a sufficient time and/or under conditions suitable and/or
25 effective to clean the item, object, or surface to a desired degree. Some such methods further comprise rinsing the item, object, or surface with water. For some such methods, the cleaning composition is a dishwashing detergent composition and the item or object to be cleaned is a dishware item or tableware item. As used herein, a “dishware item” is an item generally used in serving or eating food. A dishware item can be, but is not limited to for example, a dish, plate, cup, bowl, etc., and the like. As used herein,
30 “tableware” is a broader term that includes, but is not limited to for example, dishes, cutlery, knives, forks, spoons, chopsticks, glassware, pitchers, sauce boats, drinking vessels, serving items, etc. It is intended that “tableware item” includes any of these or similar items for serving or eating food. For some such methods, the cleaning composition is an automatic dishwashing detergent composition or a hand dishwashing detergent composition and the item or object to be cleaned is a dishware or tableware
35 item. For some such methods, the cleaning composition is a laundry detergent composition (*e.g.*, a power laundry detergent composition or a liquid laundry detergent composition), and the item to be

cleaned is a fabric item. In some other embodiments, the cleaning composition is a laundry pre-treatment composition.

[00309] In using detergent compositions that include Bleman1 in cleaning applications, the fabrics, textiles, dishes, or other surfaces to be cleaned are incubated in the presence of the Bleman1 detergent composition for a time sufficient to allow Bleman1 to hydrolyze mannan substrates including, but not limited to, locust bean gum, guar gum, and combinations thereof present in soil or stains, and then typically rinsed with water or another aqueous solvent to remove the Bleman1 detergent composition along with hydrolyzed mannans.

[00310] In some embodiments, the present invention provides methods for cleaning or washing a fabric item optionally in need of cleaning or washing, respectively. In some embodiments, the methods comprise providing a composition comprising the variant mannanase enzyme, including but not limited to fabric or laundry cleaning composition, and a fabric item or laundry item in need of cleaning, and contacting the fabric item or laundry item (or a portion of the item desired to be cleaned) with the composition under conditions sufficient or effective to clean or wash the fabric or laundry item to a desired degree.

[00311] In some embodiments, the present invention provides a method for cleaning or washing an item or surface (*e.g.*, hard surface) optionally in need of cleaning, the method comprising providing an item or surface to be cleaned or washed and contacting the item or surface (or a portion of the item or surface desired to be cleaned or washed) with at least one mannanase of the invention or a composition of the invention comprising at least one such mannanase for a sufficient time and/or under conditions sufficient or effective to clean or wash the item or surface to a desired degree. Such compositions include, but are not limited to for example, a cleaning composition or detergent composition of the invention (*e.g.*, a hand dishwashing detergent composition, hand dishwashing cleaning composition, laundry detergent or fabric detergent or laundry or fabric cleaning composition, liquid laundry detergent, liquid laundry cleaning composition, powder laundry detergent composition, powder laundry cleaning composition, automatic dishwashing detergent composition, laundry booster cleaning or detergent composition, laundry cleaning additive, and laundry pre-spotter composition, etc.). In some embodiments, the method is repeated one or more times, particularly if additional cleaning or washing is desired. For example, in some instance, the method optionally further comprises allowing the item or surface to remain in contact with the at least one variant mannanase enzyme or composition for a period of time sufficient or effective to clean or wash the item or surface to the desired degree. In some embodiments, the methods further comprise rinsing the item or surface with water and/or another liquid. In some embodiments, the methods further comprise contacting the item or surface with at least one variant mannanase enzyme of the invention or a composition of the invention again and allowing the item or surface to remain in contact with the at least one variant mannanase enzyme or composition for a period of time sufficient to clean or wash the item or surface to the desired degree. In some embodiments, the cleaning composition is a dishwashing detergent composition and the item to be

cleaned is a dishware or tableware item. In some embodiments of the present methods, the cleaning composition is an automatic dishwashing detergent composition or a hand dishwashing detergent composition and the item to be cleaned is a dishware or tableware item. In some embodiments of the methods, the cleaning composition is a laundry detergent composition and the item to be cleaned is a fabric item.

[00312] The present invention also provides methods of cleaning a tableware or dishware item in an automatic dishwashing machine, the method comprising providing an automatic dishwashing machine, placing an amount of an automatic dishwashing composition comprising at least one mannanase of the present invention or a composition of the invention sufficient to clean the tableware or dishware item in the machine (*e.g.*, by placing the composition in an appropriate or provided detergent compartment or dispenser in the machine), putting a dishware or tableware item in the machine, and operating the machine so as to clean the tableware or dishware item (*e.g.*, as per the manufacturer's instructions). In some embodiments, the methods include any automatic dishwashing composition described herein, which comprises, but is not limited to at least one mannanase provided herein. The amount of automatic dishwashing composition to be used can be readily determined according to the manufacturer's instructions or suggestions and any form of automatic dishwashing composition comprising at least one variant mannanase enzyme of the invention (*e.g.*, liquid, powder, solid, gel, tablet, etc.), including any described herein, may be employed.

[00313] The present invention also provides methods for cleaning a surface, item or object optionally in need of cleaning, the method comprises contacting the item or surface (or a portion of the item or surface desired to be cleaned) with at least one mannanase of the present invention or a cleaning composition of the invention in neat form or diluted in a wash liquor for a sufficient time and/or under conditions sufficient or effective to clean or wash the item or surface to a desired degree. The surface, item, or object may then be (optionally) washed and/or rinsed if desired. For purposes of the present invention, "washing" includes, but is not limited to for example, scrubbing and mechanical agitation. In some embodiments, the cleaning compositions are employed at concentrations of from about 500 ppm to about 15,000 ppm in solution (*e.g.*, aqueous solution). When the wash solvent is water, the water temperature typically ranges from about 5°C to about 90°C and when the surface, item or object comprises a fabric, the water to fabric mass ratio is typically from about 1:1 to about 30:1.

[00314] The present invention also provides methods of cleaning a laundry or fabric item in an washing machine, the method comprising providing an washing machine, placing an amount of a laundry detergent composition comprising at least one mannanase of the invention sufficient to clean the laundry or fabric item in the machine (*e.g.*, by placing the composition in an appropriate or provided detergent compartment or dispenser in the machine), placing the laundry or fabric item in the machine, and operating the machine so as to clean the laundry or fabric item (*e.g.*, as per the manufacturer's instructions). The methods of the present invention include any laundry washing detergent composition described herein, comprising but not limited to at least one of any mannanase provided herein. The

amount of laundry detergent composition to be used can be readily determined according to manufacturer's instructions or suggestions and any form of laundry detergent composition comprising at least one variant mannanase enzyme of the invention (*e.g.*, solid, powder, liquid, tablet, gel, etc.), including any described herein, may be employed.

- 5 **[00315]** Other aspects and embodiments of the present compositions and methods will be apparent from the foregoing description and following examples.

EXAMPLES

- [00316]** The following examples are provided to demonstrate and illustrate certain preferred
10 embodiments and aspects of the present disclosure and should not be construed as limiting.
- [00317]** In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar); μ M (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); g and gm (grams); mg (milligrams); μ g (micrograms); pg (picograms); L (liters); ml and mL (milliliters); μ l and μ L (microliters); cm (centimeters); mm (millimeters); μ m
15 (micrometers); nm (nanometers); U (units); MW (molecular weight); sec (seconds); min(s) (minute/minutes); h(s) and hr(s) (hour/hours); °C (degrees Centigrade); QS (quantity sufficient); ND (not done); rpm (revolutions per minute); H₂O (water); dH₂O (deionized water); HCl (hydrochloric acid); aa (amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); MgCl₂ (magnesium chloride); NaCl (sodium chloride); Ca (calcium); Mg (magnesium); HEPES (4-(2-hydroxyethyl)-1-
20 piperazineethanesulfonic acid); CHES (N-cyclohexyl-2-aminoethanesulfonic acid); w/v (weight to volume); v/v (volume to volume); g (gravity); OD (optical density); ppm (parts per million); *m*- (*meta*-); *o*- (*ortho*-); *p*- (*para*-); Bleman1 (*Bacillus lentus* mannanase1); SRI (stain removal index).

EXAMPLE 1

25 Mannanase activity of Bleman1

A. Activity on AZO-Carob Galactomannan substrate

- [00318]** The mannanase activity of a post fermentation concentrate of *Bacillus lentus* expressing a mannanase (Bleman1) was determined by assaying enzyme activity at a concentration of 60ppm at 40°C for 10 minutes in 100mM HEPES pH 7.5 using AZO-Carob Galactomannan (partially depolymerised and
30 dyed carob Galactomannan; Carob galactomannan is dyed with Remazolbrilliant Blue R to an extent of approx. one dye molecule per 30 sugar Residues) as substrate (Megazyme International; S-ACGLM ; Wicklow, Ireland). The assay was performed by initially pre-equilibrating varying dilutions of fermentation concentrate and 0.25mL aliquots of 2%w/v substrate solution at 40°C in a water bath for 10 min. The reaction was initiated by the addition of 0.25mL of 30ppm Bleman1 enzyme solution into the
35 pre-equilibrated substrate solution, mixed by vortexing for six seconds, and incubated at 40°C in a water bath for 10 minutes. The reaction was terminated with the addition of 1.25mL of 95% v/v ethanol and the contents vortexed for 10 seconds. The reaction tubes were then centrifuged for 10 minutes at 3,000 rpm

and the supernatant was transferred to a disposable polystyrene cuvette. The absorbance of the supernatant was measured at 590nm using a Varian Cary 100-Bio UV-Visible Spectrophotometer. Enzyme activity is reported as absorbance of released Remazolbrilliant Blue R dye at 590nm and is shown in Figure 1-1.

5

B. Activity on Locust Bean Gum substrate

[00319] The mannanase activity of Bleman1 was also determined on Locust Bean Gum substrate (Sigma-Aldrich, #G0753; St. Louis, MO) by assaying for enzyme activity at a concentration of 3.0ppm at 40°C for 10 minutes in 50mM Tris-HCl pH 7.5. This procedure is a colorimetric method that measures the release of reducing sugars by the action of endo-1,4-β-D-mannanase on Locust Bean Gum. Dinitrosalicylic acid reaction is used to determine the increase in reducing sugar content, which is proportional to the enzyme activity reported as absorbance at 540nm. The assay was performed by initially pre-equilibrating varying dilutions of fermentation concentrate and 0.4mL aliquots of 2.8g/L Locust Bean Gum in 50mM Tris-HCl pH 7.5 substrate solution at 40°C in a waterbath for 10 min. The reaction was initiated by the addition of 0.1mL of 3.0ppm Bleman1 enzyme solution into the pre-equilibrated substrate solution, mixed by vortexing for six seconds, and incubated at 40°C in a water bath for 10 minutes. The reaction was terminated after exactly 10 minutes with the addition of 0.6mL of 3,5-dinitrosalicylic acid reagent (43.8mM 3,5-dinitrosalicylic acid, 400mM sodium hydroxide, 1.063M potassium sodium tartrate tetrahydrate) and the contents vortexed to mix. For substrate blanks, 0.6mL of 3,5-dinitrosalicylic acid reagent was added to corresponding tubes containing only buffer after the 10 minute incubation at 40°C and then 0.1mL of 3.0ppm Bleman1 enzyme solution was added. All reaction tubes after addition of 3,5-dinitrosalicylic acid reagent were capped and placed in a 100°C water bath for exactly 15 minutes following which the tubes were transferred to an ice bath for 5 minutes. Finally the reaction tubes were allowed to equilibrate to room temperature for 10 minutes and then 0.2mL of the reaction mixture from each tube was transferred in triplicate to a 96 well microtiter plate (Costar 9017) and absorbance at 540nm measured using a Bio-TEK Synergy HT plate reader. Enzyme activity is reported as absorbance at 540nm and is shown in Figure 1-2.

30

EXAMPLE 2

pH profile of Bleman1

[00320] The pH profile of Bleman1 on AZO-Carob Galactomannan substrate was determined by assaying enzyme activity at a concentration of 60ppm at 40°C for 10 minutes in 100mM HEPES, TAPS, Sodium Carbonate tri-buffer pH 7, 8, 9, 10, 11. The assay was performed by first pre-equilibrating enzyme dilutions and 0.125mL aliquots of 2%w/v AZO-Carob Galactomannan substrate solutions prepared in the different buffers at 40°C for 10 minutes. The reaction was initiated by the addition of 0.125mL of 60ppm Bleman1 enzyme solution into the pre-equilibrated substrate solution, mixed by

35

vortexing for six seconds, and incubated at 40°C in a thermomixer for 10 minutes. The reaction was then terminated with the addition of 0.625mL of 95% v/v ethanol and the contents vortexed to mix for 10 seconds. The reaction tubes were then centrifuged for 10 minutes at 3,000 rpm and then 0.2mL of the supernatant was transferred to a 96 well microtiter plate (Costar 9017) in triplicate and absorbance at 590nm measured using a Bio-TEK Synergy HT plate reader. The enzyme activity at pH 7 was normalized to 100% and enzyme activity obtained at the other pH values was calculated as % remaining activity of the activity at pH 7. The calculation was as follows: %Remaining Activity = (Avg Absorbance at 590nm for pH value/ Avg Absorbance at 590nm for pH7) * 100. Results are shown in Figure 2-1. The pH profile of Bleman1 was also determined using Locust Bean Gum substrate. The assay was performed as described in Example 1 except that the enzyme dilutions and 0.4mL aliquots of 2.8g/L Locust Bean Gum solutions were pre-equilibrated in 100mM HEPES, TAPS, Sodium carbonate tri-buffer pH 7, 8, 9, 10, 11 at 40°C in a waterbath for 10 min. The enzyme activity at pH 7 was normalized to 100% and enzyme activity obtained at the other pH values was calculated as % remaining activity of the activity at pH 7. The calculation was as follows: %Remaining Activity = (Avg Absorbance at 540nm for pH value/ Avg Absorbance at 540nm for pH7) * 100. Results are shown in Figure 2-1. Bleman1 pH profile on both AZO-Carob Galactomannan and Locust Bean Gum indicate that the mannanase activity at 40°C decreases as the pH increases with maximum activity at pH 7-8 and lowest activity levels found in this experiment at pH 10-11.

