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(54) **DETERGENT COMPOSITION COMPRISING PROTEASE**

REINIGUNGSZUSAMMENSETZUNG MIT PROTEASE

COMPOSITION DÉTERGENTE COMPRENANT DE LA PROTÉASE

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EP 3 717 616 B1

Description

Field of Invention

[0001] The invention concerns a detergent composition comprising a surfactant that incorporates a new protease enzyme.

Background of the Invention

[0002] Water can be a scare resource. Consumers who wish to use detergent compositions on a substrate, particularly laundry detergents on textiles may only be able to use water that is not optimum for cleaning. One example of this is that salty water (water with a significant sodium chloride content) such as sea water is sometimes used.

[0003] Proteases are common ingredients in cleaning compositions. One problem with commercial proteases is that they work poorly in salty water conditions. EP0319460 A2 relates to cleaning compositions, and to a method of cleaning using such compositions, which contain certain proteases produced by microorganisms of the genus Vibrio.

[0004] WO2016207275 A1 concerns the use of one or more enzymes, such as proteases, for washing or rinsing a laundry item with water having a salt content of at least 0.05 % at 20°C and/or a BOD value of at least 1 mg/L at 20°C.

Summary of the Invention

[0005] We have found that the incorporation of the new protease enzyme according to claim 1 in detergent compositions shows enhanced cleaning.

[0006] In one aspect the present invention provides a detergent composition comprising:

(i) from 1 to 60 wt.%, preferably from 2 to 50, more preferably from 4 to 50 wt.% of surfactant;

(ii) from 0.0005 to 1 wt.%, preferably from 0.005 to 0.6 wt.% of a protease enzyme having at least 90% sequence identity to SEQ ID NO: 1.

[0007] Preferably the protease enzyme has at least 95%, more preferably 97% sequence identity to SEQ ID NO: 1. Most preferably the protease enzyme has 100% sequence identity to SEQ ID NO: 1.

[0008] A preferred detergent composition is a laundry detergent composition. Preferably the laundry detergent composition is a liquid or a powder, more preferably the detergent is a liquid detergent.

[0009] Preferably the laundry detergent composition comprises anionic and/or nonionic surfactant, more preferably the laundry detergent composition comprises both anionic and nonionic surfactant.

[0010] The laundry detergent preferably comprises an alkoxylated polyamine.

[0011] The laundry detergent preferably comprises a soil release polymer, more preferably a polyester based soil released polymer.

[0012] Preferably the laundry detergent comprises phosphonic acid (or salt thereof) chelating agent at a level that is less than 0.1 wt.%, more preferably less than 0.01 wt.%, most preferably the composition is free from phosphonic acid (or salt thereof) chelating agent.

[0013] Preferred detergent compositions, particularly laundry detergent compositions additionally comprise a further enzyme selected from the group consisting of: lipases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, mannanases, and/or additional proteases.

[0014] In another aspect the present invention provides a method of improving enzymatic cleaning in water having a sodium chloride content of from 0.1 to 4%, preferably from 0.25 to 3 wt.% at 20°C, said method comprising incorporation of a protease enzyme having at least 90% sequence identity to SEQ ID NO: 1 into a detergent composition comprising from 1 to 60 wt.% of a surfactant; and subsequent treatment of a substrate, preferably textiles, with said composition.

[0015] In another aspect the present invention provides the use of a protease enzyme having at least 90%, preferably 95%, more preferably 97%, most preferably 100%, sequence identity to SEQ ID NO: 1 to improve enzymatic cleaning in water having a sodium chloride content of from 0.1 to 4%, at a temperature of from 15°C to 45°C.

Detailed Description of the Invention

[0016] The indefinite article "a" or "an" and its corresponding definite article "the" as used herein means at least one, or one or more, unless specified otherwise.

[0017] All % levels of ingredients in compositions (formulations) listed herein are in wt.% based on total formulation unless other stated.

[0018] The detergent composition may take any suitable form, for example liquids, solids (including powders) or gels.

[0019] The detergent composition can be applied to any suitable substrate. Particularly preferred substrates are textiles. Particularly preferred detergent compositions are laundry detergent compositions.

[0020] Laundry detergent compositions may take any suitable form. Preferred forms are liquid or powder, with liquid being most preferred.

Surfactant

[0021] The detergent composition comprises surfactant (which includes a mixture of two or more surfactants). The composition comprises from 1 to 60 wt.%, preferably from 2 to 50 wt.%, more preferably from 4 to 50 wt.% of surfactant. Even more preferred levels of surfactant are from 6 to 30 wt.%, more preferably from 8 to 20 wt.%.

[0022] The detergent composition (preferably a laundry detergent composition) comprises anionic and/or nonionic surfactant, preferably comprising both anionic and nonionic surfactant.

[0023] Suitable anionic detergent compounds which may be used are usually water-soluble alkali metal salts of organic sulphates and sulphonates having alkyl radicals containing from about 8 to about 22 carbon atoms, the term alkyl being used to include the alkyl portion of higher alkyl radicals.

[0024] Examples of suitable synthetic anionic detergent compounds are sodium and potassium alkyl sulphates, especially those obtained by sulphating higher C_8 to C_{18} alcohols, produced for example from tallow or coconut oil, sodium and potassium alkyl C_9 to C_{20} benzene sulphonates, particularly sodium linear secondary alkyl C_{10} to C_{15} benzene sulphonates; and sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum.

[0025] The anionic surfactant is preferably selected from: linear alkyl benzene sulphonate; alkyl sulphates; alkyl ether sulphates; soaps; alkyl (preferably methyl) ester sulphonates, and mixtures thereof.

[0026] The most preferred anionic surfactants are selected from: linear alkyl benzene sulphonate; alkyl sulphates; alkyl ether sulphates and mixtures thereof. Preferably the alkyl ether sulphate is a C_{12}-C_{14} n-alkyl ether sulphate with an average of 1 to 3EO (ethoxylate) units.

[0027] Sodium lauryl ether sulphate is particularly preferred (SLES). Preferably the linear alkyl benzene sulphonate is a sodium C_{11} to C_{15} alkyl benzene sulphonates. Preferably the alkyl sulphates is a linear or branched sodium C_{12} to C_{18} alkyl sulphates. Sodium dodecyl sulphate is particularly preferred, (SDS, also known as primary alkyl sulphate).

[0028] In liquid formulations preferably two or more anionic surfactant are present, for example linear alkyl benzene sulphonate together with an alkyl ether sulphate.

[0029] In liquid formulations, preferably the laundry composition in addition to the anionic surfactant comprises alkyl exthoylated non-ionic surfactant, preferably from 2 to 8 wt.% of alkyl ethoxylated non-ionic surfactant.