EXAMPLE 3

Temperature profile of Bleman1

[00321] The temperature profile of Bleman1 was determined by assaying enzyme activity on AZO-Carob Galactomannan substrate at a concentration of 60ppm at temperatures varying between 10°C and 60°C for 10 minutes in 100mM HEPES pH 7 & 8, 100mM TAPS pH 9, and 100mM Sodium Carbonate pH 10. The assay was performed by first pre-equilibrating enzyme dilutions and 0.125mL aliquots of 2%w/v AZO-Carob Galactomannan substrate solution at the assay temperature for 10 min. The reaction was initiated by the addition of 0.125mL of 60ppm Bleman1 enzyme solution into the pre-equilibrated substrate solution, mixed by vortexing for six seconds, and incubated at the assay temperature in a thermomixer for 10 minutes. The reaction was then terminated with the addition of 0.625mL of 95% v/v ethanol and the contents vortexed to mix for 10 seconds. The reaction tubes were then centrifuged for 10 minutes at 3,000 rpm and then 0.2mL of the supernatant was transferred to a 96 well microtiter plate (Costar 9017) in triplicate and absorbance at 590nm measured using a Bio-TEK Synergy HT plate reader. Enzyme activity is reported as absorbance of released Remazolbrilliant Blue R dye at 590nm and is shown in Figure 3-1. Bleman1 temperature profile on AZO-Carob Galactomannan indicates that the mannanase activity increases with temperature most significantly at pH 7, with maximum activity found here at 60°C.

EXAMPLE 4

Dose response of cleaning performance of Bleman1 in liquid laundry detergent at 32°C

[00322] A dose response for the cleaning performance of Bleman1 was conducted at full scale using Maytag Centennial (W10140921 D) washing machines set at Water Level: Medium; Temperature: Cold; Wash Cycle: Normal, Heavy; Extra rinse: Off. Stain removal experiments were carried out using CS-73 Locust bean gum and GC10-12 ASDA Chocolate Ice Cream pre-stained cotton swatches (Center for Testmaterials 30 (CFT), the Netherlands). The performance of Bleman1 was tested in the presence of commercially available heat inactivated Tide® HE detergent in top loading Maytag machines. Bleman1 was dosed at 0.5X, 1X, and 2X the activity measured in commercially available Tide® HE. These dosing levels were 0.055 wt%, 0.11 wt%, and 0.22 wt% of Bleman1 in 0.72g/L Tide(R) HE, respectively. The mannanase activity in commercially available Tide® HE detergent was determined using the assay method described in Example 1-A at a dilution of 1:25 and allowed to incubate for 60 minutes instead of 10 minutes. The cleaning performance testing was done at 32°C in deionized water with water hardness adjusted to a final concentration of 150ppm 3:1 Ca:Mg. Detergent, enzymes, and swatches were added to the machine, and the wash cycle was run for exactly 12 minutes. The machines were then drained and allowed to spin for extraction. Next the machines were refilled with 16°C deionized water with water hardness adjusted to a final concentration of 150ppm 3:1 Ca:Mg and allowed to agitate for 2.5 minutes following which the machines drained again and allowed to spin for extraction. Finally the swatches were transferred from the washing machine to the automatic dryer (Miele Touchtronic T8013C) and dried on the normal/gentle setting prior to post-wash surface spectrophotometric analysis. Stain removal performance for each swatch was evaluated by surface spectroscopy as the difference of the post- and pre cleaning L*a*b*(three coordinates of CIELAB) color values measurements and is reported as %SRI (soil removal index) as described below. The dose response for the cleaning performance is shown in Figure 4-1. Performance on Locust Bean Gum and ASDA Chocolate Ice Cream stains shows a benefit over heat inactivated Tide® HE detergent. This test shows that Bleman1, when dosed at equal dosing compared to the dosing of Tide® HE mannanase as measured by Locust Bean Gum activity assay, showed comparable performance compared to activity measured in Tide® HE on the performance on these two stains. In further performance testing in liquid detergent, a 1X activity based on mannanase activity in commercially available Tide® HE was used.

[00323] Stain removal was quantified by measuring the L*a*b*color values using a Konica Minolta CM-600d Spectro Photo Meter on a black background. Stain removal was calculated using the L*a*b*color values as the difference of the post and pre cleaning L*a*b*color measurements for each swatch.

ΔSRI (change in Soil Removal Index) values of the washed fabric were calculated in relation to the unwashed fabrics using the formula:

$$\% \text{ Soil Removal } dE(L^*a^*b^*) = (\text{soil removal } dE(L^*a^*b^*) / \text{initial soil } dE(L^*a^*b^*)) \times 100\%$$

Where:

Soil Removal dE ($L^*a^*b^*$) = $\text{SQRT} ((L^* \text{ after} - L^* \text{ before})^2 + (a^* \text{ after} - a^* \text{ before})^2 + (b^* \text{ after} - b^* \text{ before})^2)$

and

5 Initial soil dE ($L^*a^*b^*$) = $\text{SQRT} ((L^* \text{ ref} - L^* \text{ before})^2 + (a^* \text{ ref} - a^* \text{ before})^2 + (b^* \text{ ref} - b^* \text{ before})^2)$

$L^*a^*b^*\text{ref}$ values are the values of the unsoiled cotton (white).

EXAMPLE 5

10 Cleaning performance of Bleman1 in liquid laundry detergent at 16°C and 32°C

[00324] The cleaning performance of Bleman1 was tested at full scale as described in Example 4 except that the temperature during cleaning cycle was set at either 16°C or 32°C. Stain removal experiments were carried out using GC10-12 ASDA Chocolate Ice Cream, and CS-73 Locust bean gum with pigment pre-stained cotton swatches (Center For Testmaterials 30 (CFT), the Netherlands). The
15 mannanase was dosed based on equal active levels of the activity measured in commercially available Tide® HE liquid detergent, at a measure of 0.11 wt% of the 0.72 g/L dose of heat inactivated Tide® HE. Results are shown in Figure 5-1. At both 16°C and 32°C, the addition of Bleman1 alone provide a cleaning benefit over heat inactivated Tide® HE liquid detergent.

20

EXAMPLE 6

Cleaning performance of Bleman1 in combination with protease in liquid laundry detergent at 16°C and 32°C

[00325] The cleaning performance of Bleman1 was tested at 16°C and 32°C in combination with
25 protease (PREFERENZ™ P 110, DuPont) at full scale as described in Example 4. Stain removal experiments were carried out using CS-68 Chocolate ice cream and CS-73 Locust bean gum with pigment pre-stained cotton swatches (Center For Testmaterials 30 (CFT), the Netherlands). Bleman1 and PREFERENZ™ P 110 were dosed at 1X the activity measured in commercially available Tide® HE. The mannanase and protease were dosed based on equal active levels of the activity measured in
30 commercially available Tide® HE liquid detergent, at a measure of 0.11 wt% and the protease was dosed at 0.4 wt% of the 0.72 g/L dose of heat inactivated Tide® HE. Results are shown in Figure 6-1. The increased cleaning effects of the mannanase/protease combination are best seen on the Locust Bean Gum stain. On Chocolate Ice Cream, there is some effect of the mannanase/protease combination at 32°C.

EXAMPLE 7

A. Purification of Bleman1

[00326] The sample used was a concentrated fermentation product (uf concentrate) which contained suspended solids. This material was centrifuged at 12,000rpm for 30 minutes to remove the precipitate. Two 2.5 mL aliquots were taken from this clarified product and applied to two 10mL PD10 desalting columns (GE Healthcare PN 17-0851-01) which were equilibrated with 10 millimolar Tris(hydroxymethyl)aminomethane (tris) buffer, pH = 8.0. The target protein was eluted from the column using 3 mL of the same buffer on each column. The 6mL of desalted eluate was diluted to 60mL with 25 millimolar N-(Tris(hydroxymethyl)methyl)-2-aminoethane sulfonic acid (tris), pH = 6.8 buffer. The diluted sample was applied to a 6 mL Resource Q (GE Healthcare PN 17-1179-01) column which was equilibrated with 25 millimolar N-(Tris(hydroxymethyl)methyl)-2-aminoethane sulfonic acid (tris), pH = 6.8 buffer (loading buffer). The column was then washed with 5 column volumes of loading buffer. The mannanase protein was eluted from the column using a 20 column volume linear gradient from 50 millimolar N-(Tris(hydroxymethyl)methyl)-2-aminoethane sulfonic acid, pH = 6.8 to 50 millimolar N-(Tris(hydroxymethyl)methyl)-2-aminoethane sulfonic acid + 250 mM sodium chloride, pH = 6.8. Fractions were collected in 6 mL volumes across the gradient. An SDS-PAGE gel was run on these fractions and they showed that several fractions had a pure mannanase (32.6 KDa) evident in the fractions. The purified Bleman1 was evaluated for cleaning performance in laundry applications.

B. Cleaning performance of purified Bleman1 in liquid laundry detergent at 16°C and 32°C

[00327] The cleaning performance of purified Bleman1 was tested at 16°C and 32°C at full scale as described in Example 4. Stain removal experiments were carried out using GC10-12 ASDA Chocolate ice cream and CS-73 Locust bean gum with pigment pre-stained cotton swatches (Center For Testmaterials 30 (CFT), the Netherlands). Purified Bleman1 was dosed at 1X the activity measured in commercially available Tide® HE. The mannanase was dosed based on equal active levels of the activity measured in commercially available Tide® HE liquid detergent. This was a 0.2 wt% enzyme dose of the purified sample in the 0.72 g/L dose of heat inactivated Tide® HE. Results are shown in Figure 7-1. At 16°C, the addition of Bleman1 alone provides a cleaning benefit over heat inactivated Tide® HE liquid detergent. Based on this set of data, we can see that there is no difference in cleaning performance when using the Bleman1 product versus a purified sample of Bleman1.

EXAMPLE 8

Cleaning performance of purified Bleman1 in combination with protease in liquid laundry detergent at 16°C and 32°C

[00328] The cleaning performance of purified Bleman1 was tested at 16°C and 32°C in combination with protease (PREFERENZ™ P 110, DuPont) at full scale as described in Example 4. Stain removal

experiments were carried out using CS-68 Chocolate ice cream, CS-73 Locust bean gum with pigment, and EMPA-165 Chocolate pudding pre-stained cotton swatches (Center For Testmaterials 30 (CFT), the Netherlands). Purified Bleman1 and PREFERENZ™ P 110 were dosed at 1X the activity measured in commercially available Tide® HE. . The mannanase and protease were dosed based on equal active levels of the activity measured in commercially available Tide® HE liquid detergent, at a measure of 0.2 wt% and the protease was dosed at 0.4 wt% of the 0.72 g/L dose of heat inactivated Tide® HE.

EXAMPLE 9

Cleaning performance of Bleman1 in combination with protease/amylase/cellulase in powder laundry detergent with and without bleach

[00329] The cleaning performance of purified Bleman1 was tested alone and in combination with protease/amylase/cellulase (SMARTENZ™ 1050, DuPont) in industry standard powder laundry detergent (ECE-2; wfk Testgewebe GmbH; Bruggen, Germany) with or without the presence of bleach Tetraacetylenediamine (TAED) (wfk Testgewebe GmbH; Bruggen, Germany) and Sodium Percarbonate (Solvay Chemicals; Brussels, Belgium) at a composition of 82.6% (w/w) ECE-2, 14.2% (w/w) sodium percarbonate and 3.2% (w/w) TAED. The cleaning was conducted at full scale as described in Example 4 using CS-06 Salad dressing with natural black, CS-73 Locust bean gum with pigment, and CS-69 Chocolate pudding pre-stained cotton swatches (Center for Testmaterials 30 (CFT), the Netherlands). Bleman1 was added at a dose of 0.2 wt% and SMARTENZ™ 1050 was added at a dose of 2.0 wt%. The Bleman1 and SMARTENZ™ 1050 granule were dosed as a weight % of the 2 g/L dose of powder detergent. Results are shown in Figure 9-1. The addition of bleach in ECE-2 powder detergent does not negatively impact wash performance of the Bleman1 mannanase. The addition of a protease/amylase/cellulase granule blend does provide an increased cleaning benefit with Bleman1 especially on the Locust Bean Gum stain.

EXAMPLE 10

Liquid Laundry Detergent Compositions Comprising Bleman1

[00330] In this example, various formulations for liquid laundry detergent compositions are provided. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

Table 10-1. Liquid Laundry Detergent Compositions

Compound	Formulations				
	I	II	III	IV	V
LAS	24.0	32.0	6.0	3.0	6.0
NaC ₁₆ -C ₁₇ HSAS	-	-	-	5.0	-
C ₁₂ -C ₁₅ AE _{1.8} S	-	-	8.0	7.0	5.0
C ₈ -C ₁₀ propyl dimethyl amine	2.0	2.0	2.0	2.0	1.0
C ₁₂ -C ₁₄ alkyl dimethyl amine oxide	-	-	-	-	2.0

Table 10-1. Liquid Laundry Detergent Compositions					
Compound	Formulations				
	I	II	III	IV	V
C ₁₂ -C ₁₅ AS	-	-	17.0	-	8.0
CFAA	-	5.0	4.0	4.0	3.0
C ₁₂ -C ₁₄ Fatty alcohol ethoxylate	12.0	6.0	1.0	1.0	1.0
C ₁₂ -C ₁₈ Fatty acid	3.0	-	4.0	2.0	3.0
Citric acid (anhydrous)	4.5	5.0	3.0	2.0	1.0
DETPMP	-	-	1.0	1.0	0.5
Monoethanolamine	5.0	5.0	5.0	5.0	2.0
Sodium hydroxide	-	-	2.5	1.0	1.5
1 N HCl aqueous solution	#1	#1	-	-	-
Propanediol	12.7	14.5	13.1	10.	8.0
Ethanol	1.8	2.4	4.7	5.4	1.0
DTPA	0.5	0.4	0.3	0.4	0.5
Pectin Lyase	-	-	-	0.005	-
Amylase	0.001	0.002	-		-
Cellulase	-	-	0.0002		0.0001
Lipase	0.1	-	0.1	-	0.1
NprE (optional)	0.05	0.3	-	0.5	0.2
PMN	-	-	0.08	-	-
Protease A (optional)	-	-	-	-	0.1
Aldose Oxidase	-	-	0.3	-	0.003
ZnCl ₂	0.1	0.05	0.05	0.05	0.02
Ca formate	0.05	0.07	0.05	0.06	0.07
DETBCHD	-	-	0.02	0.01	-
SRP1	0.5	0.5	-	0.3	0.3
Boric acid	-	-	-	-	2.4
Sodium xylene sulfonate	-	-	3.0	-	-
Sodium cumene sulfonate	-	-	-	0.3	0.5
DC 3225C	1.0	1.0	1.0	1.0	1.0
2-butyl-octanol	0.03	0.04	0.04	0.03	0.03
Brightener 1	0.12	0.10	0.18	0.08	0.10
Balance to 100% perfume / dye and/or water					

#1: Add 1N HCl aq. soln to adjust the neat pH of the formula in the range from about 3 to about 5. The pH of Examples 9(I)-(II) is about 5 to about 7, and of 9(III)-(V) is about 7.5 to about 8.5.

EXAMPLE 11

5

Liquid Hand Dishwashing Detergent Compositions Comprising Bleman1

[00331] In this example, various hand dish liquid detergent formulations are provided. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

10

Table 11-1. Liquid Hand Dishwashing Detergent Compositions						
Compound	Formulations					
	I	II	III	IV	V	VI
C ₁₂ -C ₁₅ AE _{1.8} S	30.0	28.0	25.0	-	15.0	10.0
LAS	-	-	-	5.0	15.0	12.0
Paraffin Sulfonate	-	-	-	20.0	-	-
C ₁₀ -C ₁₈ Alkyl Dimethyl Amine Oxide	5.0	3.0	7.0	-	-	-
Betaine	3.0	-	1.0	3.0	1.0	-
C ₁₂ poly-OH fatty acid amide	-	-	-	3.0	-	1.0
C ₁₄ poly-OH fatty acid amide	-	1.5	-	-	-	-
C ₁₁ E ₉	2.0	-	4.0	-	-	20.0
DTPA	-	-	-	-	0.2	-
Tri-sodium Citrate dehydrate	0.25	-	-	0.7	-	-
Diamine	1.0	5.0	7.0	1.0	5.0	7.0
MgCl ₂	0.25	-	-	1.0	-	-
nprE (optional)	0.02	0.01	-	0.01	-	0.05
PMN	-	-	0.03	-	0.02	-
Protease A (optional)	-	0.01	-	-	-	-
Amylase	0.001	-	-	0.002	-	0.001
Aldose Oxidase	0.03	-	0.02	-	0.05	-
Sodium Cumene Sulphonate	-	-	-	2.0	1.5	3.0
PAAC	0.01	0.01	0.02	-	-	-
DETBCHD	-	-	-	0.01	0.02	0.01
Balance to 100% perfume / dye and/or water						

The pH of Examples 10(I)-(VI) is about 8 to about 11

EXAMPLE 12

5 Liquid Automatic Dishwashing Detergent Compositions Comprising Bleman1

[00332] In this example, various liquid automatic dishwashing detergent formulations are provided. In each of these formulations, Bleman1 polypeptide is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

10

Table 12-1. Liquid Automatic Dishwashing Detergent Compositions					
Compound	Formulations				
	I	II	III	IV	V
STPP	16	16	18	16	16
Potassium Sulfate	-	10	8	-	10
1,2 propanediol	6.0	0.5	2.0	6.0	0.5
Boric Acid	-	-	-	4.0	3.0
CaCl ₂ dihydrate	0.04	0.04	0.04	0.04	0.04
Nonionic	0.5	0.5	0.5	0.5	0.5
nprE (optional)	0.1	0.03	-	0.03	-
PMN	-	-	0.05	-	0.06
Protease B (optional)	-	-	-	0.01	-

Table 12-1. Liquid Automatic Dishwashing Detergent Compositions					
Compound	Formulations				
	I	II	III	IV	V
Amylase	0.02	-	0.02	0.02	-
Aldose Oxidase	-	0.15	0.02	-	0.01
Galactose Oxidase	-	-	0.01	-	0.01
PAAC	0.01	-	-	0.01	-
DETBCHD	-	0.01	-	-	0.01
Balance to 100% perfume / dye and/or water					

EXAMPLE 13

Granular and/or Tablet Laundry Compositions Comprising Bleman1

- [00333]** This example provides various formulations for granular and/or tablet laundry
5 detergents. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

Table 13-1. Granular and/or Tablet Laundry Compositions					
Compound	Formulations				
	I	II	III	IV	V
Base Product					
C ₁₄ -C ₁₅ AS or TAS	8.0	5.0	3.0	3.0	3.0
LAS	8.0	-	8.0	-	7.0
C ₁₂ -C ₁₅ AE ₃ S	0.5	2.0	1.0	-	-
C ₁₂ -C ₁₅ E ₃ or E ₃	2.0	-	5.0	2.0	2.0
QAS	-	-	-	1.0	1.0
Zeolite A	20.0	18.0	11.0	-	10.0
SKS-6 (dry add)	-	-	9.0	-	-
MA/AA	2.0	2.0	2.0	-	-
AA	-	-	-	-	4.0
3Na Citrate 2H ₂ O	-	2.0	-	-	-
Citric Acid (Anhydrous)	2.0	-	1.5	2.0	-
DTPA	0.2	0.2	-	-	-
EDDS	-	-	0.5	0.1	-
HEDP	-	-	0.2	0.1	-
PB1	3.0	4.8	-	-	4.0
Percarbonate	-	-	3.8	5.2	-
NOBS	1.9	-	-	-	-
NACA OBS	-	-	2.0	-	-
TAED	0.5	2.0	2.0	5.0	1.00
BB1	0.06	-	0.34	-	0.14
BB2	-	0.14	-	0.20	-
Anhydrous Na Carbonate	15.0	18.0	-	15.0	15.0
Sulfate	5.0	12.0	5.0	17.0	3.0
Silicate	-	1.0	-	-	8.0
nprE (optional)	0.03	-	0.1	0.06	-
PMN	-	0.05	-	-	0.1
Protease B (optional)	-	0.01	-	-	-

Table 13-1. Granular and/or Tablet Laundry Compositions					
Compound	Formulations				
	I	II	III	IV	V
Protease C (optional)	-	-	-	0.01	-
Lipase	-	0.008	-	-	-
Amylase	0.001	-	-	-	0.001
Cellulase	-	0.0014	-	-	-
Pectin Lyase	0.001	0.001	0.001	0.001	0.001
Aldose Oxidase	0.03	-	0.05	-	-
PAAC	-	0.01	-	-	0.05
Balance to 100% Moisture and/or Minors*					

* Perfume, dye, brightener / SRP1 / Na carboxymethylcellulose/ photobleach / MgSO₄ / PVPVI/ suds suppressor /high molecular PEG/clay.

EXAMPLE 14

5

Additional Liquid Laundry Detergents Comprising Bleman1

[00334] This example provides further formulations for liquid laundry detergents. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

10

Table 14-1. Liquid Laundry Detergents						
Compound	Formulations					
	IA	IB	II	III	IV	V
LAS	11.5	11.5	9.0	-	4.0	-
C ₁₂ -C ₁₅ AE _{2.85} S	-	-	3.0	18.0	-	16.0
C ₁₄ -C ₁₅ E _{2.5} S	11.5	11.5	3.0	-	16.0	-
C ₁₂ -C ₁₃ E ₉	-	-	3.0	2.0	2.0	1.0
C ₁₂ -C ₁₃ E ₇	3.2	3.2	-	-	-	-
CFAA	-	-	-	5.0	-	3.0
TPKFA	2.0	2.0	-	2.0	0.5	2.0
Citric Acid (Anhy.)	3.2	3.2	0.5	1.2	2.0	1.2
Ca formate	0.1	0.1	0.06	0.1	-	-
Na formate	0.5	0.5	0.06	0.1	0.05	0.05
ZnCl ₂	0.1	0.05	0.06	0.03	0.05	0.05
Na Culmene Sulfonate	4.0	4.0	1.0	3.0	1.2	-
Borate	0.6	0.6	1.5	-	-	-
Na Hydroxide	6.0	6.0	2.0	3.5	4.0	3.0
Ethanol	2.0	2.0	1.0	4.0	4.0	3.0
1,2 Propanediol	3.0	3.0	2.0	8.0	8.0	5.0
Monoethanolamine	3.0	3.0	1.5	1.0	2.5	1.0
TEPAE	2.0	2.0	-	1.0	1.0	1.0
nprE (optional)	0.03	0.05	-	0.03	-	0.02
PMN	-	-	0.01	-	0.08	-
Protease A (optional)	-	-	0.01	-	-	-
Lipase	-	-	-	0.002	-	-
Amylase	-	-	-	-	0.002	-
Cellulase	-	-	-	-	-	0.0001

Table 14-1. Liquid Laundry Detergents						
Compound	Formulations					
	IA	IB	II	III	IV	V
Pectin Lyase	0.005	0.005	-	-	-	-
Aldose Oxidase	0.05	-	-	0.05	-	0.02
Galactose oxidase	-	0.04	-	-	-	-
PAAC	0.03	0.03	0.02	-	-	-
DETBCHD	-	-	-	0.02	0.01	-
SRP 1	0.2	0.2	-	0.1	-	-
DTPA	-	-	-	0.3	-	-
PVNO	-	-	-	0.3	-	0.2
Brightener 1	0.2	0.2	0.07	0.1	-	-
Silicone antifoam	0.04	0.04	0.02	0.1	0.1	0.1
Balance to 100% perfume/dye and/or water						

EXAMPLE 15

High Density Dishwashing Detergents Comprising Bleman1

[00335] This example provides various formulations for high density dishwashing detergents.

- 5 In each of these compact formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

Table 15-1. High Density Dishwashing Detergents						
Compound	Formulations					
	I	II	III	IV	V	VI
STPP	-	45.0	45.0	-	-	40.0
3Na Citrate 2H ₂ O	17.0	-	-	50.0	40.2	-
Na Carbonate	17.5	14.0	20.0	-	8.0	33.6
Bicarbonate	-	-	-	26.0	-	-
Silicate	15.0	15.0	8.0	-	25.0	3.6
Metasilicate	2.5	4.5	4.5	-	-	-
PB1	-	-	4.5	-	-	-
PB4	-	-	-	5.0	-	-
Percarbonate	-	-	-	-	-	4.8
BB1	-	0.1	0.1	-	0.5	-
BB2	0.2	0.05	-	0.1	-	0.6
Nonionic	2.0	1.5	1.5	3.0	1.9	5.9
HEDP	1.0	-	-	-	-	-
DETPMP	0.6	-	-	-	-	-
PAAC	0.03	0.05	0.02	-	-	-
Paraffin	0.5	0.4	0.4	0.6	-	-
nprE (optional)	0.072	0.053	-	0.026	-	0.01
PMN	-	-	0.053	-	0.059	-
Protease B (optional)	-	-	-	-	-	0.01
Amylase	0.012	-	0.012	-	0.021	0.006
Lipase	-	0.001	-	0.005	-	-
Pectin Lyase	0.001	0.001	0.001	-	-	-
Aldose Oxidase	0.05	0.05	0.03	0.01	0.02	0.01
BTA	0.3	0.2	0.2	0.3	0.3	0.3

Table 15-1. High Density Dishwashing Detergents						
Compound	Formulations					
	I	II	III	IV	V	VI
Polycarboxylate	6.0	-	-	-	4.0	0.9
Perfume	0.2	0.1	0.1	0.2	0.2	0.2
Balance to 100% Moisture and/or Minors*						

*Brightener / dye / SRP1 / Na carboxymethylcellulose/ photobleach / MgSO₄ / PVPVI/ suds suppressor /high molecular PEG/clay. The pH of Examples 14(I) through (VI) is from about 9.6 to about 11.3.

EXAMPLE 16

5 **Tablet Dishwashing Detergent Compositions Comprising Bleman1**

[00336] This example provides various tablet dishwashing detergent formulations. The following tablet detergent compositions of the present disclosure are prepared by compression of a granular dishwashing detergent composition at a pressure of 13KN/cm² using a standard 12 head rotary press. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

Table 16-1. Tablet Dishwashing Detergent Compositions								
Compound	Formulations							
	I	II	III	IV	V	VI	VII	VIII
STPP	-	48.8	44.7	38.2	-	42.4	46.1	46.0
3Na Citrate 2H ₂ O	20.0	-	-	-	35.9	-	-	-
Na Carbonate	20.0	5.0	14.0	15.4	8.0	23.0	20.0	-
Silicate	15.0	14.8	15.0	12.6	23.4	2.9	4.3	4.2
Lipase	0.001	-	0.01	-	0.02	-	-	-
Protease B (optional)	0.01	-	-	-	-	-	-	-
Protease C (optional)	-	-	-	-	-	0.01	-	-
nprE (optional)	0.01	0.08	-	0.04	-	0.023	-	0.05
PMN	-	-	0.05	-	0.052	-	0.023	-
Amylase	0.012	0.012	0.012	-	0.015	-	0.017	0.002
Pectin Lyase	0.005	-	-	0.002	-	-	-	-
Aldose Oxidase	-	0.03	-	0.02	0.02	-	0.03	-
PB1	-	-	3.8	-	7.8	-	-	4.5
Percarbonate	6.0	-	-	6.0	-	5.0	-	-
BB1	0.2	-	0.5	-	0.3	0.2	-	-
BB2	-	0.2	-	0.5	-	-	0.1	0.2
Nonionic	1.5	2.0	2.0	2.2	1.0	4.2	4.0	6.5
PAAC	0.01	0.01	0.02	-	-	-	-	-
DETBCHD	-	-	-	0.02	0.02	-	-	-
TAED	-	-	-	-	-	2.1	-	1.6
HEDP	1.0	-	-	0.9	-	0.4	0.2	-
DETPMP	0.7	-	-	-	-	-	-	-
Paraffin	0.4	0.5	0.5	0.5	-	-	0.5	-
BTA	0.2	0.3	0.3	0.3	0.3	0.3	0.3	-
Polycarboxylate	4.0	-	-	-	4.9	0.6	0.8	-
PEG 400-30,000	-	-	-	-	-	2.0	-	2.0
Glycerol	-	-	-	-	-	0.4	-	0.5

Table 16-1. Tablet Dishwashing Detergent Compositions								
Compound	Formulations							
	I	II	III	IV	V	VI	VII	VIII
Perfume	-	-	-	0.05	0.2	0.2	0.2	0.2
Balance to 100% Moisture and/or Minors*								

*Brightener / SRP1 / Na carboxymethylcellulose/ photobleach / MgSO₄ / PVPVI/ suds suppressor /high molecular PEG/clay. The pH of Examples 15(I) through 15(VII) is from about 10 to about 11.5; pH of 15(VIII) is from 8-10. The tablet weight of Examples 15(I) through 15(VIII) is from about 20 grams to about 30 grams.

5

EXAMPLE 17

Liquid Hard Surface Cleaning Detergents Comprising Bleman1

[00337] This example provides various formulations for liquid hard surface cleaning detergents. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.

10

Table 17-1. Liquid Hard Surface Cleaning Detergents							
Compound	Formulations						
	I	II	III	IV	V	VI	VII
C ₉ -C ₁₁ E ₅	2.4	1.9	2.5	2.5	2.5	2.4	2.5
C ₁₂ -C ₁₄ E ₅	3.6	2.9	2.5	2.5	2.5	3.6	2.5
C ₇ -C ₉ E ₆	-	-	-	-	8.0	-	-
C ₁₂ -C ₁₄ E ₂₁	1.0	0.8	4.0	2.0	2.0	1.0	2.0
LAS	-	-	-	0.8	0.8	-	0.8
Sodium culmene sulfonate	1.5	2.6	-	1.5	1.5	1.5	1.5
Isachem ® AS	0.6	0.6	-	-	-	0.6	-
Na ₂ CO ₃	0.6	0.13	0.6	0.1	0.2	0.6	0.2
3Na Citrate 2H ₂ O	0.5	0.56	0.5	0.6	0.75	0.5	0.75
NaOH	0.3	0.33	0.3	0.3	0.5	0.3	0.5
Fatty Acid	0.6	0.13	0.6	0.1	0.4	0.6	0.4
2-butyl octanol	0.3	0.3	-	0.3	0.3	0.3	0.3
PEG DME-2000®	0.4	-	0.3	0.35	0.5	-	-
PVP	0.3	0.4	0.6	0.3	0.5	-	-
MME PEG (2000) ®	-	-	-	-	-	0.5	0.5
Jeffamine ® ED-2001	-	0.4	-	-	0.5	-	-
PAAC	-	-	-	0.03	0.03	0.03	-
DETBCHD	0.03	0.05	0.05	-	-	-	-
nprE (optional)	0.07	-	0.08	0.03	-	0.01	0.04
PMN	-	0.05	-	-	0.06	-	-
Protease B (optional)	-	-	-	-	-	0.01	-
Amylase	0.12	0.01	0.01	-	0.02	-	0.01
Lipase	-	0.001	-	0.005	-	0.005	-

Pectin Lyase	0.001	-	0.001	-	-	-	0.002
ZnCl ₂	0.02	0.01	0.03	0.05	0.1	0.05	0.02
Calcium Formate	0.03	0.03	0.01	-	-	-	-
PB1	-	4.6	-	3.8	-	-	-
Aldose Oxidase	0.05	-	0.03	-	0.02	0.02	0.05
Balance to 100% perfume / dye and/or water							

The pH of Examples 16(I) through (VII) is from about 7.4 to about 9.5.

EXAMPLE 18

- 5 **Detergent Compositions Comprising Bleman1**
- [00338]** This example provides various formulations for cleaning detergents. In each of these formulations, Bleman1 is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative embodiments, other concentrations will find use, as determined by the formulator, based on their needs.
- 10 **[00339]** In the exemplified detergent compositions provided herein, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications therein have the following meanings:

Abbreviation	Ingredient
LAS	: Sodium linear C ₁₁₋₁₃ alkyl benzene sulfonate.
NaC ₁₆₋₁₇ HSAS	: Sodium C ₁₆₋₁₇ highly soluble alkyl sulfate
TAS	: Sodium tallow alkyl sulphate.
C _x yAS	: Sodium C _{1x} - C _{1y} alkyl sulfate.
C _x yEz	: C _{1x} - C _{1y} predominantly linear primary alcohol condensed with an average of z moles of ethylene oxide.
C _x yAEzS	: C _{1x} - C _{1y} sodium alkyl sulfate condensed with an average of z moles of ethylene oxide. Added molecule name in the examples.
Nonionic	: Mixed ethoxylated/propoxylated fatty alcohol e.g. Plurafac LF404 being an alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5.
QAS	: R ₂ .N+(CH ₃) ₂ (C ₂ H ₄ OH) with R ₂ = C ₁₂ -C ₁₄ .
Silicate	: Amorphous Sodium Silicate (SiO ₂ :Na ₂ O ratio = 1.6-3.2:1).
Metasilicate	: Sodium metasilicate (SiO ₂ :Na ₂ O ratio = 1.0).
Zeolite A	: Hydrated aluminosilicate of formula Na ₁₂ (AlO ₂ SiO ₂) ₁₂ . 27H ₂ O
SKS-6	: Crystalline layered silicate of formula δ-Na ₂ Si ₂ O ₅ .
Sulfate	: Anhydrous sodium sulphate.
STPP	: Sodium Tripolyphosphate.
MA/AA	: Random copolymer of 4:1 acrylate/maleate, average molecular weight about 70,000-80,000.
AA	: Sodium polyacrylate polymer of average molecular weight 4,500.

Abbreviation	Ingredient
Polycarboxylate	: Copolymer comprising mixture of carboxylated monomers such as acrylate, maleate and methacrylate with a MW ranging between 2,000-80,000 such as Sokolan commercially available from BASF, being a copolymer of acrylic acid, MW4,500.
BB1	: 3-(3,4-Dihydroisoquinolinium)propane sulfonate
BB2	: 1-(3,4-dihydroisoquinolinium)-decane-2-sulfate
PB1	: Sodium perborate monohydrate.
PB4	: Sodium perborate tetrahydrate of nominal formula $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$.
Percarbonate	: Sodium percarbonate of nominal formula $2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$.
TAED	: Tetraacetyl ethylene diamine.
NOBS	: Nonanoyloxybenzene sulfonate in the form of the sodium salt.
DTPA	: Diethylene triamine pentaacetic acid.
HEDP	: 1,1-hydroxyethane diphosphonic acid.
DETPMP	: Diethyltriamine penta (methylene) phosphonate, marketed by Monsanto under the Trade name Dequest 2060.
EDDS	: Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer in the form of its sodium salt
Diamine	: Dimethyl aminopropyl amine; 1,6-hexane diamine; 1,3-propane diamine; 2-methyl-1,5-pentane diamine; 1,3-pentanediamine; 1-methyl-diaminopropane.
DETBCHD	: 5, 12- diethyl-1,5,8,12-tetraazabicyclo [6,6,2] hexadecane, dichloride, Mn(II) SALT
PAAC	: Pentaamine acetate cobalt(III) salt.
Paraffin	: Paraffin oil sold under the tradename Winog 70 by Wintershall.
Paraffin Sulfonate	: A Paraffin oil or wax in which some of the hydrogen atoms have been replaced by sulfonate groups.
Aldose oxidase	: Oxidase enzyme sold under the tradename Aldose Oxidase by Novozymes A/S
Galactose oxidase	: Galactose oxidase from Sigma
nprE	: The recombinant form of neutral metalloprotease enzyme expressed in <i>Bacillus subtilis</i> (See e.g., WO 07/044993)
PMN	: Purified neutral metalloprotease enzyme from <i>Bacillus amyloliquefaciens</i> .
Amylase	: A suitable amylolytic enzyme, such as those sold under the tradenames PURAFECT® Ox described in WO 94/18314, WO96/05295 sold by Genencor; NATALASE®, TERMAMYL®, FUNGAMYI® and DURAMYL™, all available from Novozymes A/S.
Lipase	: A suitable lipolytic enzyme such as those sold under the tradenames LIPEX®, LIPOLASE®, LIPOLASE® Ultra by Novozymes A/S and Lipomax™ by Gist-Brocades.
Cellulase	: A suitable cellulolytic enzyme such as those sold under the tradenames CAREZYME®, CELLUZYME®, and/or ENDOLASE® by Novozymes A/S add ours.
Pectin Lyase	: A suitable pectin lyase, such as those sold under the tradenames PECTAWAY® and PECTAWASH® available from Novozymes A/S.

Abbreviation	Ingredient
PVP	: Polyvinylpyrrolidone with an average molecular weight of 60,000
PVNO	: Polyvinylpyridine-N-Oxide, with an average molecular weight of 50,000.
PVPVI	: Copolymer of vinylimidazole and vinylpyrrolidone, with an average molecular weight of 20,000.
Brightener 1	: Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
Silicone antifoam	: Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
Suds Suppressor	: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form.
SRP 1	: Anionically end capped poly esters.
PEG X	: Polyethylene glycol, of a molecular weight of x.
PVP K60 ®	: Vinylpyrrolidone homopolymer (average MW 160,000)
Jeffamine ® ED-2001	: Capped polyethylene glycol from Huntsman
Isachem ® AS	: A branched alcohol alkyl sulphate from Enichem
MME PEG (2000)	: Monomethyl ether polyethylene glycol (MW 2000) from Fluka Chemie AG.
DC3225C	: Silicone suds suppresser, mixture of Silicone oil and Silica from Dow Corning.
TEPAE	: Tetraethylenepentamine ethoxylate.
BTA	: Benzotriazole.
Betaine	: $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COO}^-$
Sugar	: Industry grade D-glucose or food grade sugar
CFAA	: C_{12} - C_{14} alkyl N-methyl glucamide
TPKFA	: C_{12} - C_{14} topped whole cut fatty acids.
Clay	: A hydrated aluminum silicate in a general formula $\text{Al}_2\text{O}_3\text{SiO}_2 \cdot x\text{H}_2\text{O}$. Types: Kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite.
pH	: Measured as a 1% solution in distilled water at 20°C.

[00340] For North American (NA) and Western European (WE) heavy duty liquid laundry (HDL) detergents, heat inactivation of the enzymes present in commercially-available detergents is performed by placing pre-weighed liquid detergent (in a glass bottle) in a water bath at 95°C for 4 hours. The incubation time for heat inactivation of NA and WE auto dish washing (ADW) detergents is 8 hours.

- 5 Both un-heated and heated detergents are assayed within 5 minutes of dissolving the detergent to accurately determine percentage deactivated. Enzyme activity is tested by using an appropriate enzyme assay (e.g. AZO-Carob Galactomannan assay).

[00341] For testing of enzyme activity in heat-inactivated detergents, working solutions of detergents are made from the heat inactivated stocks. Appropriate amounts of water hardness (e.g., 6 gpg or 12 gpg) and buffer are added to the detergent solutions to match the desired conditions. The solutions are mixed by vortexing or inverting the bottles. The following Table provides information regarding some of the commercially-available detergents and test conditions used herein. In some experiments, additional and/or other commercially available detergents find use in the following Examples.

10

Table A. Laundry and Dish Washing Conditions							
Region	Form	Dose	Detergent*	Buffer	Gpg	pH	T (°C)
Laundry (Heavy Duty Liquid and Granular)							
NA	HDL	0.78 g/l	P&G TIDE® 2X	5 mM HEPES	6	8.0	20
WE	HDL	5.0 g/L	Henkel PERSIL™	5 mM HEPES	12	8.2	40
WE	HDG	8.0 g/L	P&G ARIEL®	2 mM Na ₂ CO ₃	12	10.5	40
JPN	HDG	0.7 g/L	P&G TIDE®	2 mM Na ₂ CO ₃	6	10.0	20
NA	HDG	1.0 g/L	P&G TIDE®	2 mM Na ₂ CO ₃	6	10.0	20
Automatic Dish Washing							
WE	ADW	3.0 g/L	RB CALGONIT™	2 mM Na ₂ CO ₃	21	10.0	40
NA	ADW	3.0 g/L	P&G CASCADE®	2 mM Na ₂ CO ₃	9	10.0	40

[00342] In some additional Examples, the following solutions find use:

Table B Working Detergent Solutions					
Detergent	Temp (C)	Detergent g/L	pH	Buffer	Gpg
TIDE® 2X Cold	16	0.98	8	5mM HEPES	6
TIDE® 2X Cold	32	0.98	8	5mM HEPES	6
TIDE® 2X Cold	16	0.98	7	5mM MOPS	6

[00343] Table C provides granular laundry detergent compositions produced in accordance with the invention suitable for laundering fabrics. The mannanase of the present invention is added at 0.1-10%.

Table C. Granular Laundry Detergent Compositions and Their Components						
Component	Detergent Compositions					
	1	2	3	4	5	6
Linear alkylbenzenesulfonate with aliphatic carbon chain length C ₁₁ -C ₁₂	15	12	20	10	12	13
Other surfactants	1.6	1.2	1.9	3.2	0.5	1.2
Phosphate builder(s)	2	3	4			
Zeolite		1		1	4	1
Silicate	4	5	2	3	3	5
Sodium Carbonate	2	5	5	4	0	3
Polyacrylate (MW 4500)	1	0.6	1	1	1.5	1
Carboxymethyl cellulose (Finnfix BDA ex CPKelco)	1	-	0.3	-	1.1	-
Celluclean® (15.6mg/g)	0.23	0.17	0.5	0.2	0.2	0.6
Lipase (20mg/g)	0.2		0.1		0.3	
Stainzyme Plus® (14mg/g)	0.23	0.17	0.5	0.2	0.2	0.6
Fluorescent Brightener(s)	0.16	0.06	0.16	0.18	0.16	0.16

Table C. Granular Laundry Detergent Compositions and Their Components

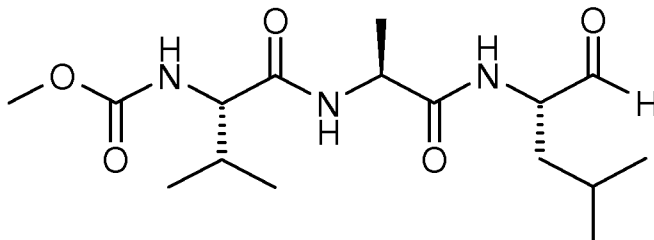
Component	Detergent Compositions					
	1	2	3	4	5	6
Diethylenetriamine pentaacetic acid or Ethylene diamine tetraacetic acid	0.6		0.6	0.25	0.6	0.6
MgSO ₄	1	1	1	0.5	1	1
Bleach(es) and Bleach activator(s)	6.88		6.12	2.09	1.17	4.66
Ethoxylated thiophene Hueing Dye ⁵	0.002	0.001	0.003	0.003	-	-
Direct Violet 9 ex Ciba Specialty Chemicals				0.0006	0.0004	0.0006
Sulfate/Citric Acid/ Sodium Bicarbonate/ Moisture/perfume	Balance to 100%					

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 Polyethylenimine (MW = 600) with 20 ethoxylate groups per -NH.

³ Amphiphilic alkoxyated grease cleaning polymer is a polyethylenimine (MW = 600) with 24 ethoxylate groups per -NH and 16 propoxylate groups per -NH

⁴ Reversible protease enzyme inhibitor of structure:



⁵Ethoxylated thiophene Hueing Dye is as described in US 7,208,459 B2.

[00344] In Table C, all enzyme levels expressed as % enzyme raw material, except for mannanase enzyme (of this invention) which is expressed as % of active protein added to the product.

[00345] Table D provides granular laundry detergent compositions suitable for top-loading automatic washing machines (detergent compositions 7-9) and front loading washing machines (detergent compositions 10-11). The mannanase enzyme tested and/or mannanase enzyme of the present invention is added separately to these formulations so that the final concentration in the wash liquor is between 0.01ppm and 10ppm.

Table D. Granular Laundry Detergent Compositions and Their Components

Component	Detergent Composition				
	7	8	9	10	11
Surfactants					
C ₁₆₋₁₇ Branched alkyl sulfate	3.55	15.8			
C ₁₂₋₁₄ alkyl sulphate			1.5		

Table D. Granular Laundry Detergent Compositions and Their Components					
Component	Detergent Composition				
	7	8	9	10	11
Sodium linear alkylbenzenesulfonate with aliphatic chain length C ₁₁ -C ₁₂	9.6		10.6	7.5	9
Sodium C _{14/15} alcohol ethoxy – 3 - sulfate	1.15			2.88	
Sodium C _{14/15} alkyl sulphate	2.37				
C _{14/15} alcohol ethoxylate with average 7 moles of ethoxylation				1.17	1
mono-C ₈₋₁₀ alkyl mono-hydroxyethyl dimethyl quaternary ammonium chloride					0.45
Di methyl hydroxyl ethyl lauryl ammonium chloride			0.18		
Zeolite A	13.9	4.7	0.01	2.9	1.8
Sodium Silicate 1.6.ratio	4	0.2		4	4
Sodium Silicate 2.35.ratio			8		
Citric Acid				2.5	1.4
Sodium tripolyphosphate			5		
Sodium Carbonate	24.1	30	16.9	24.4	21
Nonanoyloxybenzenesuphfonate	5.78	2.81	0.96		
Oxaziridinium-based bleach booster				0.03	0.017
Tetrasodium S,S,-ethylenediaminedisuccinate				0.2	
Diethylenetriamine penta (methylene phosphonic acid), heptasodium salt	0.61				0.33
Hydroxyethane dimethylene phosphonic acid				0.29	0.45
Ethylene diamine tetraacetate			0.27		
MgSO ₄			0.47	0.5994	0.782
Sodium Percarbonate	7	4.4		15.9	19.1
Tetra Acetyl Ethylene Diamine				3.3	4.6
Sodium Perborate Monohydrate			1.2		
Carboxymethyl cellulose (e.g. Finnfix BDA ex CPKelco)	0.1		0.17	1.69	0.23
Sodium Acrylic acid/maleic acid copolymer (70/30)	0.0236	3.8		2	2.5
Sodium polyacrylate (Sokalan PA30 CL)	4		0.84		
Terephthalate polymer				0.23	
Polyethylene glycol/vinyl acetate random graft co polymer			0.89	0.89	0.91
Photobleach- zinc phthalocyanine tetrasulfonate			0.005	0.001	0.002
C.I.Fluorescent Brightener 260	0.11	0.15	0.04	0.23	0.15
C.I.Fluorescent Brightener 351 (Tinopal ® CBS)			0.1		
Suds suppressor granule		0.25		0.07	0.04
Hydrophobically modified carboxy methyl cellulose (Finnifix ® SH-1)			0.019	0.028	
Bentonite			8.35		

Table D. Granular Laundry Detergent Compositions and Their Components					
Component	Detergent Composition				
	7	8	9	10	11
Miscellaneous (Dyes, perfumes, process aids, moisture and sodium sulphate)	Balance	Balance	Balance	Balance	Balance

[00346] In Table D, surfactant ingredients can be obtained from any suitable supplier, including but not limited to BASF (*e.g.*, LUTENSOL®), Shell Chemicals, Stepan, Huntsman, and Clariant (*e.g.*, PRAEPAGEN®). Zeolite can be obtained from sources such as Industrial Zeolite. Citric acid and sodium citrate can be obtained from sources such as Jungbunzlauer. Sodium percarbonate, sodium carbonate, sodium bicarbonate and sodium sesquicarbonate can be obtained from sources such as Solvay. Acrylate/maleate copolymers can be obtained from sources such as BASF. Carboxymethylcellulose and hydrophobically modified carboxymethyl cellulose can be obtained from sources such as CPKelco. C.I. Fluorescent Brightener 260 can be obtained from 3V Sigma (*e.g.*, OPTIBLANC®, OPTIBLANC® 2M/G, OPTIBLANC® 2MG/LT Extra, or OPTIBLANC® Ecobright. Tetrasodium S,S-ethylenediamine disuccinate can be obtained from sources such as Innospec. Terephthalate co-polymer can be obtained from Clariant (*e.g.*, REPELOTEx SF 2). In addition, 1-Hydroxyethane -1,1-diphosphonic acid can be obtained from Thermphos. Oxaziridinium-based bleach booster has the following structure, where R1 = 2-butyloctyl, and was produced according to US 2006/0089284A1.



[00347] The enzymes NATALASE®, TERMAMYL®, STAINZYME PLUS®, CELLUCLEAN® and MANNAWAY®, can be obtained from Novozymes. Zinc phthalocyanine tetrasulfonate can be obtained from Ciba Specialty Chemicals (*e.g.*, TINOLUX® BMC). Suds suppressor granule can be obtained from Dow Corning. In these detergent compositions, 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.

[00348] Tables E-G provide additional granular detergent compositions suitable for washing machines (detergents 36a-n). The Bleman1 mannanase enzyme of the present invention is added separately to these formulations.

Table E. Additional Granular Laundry Detergent Compositions and Their Components					
Component	Detergent Composition				
	36a	36b	36c	36d	36e
Surfactants					
C ₁₀ Nonionic				0.1843	
C ₁₆₋₁₇ Branched alkyl sulfate	3.53	3.53	3.53		
C ₁₂₋₁₄ alkyl sulphate					
Sodium linear alkylbenzenesulfonate with aliphatic chain length C ₁₁ -C ₁₂	8.98	8.98	8.98	13.58	14.75
Sodium C _{14/15} alcohol ethoxy – 3 - sulfate	1.28	1.28	1.28		
Sodium C _{14/15} alkyl sulphate	2.36	2.36	2.36		
C _{14/15} alcohol ethoxylate with average 7 moles of ethoxylation					
mono-C ₈₋₁₀ alkyl mono-hydroxyethyl di-methyl quaternary ammonium chloride					
Di methyl hydroxyl ethyl lauryl ammonium chloride				0.1803	
Zeolite A	15.31	15.31	15.31		4.47
Bentonite				8.35	
Sodium Silicate 1.6.ratio					0.16
Sodium Silicate 2.0.ratio	3.72	3.72	3.72	8.41	
Sodium Silicate 2.35.ratio					
Citric Acid				0.0066	
Sodium tripolyphosphate				5.06	
Sodium Carbonate	26.1	26.18	26.1	15.9	29.0
Nonanoyloxybenzenesuphphonate	5.78	5.78	5.78	1.17	1.86
Oxaziridinium-based bleach booster	0.037	0.037	0.037		
Tetrasodium S,S,-ethylenediaminedisuccinate					
Diethylenetriamine penta (methylene phosphonic acid), heptasodium salt	0.62	0.62	0.62		
Hydroxyethane dimethylene phosphonic acid					
Ethylene diamine tetraacetate				0.2701	
MgSO ₄	0.056	0.056	0.056	0.47	
Sodium Percarbonate		7.06	7.06		3.64
Tetra Acetyl Ethylene Diamine					
Sodium Perborate Monohydrate				1.47	
Carboxymethyl cellulose (e.g. Finnfix BDA ex CPKelco)	0.38	0.38	0.38	0.173	
Sodium Acrylic acid/maleic acid co-polymer (70/30)	3.79	3.78	3.79		3.64
Sodium polyacrylate (Sokalan PA30 CL)	3.78	3.78	3.78	0.842	
Terephthalate polymer					
Polyethylene glycol/vinyl acetate random graft co polymer				0.89	
Photobleach- zinc phthalocyanine tetrasulfonate					
C.I.Fluorescent Brightener 260	0.1125	0.1125	0.1125	0.043	0.15
C.I.Fluorescent Brightener 351 (Tinopal ® CBS)				0.0952	

Suds suppressor granule	0.015	0.015	0.015		0.031
Hydrophobically modified carboxy methyl cellulose (Finnifix ® SH-1)					
Bentonite					
Miscellaneous (Dyes, perfumes, process aids, moisture and sodium sulphate)	Balance	Balance	Balance	Balance	Balance

Table F. Additional Granular Laundry Detergent Compositions and Their Components					
	Detergent Composition				
Component	36f	36g	36h	36i	36j
Surfactants					
C ₁₀ Nonionic	0.1142	0.2894	0.1885	0.1846	0.1885
C ₁₆₋₁₇ Branched alkyl sulfate					
C ₁₂₋₁₄ alkyl sulphate					
Sodium linear alkylbenzenesulfonate with aliphatic chain length C ₁₁ -C ₁₂	12.94	15.69	9.01	8.42	9.51
Sodium C _{14/15} alcohol ethoxy – 3 - sulfate					
Sodium C _{14/15} alkyl sulphate					
C _{12/14} alcohol ethoxylate with average 7 moles of ethoxylation	2.9				
C _{12/14} alcohol ethoxylate with average 3 moles of ethoxylation				2.44	
C _{14/15} alcohol ethoxylate with average 7 moles of ethoxylation			0.97	1.17	0.97
mono-C ₈₋₁₀ alkyl mono-hydroxyethyl dimethyl quaternary ammonium chloride			0.45		
Di methyl hydroxyl ethyl lauryl ammonium chloride		0.195			0.45
Zeolite A	2.01	0.39	1.83	2.58	0.59
Sodium Silicate 1.6.ratio			4.53	5.62	4.53
Sodium Silicate 2.0.ratio		10.1			
Sodium Silicate 2.35.ratio	7.05				
Citric Acid			1.4	1.84	1.0
Sodium tripolyphosphate		5.73			
Sodium Carbonate	12.65	15.93	21.0	27.31	20.2
Nonanoyloxybenzenesulphonate		1.73			
Oxaziridinium-based bleach booster			0.0168	0.0333	0.024
Tetrasodium S,S'-ethylenediaminedisuccinate					
Diethylenetriamine penta (methylene phosphonic acid), heptasodium salt			0.327		0.3272
Hydroxyethane dimethylene phosphonic acid			0.45	0.2911	0.45
Ethylene diamine tetraacetate		0.28		0.1957	
MgSO ₄		0.54	0.79	0.6494	0.793
Sodium Percarbonate			19.1	15.85	22.5
Tetra Acetyl Ethylene Diamine			4.554	3.71	5.24
Sodium Perborate Monohydrate		5.55			

Carboxymethyl cellulose (e.g. Finnfix BDA ex CPKelco)	0.62	0.21	0.23	1.07	0.2622
Sodium Acrylic acid/maleic acid co- polymer (70/30)	0.40	2.61	2.5	2.00	1.75
Sodium polyacrylate (Sokalan PA30 CL)			0.0055	0.011	0.008
Terephthalate polymer				0.231	
Polyethylene glycol/vinyl acetate random graft co polymer	0.55	1.40	0.911	0.8924	0.911
Photobleach- zinc phthalocyanine tetrasulfonate					
C.I.Fluorescent Brightener 260	0.1174	0.048	0.1455	0.2252	0.1455
C.I.Fluorescent Brightener 351 (Tinopal ® CBS)		0.1049			
Suds suppressor granule			0.04	0.0658	0.04
Hydrophobically modified carboxy methyl cellulose (Finnifix ® SH-1)					
Bentonite					
Miscellaneous (Dyes, perfumes, process aids, moisture and sodium sulphate)	Balance	Balance	Balance	Balance	Balance

Table G. Additional Granular Laundry Detergent Compositions and Their Components					
	Detergent Composition				
Component	36k	36l	36m	36n	
Surfactants					
C ₁₀ Nonionic	0.1979	0.1979	0.1979	0.1979	
C ₁₆₋₁₇ Branched alkyl sulfate					
C ₁₂₋₁₄ alkyl sulphate					
Sodium linear alkylbenzenesulfonate with aliphatic chain length C ₁₁ -C ₁₂	8.92	8.92	11.5	11.5	
Sodium C _{14/15} alcohol ethoxy – 3 - sulfate	1.62	1.62	1.125	1.125	
Sodium C _{14/15} alkyl sulphate					
C _{14/15} alcohol ethoxylate with average 7 moles of ethoxylation	1.0	1.0	1.5	1.5	
mono-C ₈₋₁₀ alkyl mono-hydroxyethyl di- methyl quaternary ammonium chloride					
Di methyl hydroxyl ethyl lauryl ammonium chloride					
Zeolite A	1.63	1.63	2.0	2.0	
Sodium Silicate 1.6.ratio	4.75	4.75	4.75	4.75	
Sodium Silicate 2.0.ratio			0.06	0.06	
Sodium Silicate 2.35.ratio					
Citric Acid	1.10	1.10	1.1	1.1	
Sodium tripolyphosphate					
Sodium Carbonate	23.3	23.3	23.3	23.3	
Nonanoyloxybenzenesuphlonate					
Oxaziridinium-based bleach booster	0.021	0.021	0.015	0.015	
Tetrasodium S,S,- ethylenediaminedisuccinate	0.26	0.26	0.26	0.26	

Diethylenetriamine penta (methylene phosphonic acid), heptasodium salt					
Hydroxyethane dimethylene phosphonic acid	0.47	0.47	0.47	0.47	
Ethylene diamine tetraacetate					
MgSO ₄	0.83	0.83	0.82	0.82	
Sodium Percarbonate	19.35	19.35	19.35	19.35	
Tetra Acetyl Ethylene Diamine	4.51	4.51	4.51	4.51	
Sodium Perborate Monohydrate					
Carboxymethyl cellulose (e.g. Finnfix BDA ex CPKelco)	1.01	1.01	1.01	1.01	
Sodium Acrylic acid/maleic acid co-polymer (70/30)	1.84	1.84	1.84	1.84	
Sodium polyacrylate (Sokalan PA30 CL)	0.007	0.007	0.005	0.005	
Terephthalate polymer	0.179	0.179	0.179	0.179	
Polyethylene glycol/vinyl acetate random graft co polymer	0.96	0.96	0.96	0.96	
Photobleach- zinc phthalocyanine tetrasulfonate					
C.I.Fluorescent Brightener 260	0.153	0.153	0.171	0.171	
C.I.Fluorescent Brightener 351 (Tinopal ® CBS)					
Suds suppressor granule	0.042	0.042	0.042	0.042	
Hydrophobically modified carboxy methyl cellulose (Finnifix ® SH-1)					
Bentonite					
Miscellaneous (Dyes, perfumes, process aids, moisture and sodium sulphate)	Balance	Balance	Balance	Balance	Balance

[00349] Notes for detergent compositions 36 a – n in Tables E, F, G:

[00350] Surfactant ingredients can be obtained from BASF, Ludwigshafen, Germany (Lutensol®); Shell Chemicals, London, UK; Stepan, Northfield, Illinois, USA; Huntsman, Huntsman, Salt Lake City, Utah, USA; Clariant, Sulzbach, Germany (Praepagen®).

[00351] Zeolite can be obtained from Industrial Zeolite (UK) Ltd, Grays, Essex, UK.

[00352] Citric acid and sodium citrate can be obtained from Jungbunzlauer, Basel, Switzerland.

[00353] Sodium percarbonate, sodium carbonate, sodium bicarbonate and sodium sesquicarbonate can be obtained from Solvay, Brussels, Belgium.

[00354] Acrylate/maleate copolymers can be obtained from BASF, Ludwigshafen, Germany.

[00355] Carboxymethylcellulose and hydrophobically modified carboxymethyl cellulose can be obtained from CPKelco, Arnhem, The Netherlands.

[00356] C.I. Fluorescent Brightener 260 can be obtained from 3V Sigma, Bergamo, Italy as Optiblanc® Optiblanc® 2M/G, Optiblanc® 2MG/LT Extra, or Optiblanc® Ecobright.

[00357] Tetrasodium S,S-ethylenediamine disuccinate can be obtained from Innospec, Ellesmere Port, UK.

[00358] Terephthalate co-polymer can be obtained from Clariant under the tradename Repelotex SF 2.

[00359] 1-Hydroxyethane -1,1-diphosphonic acid can be obtained from Thermphos, Vlissingen-Oost, The Netherlands.

[00360] Oxaziridinium-based bleach booster has the following structure, where R1 = 2-butyloctyl, and was produced according to US 2006/0089284A1.



10 [00361] Enzymes Natalase®, Termamyl®, Stainzyme Plus®, Celluclean® and Mannaway®, can be obtained from Novozymes, Bagsvaerd, Denmark.

[00362] Zinc phthalocyanine tetrasulfonate can be obtained from Ciba Specialty Chemicals, Basel, Switzerland, as Tinolux® BMC.

[00363] Suds suppressor granule can be obtained from Dow Corning, Barry, UK.

15 [00364] 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.

CLAIMS

We claim:

1. A recombinant polypeptide comprising a catalytic domain of a mannanase enzyme, wherein the catalytic domain is at least 70% identical to the amino acid sequence of
5 SEQ ID NO:2.
2. The recombinant polypeptide of Claim 1, wherein the polypeptide has mannanase activity on an AZO-Carob Galactomannan or Locust Bean Gum substrate.
3. The recombinant polypeptide of Claim 2, wherein the substrate is AZO-Carob Galactomannan.
- 10 4. The recombinant polypeptide of Claim 2, wherein the substrate is Locust Bean Gum.
5. The recombinant polypeptide of any one of claims 1-4, wherein the polypeptide has mannanase activity at pH values of between 7 and 11.
6. The recombinant polypeptide of any one of claims 1-5, wherein the polypeptide
15 retains greater than 50% mannanase activity at pH values of between 7 and 9.
7. The recombinant polypeptide of any one of claims 1-6, wherein the polypeptide has mannanase activity at temperature values of between 10°C and 60°C.
8. The recombinant polypeptide of Claim 1, wherein the polypeptide has mannanase activity in the presence of detergent.
- 20 9. The recombinant polypeptide of Claim 1 or 2, wherein the polypeptide has mannanase activity in the presence of a protease.
10. The recombinant polypeptide of any one of Claims 1-9, further comprising a native or non-native signal peptide.
11. The recombinant polypeptide of any one of Claims 1-10, wherein the polypeptide
25 does not further comprise a carbohydrate-binding module.
12. A detergent composition comprising the recombinant polypeptide of any one of Claims 1-11.

13. The detergent composition of Claim 12, wherein the polypeptide is capable of hydrolyzing a substrate selected from the group consisting of chocolate ice cream, chocolate pudding, guar gum, locust bean gum, and combinations thereof.

14. The detergent composition of Claim 12 or 13, wherein the polypeptide is active at
5 a temperature between 10°C and 60°C.

15. The detergent composition of any of Claims 12-14, wherein the polypeptide is active at 16°C or 32°C.

16. The detergent composition of any of Claims 12-15, wherein the polypeptide has mannanase activity in the presence of a protease.

10 17. The detergent composition of any of Claims 12-16, wherein the polypeptide retains activity in the presence of a bleaching agent.

18. The detergent composition of any of Claims 12-17, further comprising a bleaching agent.

15 19. The detergent composition of any of Claims 12-18, further comprising an amylase enzyme.

20. The detergent composition of any of Claims 12-19, further comprising a surfactant.

21. The detergent composition of Claim 20, wherein the surfactant is an ionic or non-ionic surfactant.

20 22. The detergent composition of Claim 21, wherein the ionic surfactant is selected from the group consisting of an anionic surfactant, a cationic surfactant, a zwitterionic surfactant, and a combination thereof.

23. The detergent composition of any one of Claims 12-22, further comprising an enzyme selected from the group consisting of proteases, peroxidases, cellulases, beta-glucanases, hemicellulases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases,

25

xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo- β -mannanases, exo- β -mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof.

24. The detergent composition of Claim 23, wherein the combination comprises a
5 protease and an amylase.

25. The detergent composition of any one of Claims 12-24, wherein the detergent is selected from the group consisting of a laundry detergent, dishwashing machine cleaning, hand dishwashing, a fabric softening detergent, a dishwashing detergent, and a hard-surface cleaning detergent.

10 26. The detergent composition of any one of Claims 12-25, wherein the detergent is in a form selected from the group consisting of a liquid, a powder, a granulated solid, and a tablet.

27. A method for hydrolyzing a mannan substrate present in a soil or stain on a surface, comprising: contacting the surface with the detergent composition of any one of Claims
15 12-26 to produce a clean surface.

28. A method of textile cleaning comprising: contacting a soiled textile with the detergent composition of any one of Claims 12-26 to produce a clean textile.

29. An isolated nucleic acid encoding the recombinant polypeptide of any one of Claims 1-11.

20 30. An expression vector comprising the isolated nucleic acid of Claim 29 in operable combination to a regulatory sequence.

31. A host cell comprising the expression vector of Claim 30.

32. The host cell of Claim 31, wherein the host cell is a bacterial cell, mammalian cell or a fungal cell.

25 33. A method of producing a mannanase, comprising: culturing the host cell of Claim 30 or 31 in a culture medium, under suitable conditions to produce a culture comprising the endo- β -mannanase.

34. A method for hydrolyzing a polysaccharide, comprising: contacting a polysaccharide comprising mannose with the polypeptide of Claim 1 to produce oligosaccharides comprising mannose.

5 35. The method of Claim 34, wherein the polysaccharide is selected from the group consisting of mannan, glucomannan, galactomannan, galactoglucomannan, and combinations thereof.

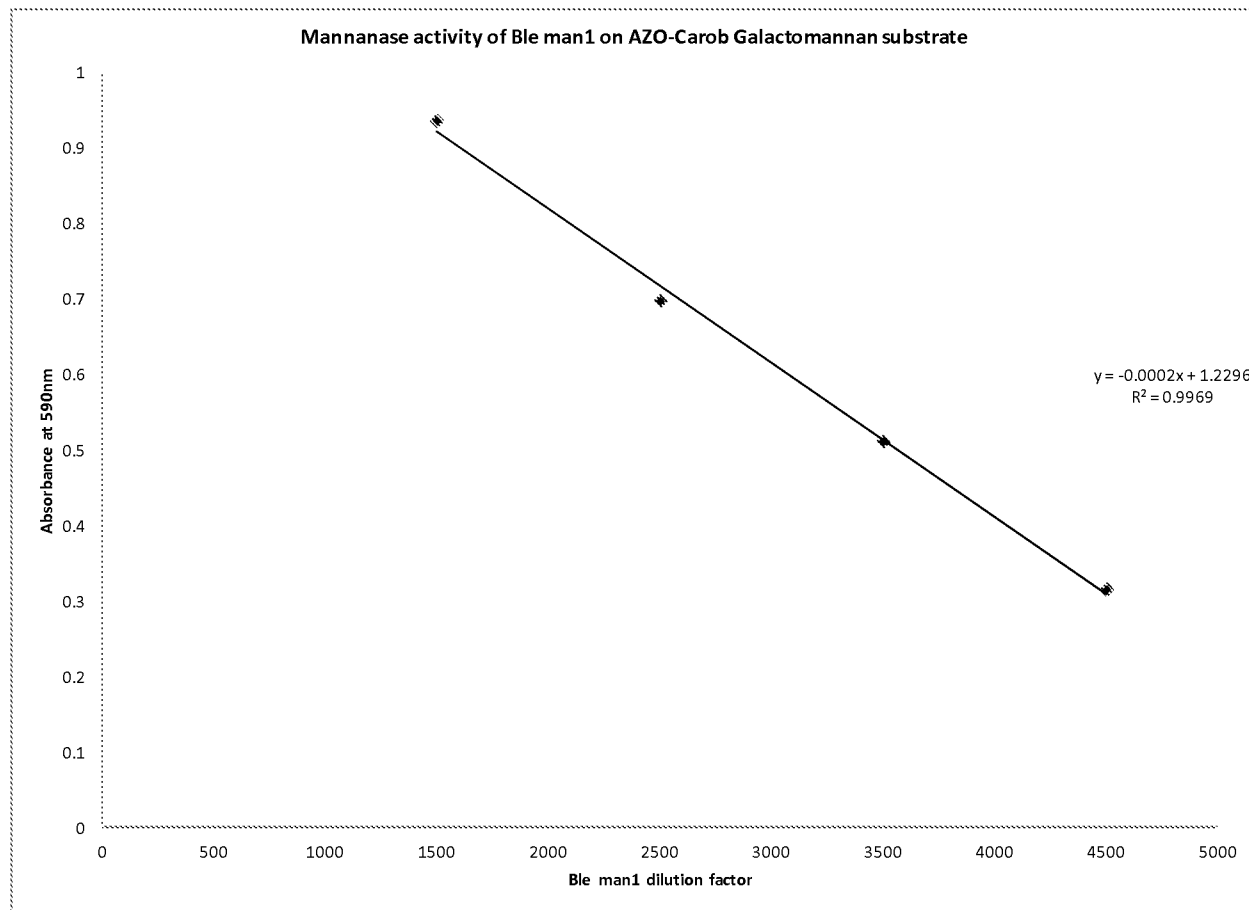


Figure 1-1: Dose response curve of Bleman1 on AZO-Carob Galactomannan

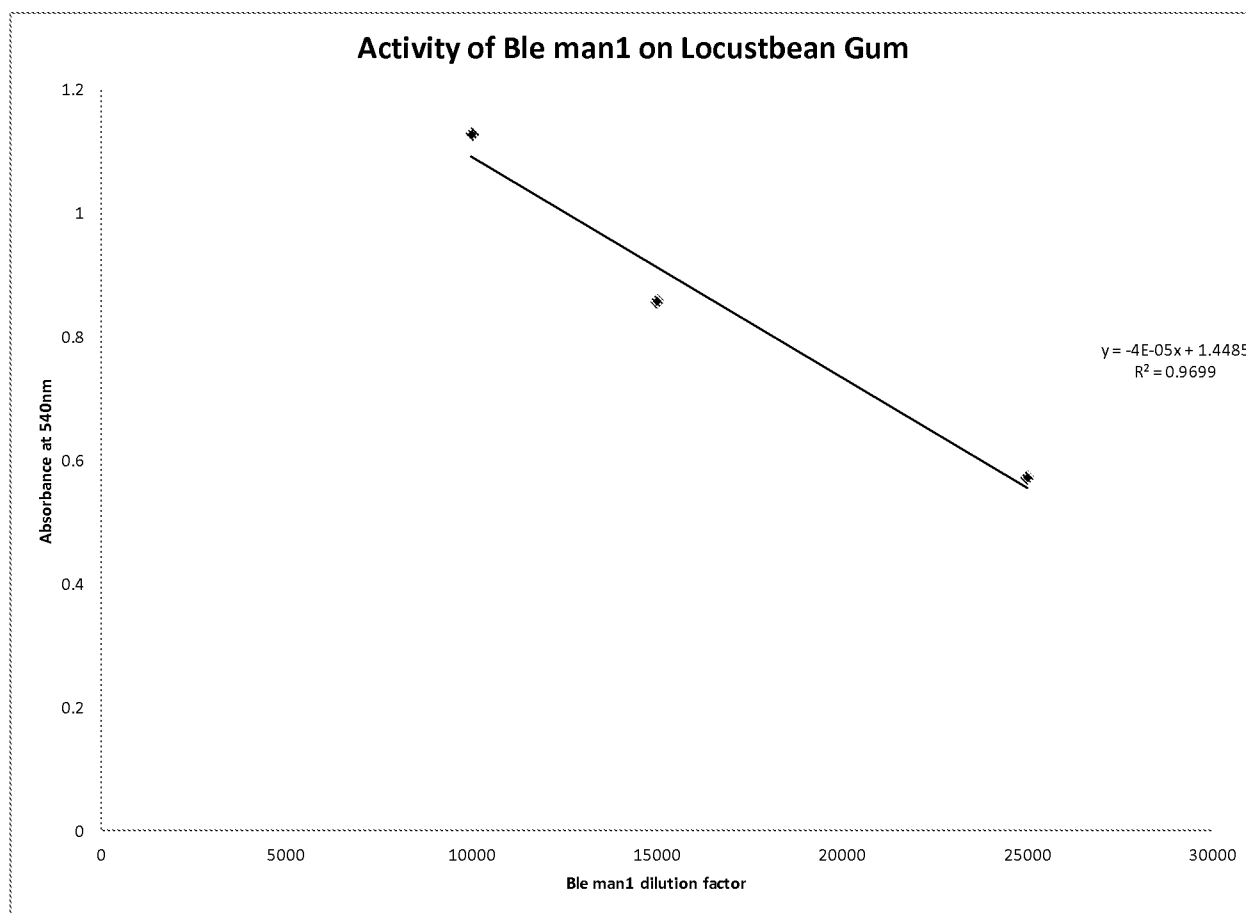


Figure 1-2: Dose response curve of Bleman1 on Locust Bean Gum

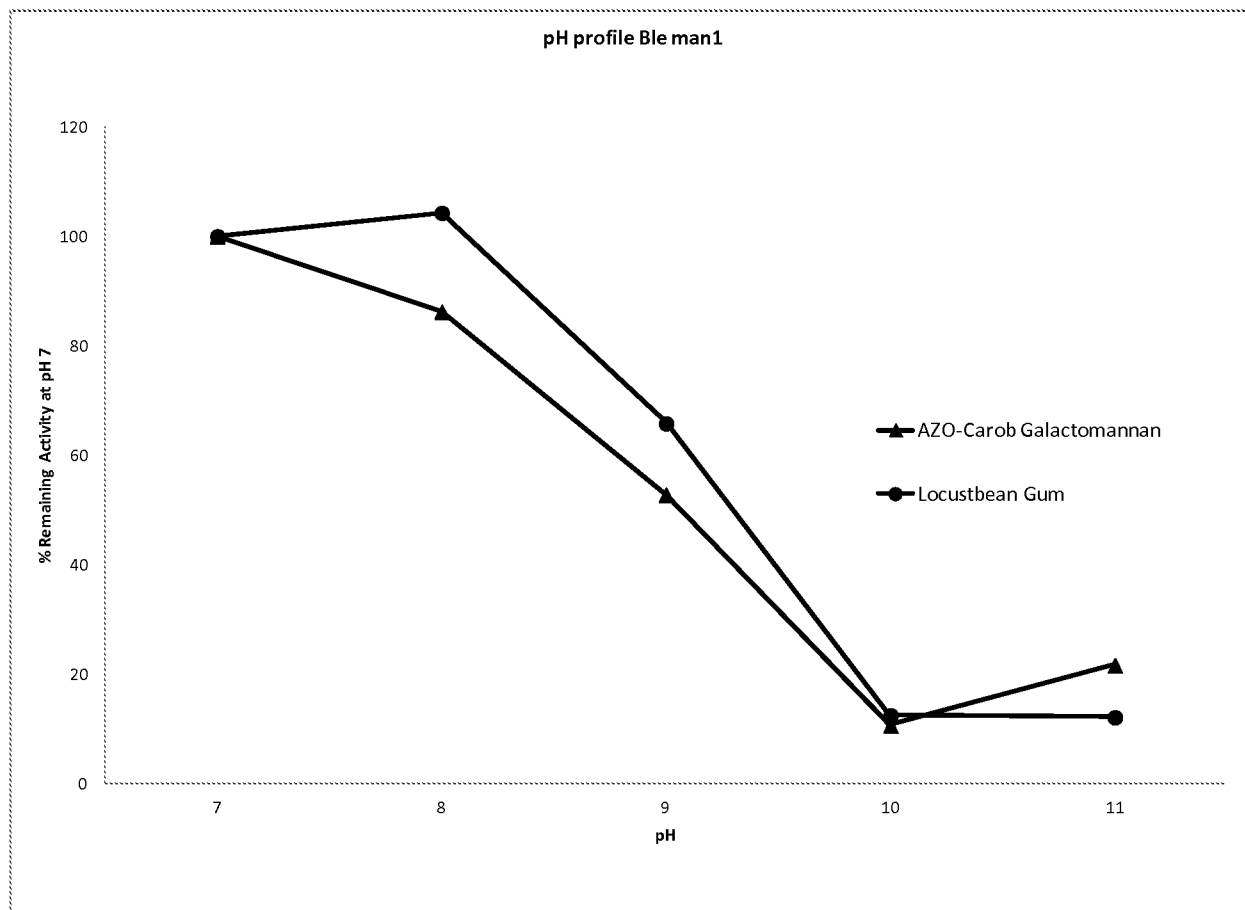
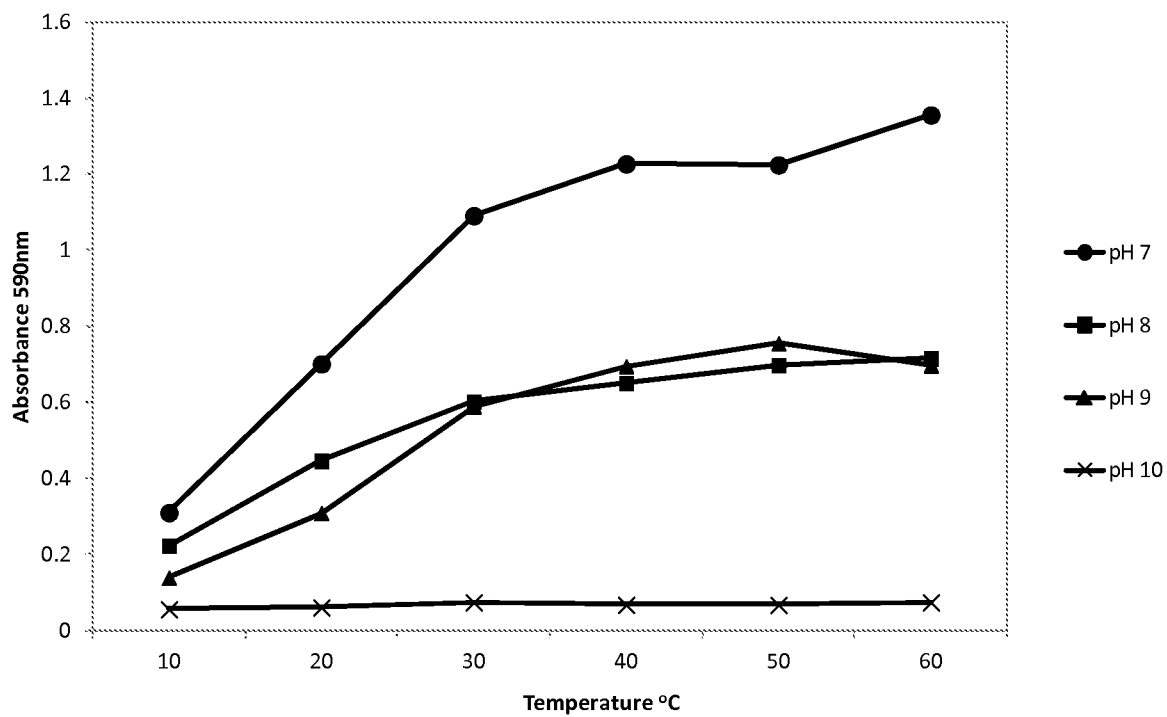


Figure 2-1: pH profile of Bleman on AZO-Carob Galactomannan

Temperature Profile of Ble man1 on AZO-Carob Galactomannan**Figure 3-1: Temperature profile of Bleman1 on AZO-Carob Galactomannan**

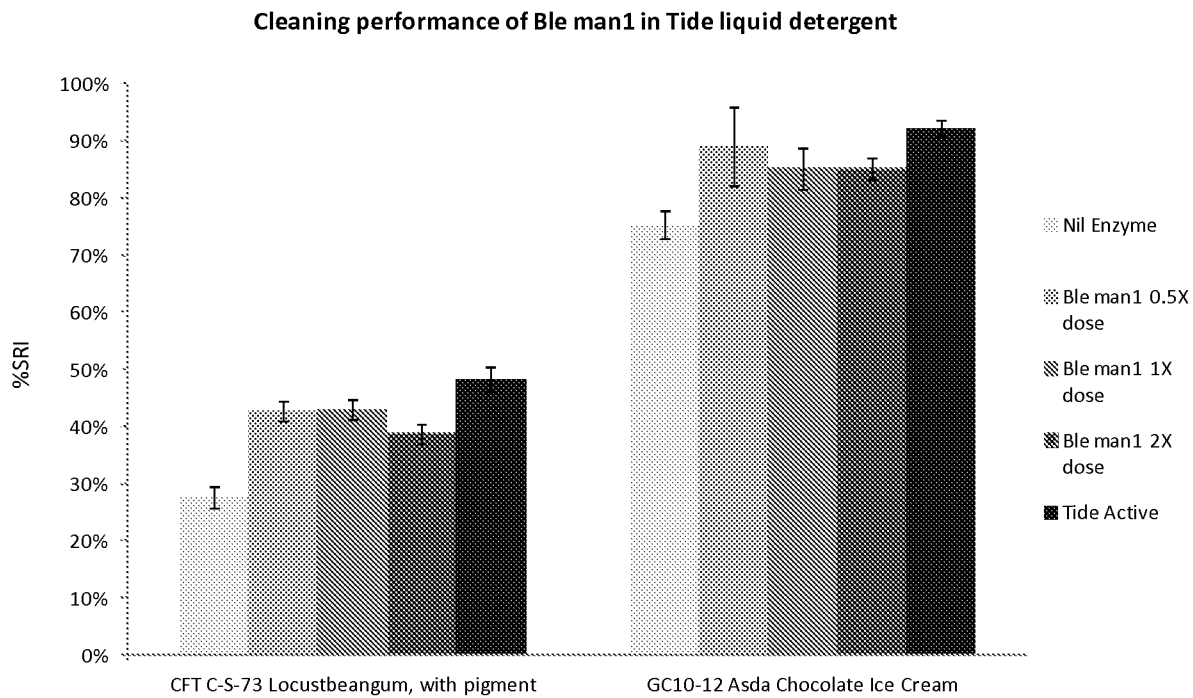


Figure 4-1. Dose response for the cleaning performance of Bleman1.

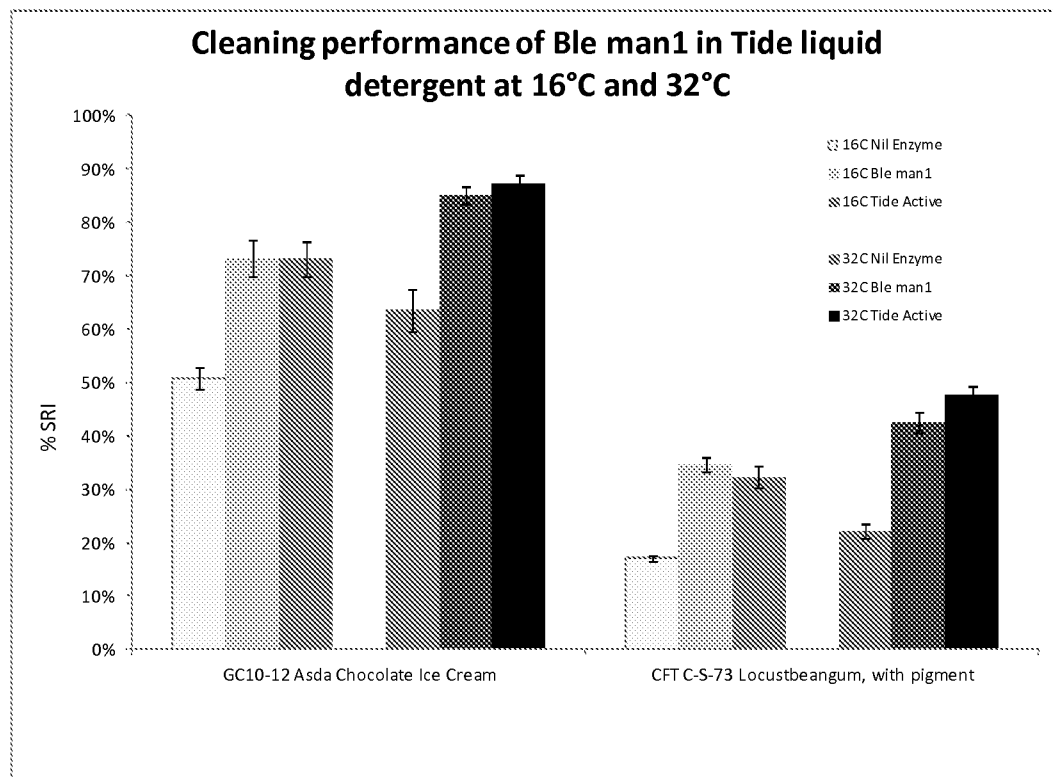


Figure 5-1. Cleaning performance of Bleman1 in heat inactivated Tide liquid detergent at 16°C and 32°C.

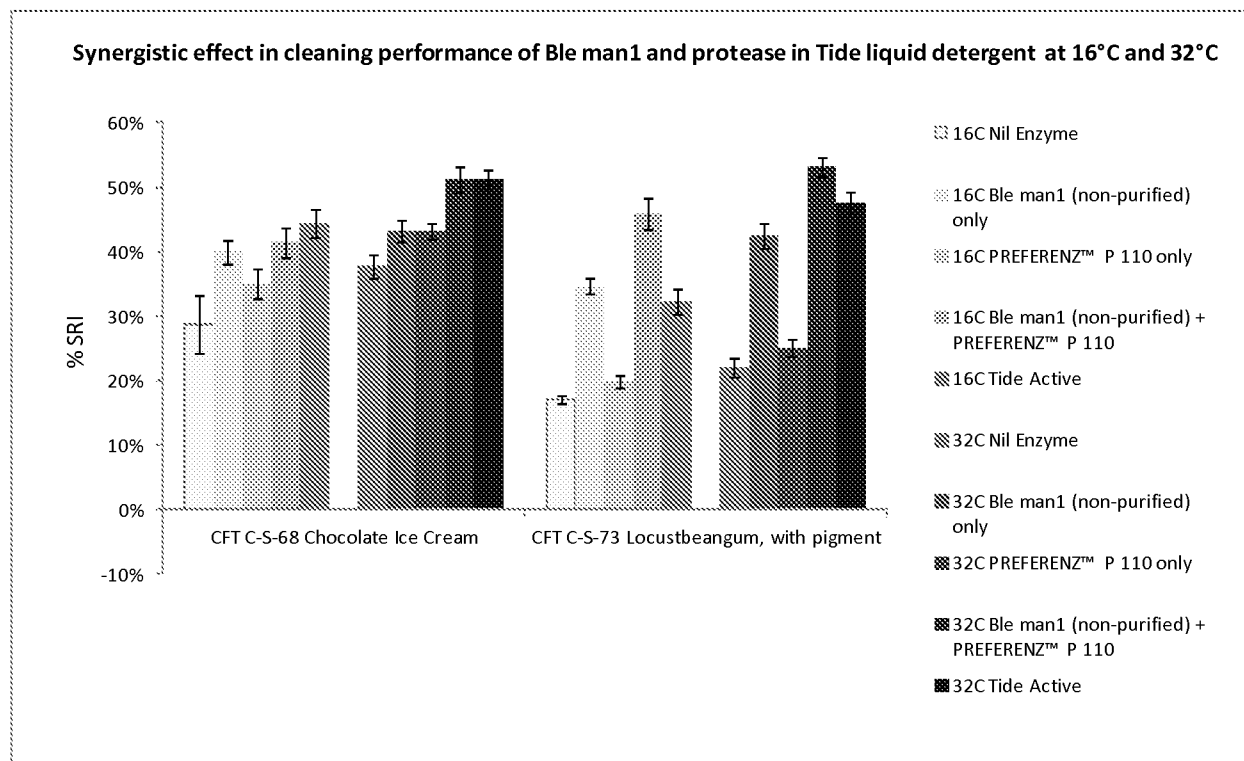


Figure 6-1. Cleaning performance of Bleman1 in the presence of protease.

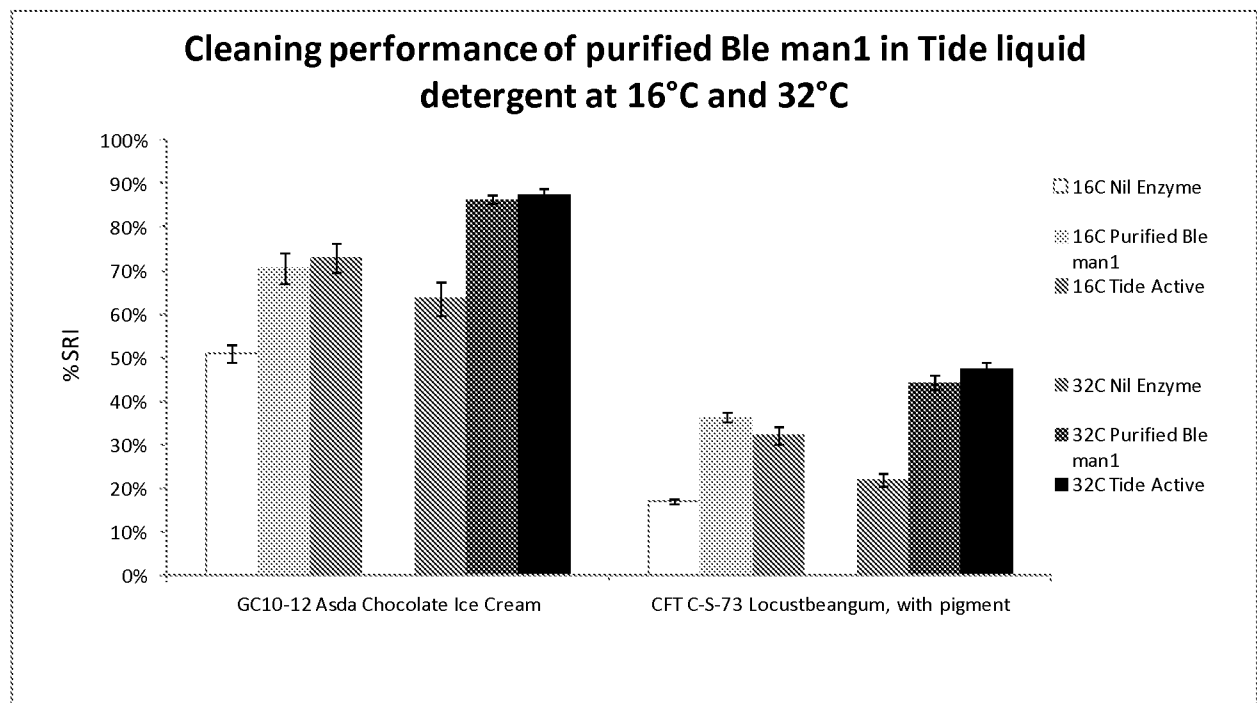


Figure 7-1. Cleaning performance of purified Bleman1 in Tide liquid detergent at 16°C and 32°C.

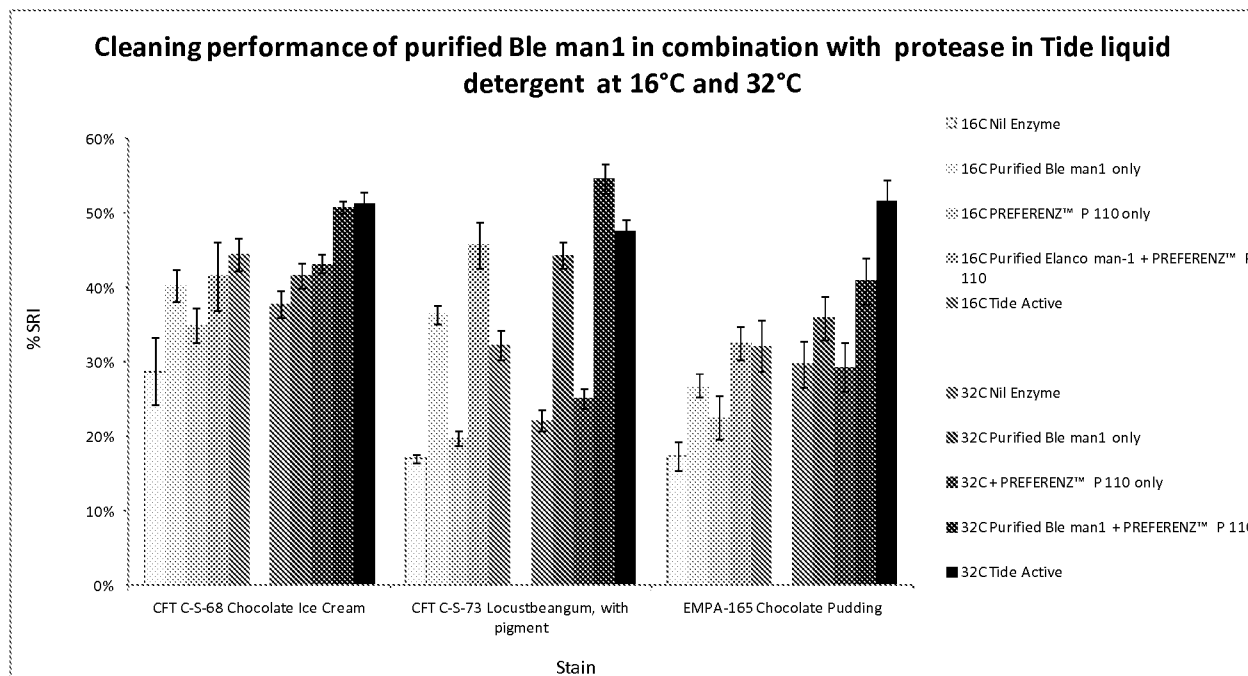


Figure 8-1. Cleaning performance of purified Bleman1 in the presence of protease.

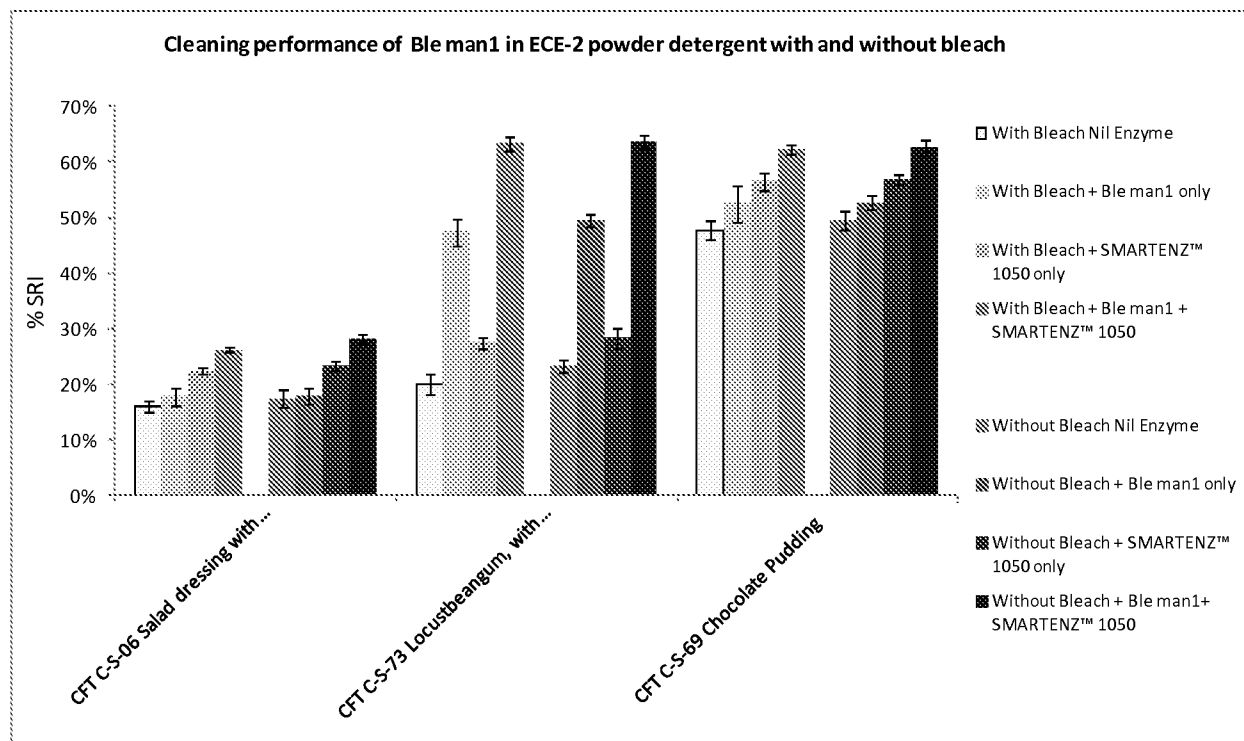


Figure 9-1. Cleaning performance of Bleman1 alone and with a protease/amylase granule blend in powder detergent with and without bleach.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/075829

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/24
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

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, CHEM ABS Data, Sequence Search, 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.
X	EP 2 404 930 A1 (VERENIUM CORP [US]; SYNGENTA PARTICIPATIONS AG [CH] VERENIUM CORP [US]) 11 January 2012 (2012-01-11) embodiments 6,24,41,45,106; paragraphs [0054], [0088], [0434], [0435], [0530] - [0541], [0548]; figure 5; sequences 388,389 -----	1-35
X	WO 91/18974 A1 (CHEMGEN CORP [US]) 12 December 1991 (1991-12-12)	1-17, 29-35
Y	the whole document claims 2,3,5,6,15-18; examples 3,10-13 -----	18-28
Y	WO 2012/149317 A1 (DANISCO US INC [US]; JONES BRIAN E [US]; KOLKMAN MARC [US]; QIAN ZHEN) 1 November 2012 (2012-11-01) the whole document ----- -/-	18-28



Further documents are listed in the continuation of Box C.



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

25 March 2014

Date of mailing of the international search report

02/04/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Pilat, Daniel

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/075829

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/149333 A1 (DANISCO US INC [US]; JONES BRIAN E [US]; KOLKMAN MARC [US]; QIAN ZHEN) 1 November 2012 (2012-11-01) the whole document	18-28
X	----- YOSHIDA S ET AL: "CLONING, SEQUENCE ANALYSIS AND EXPRESSION IN ESCHERCHIA COLI OF A GENE CODING FOR AN ENZYME FROM BACILLUS CIRCULANS K-1 THAT DEGRADATES GUAR GUM", BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOCIETY FOR BIOSCIENCE, BIOTECHNOLOGY, AND AGROCHEMISTRY, TOKYO, JAPAN, vol. 62, no. 3, 1 January 1998 (1998-01-01), pages 514-520, XP000881878, ISSN: 0916-8451, DOI: 10.1271/BBB.62.514 the whole document	1-35
A	----- JP 2006 087401 A (ALMIGHTY KK) 6 April 2006 (2006-04-06) the whole document	1-35
A	----- DHAWAN SAMRITIL ET AL: "Microbial mannanases: An overview of production and applications", CRC CRITICAL REVIEWS IN BIOTECHNOLOGY, CRC PRESS, BOCA RATON, FL, US, vol. 27, no. 4, 1 January 2007 (2007-01-01), pages 197-216, XP009161462, ISSN: 0738-8551 abstract -----	1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/075829

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2404930	A1	11-01-2012	AU 2004254640 A1 13-01-2005
			AU 2010246408 A1 16-12-2010
			BR PI0412279 A 19-09-2006
			CA 2529403 A1 13-01-2005
			CN 101370819 A 18-02-2009
			CN 103484485 A 01-01-2014
			CN 103484486 A 01-01-2014
			DK 1641910 T3 21-05-2013
			EP 1641910 A2 05-04-2006
			EP 2404928 A1 11-01-2012
			EP 2404929 A1 11-01-2012
			EP 2404930 A1 11-01-2012
			EP 2404931 A1 11-01-2012
			EP 2409981 A1 25-01-2012
			ES 2401795 T3 24-04-2013
			JP 2007529993 A 01-11-2007
			JP 2011087583 A 06-05-2011
			JP 2014027928 A 13-02-2014
			US 2008233175 A1 25-09-2008
			US 2012021490 A1 26-01-2012
			WO 2005003319 A2 13-01-2005
WO 9118974	A1	12-12-1991	AU 8060091 A 31-12-1991
			US 5476775 A 19-12-1995
			US 5551515 A 03-09-1996
			WO 9118974 A1 12-12-1991
WO 2012149317	A1	01-11-2012	AR 086214 A1 27-11-2013
			EP 2702072 A1 05-03-2014
			US 2014073030 A1 13-03-2014
			WO 2012149317 A1 01-11-2012
WO 2012149333	A1	01-11-2012	AR 086216 A1 27-11-2013
			EP 2702152 A1 05-03-2014
			US 2014073548 A1 13-03-2014
			WO 2012149333 A1 01-11-2012
JP 2006087401	A	06-04-2006	NONE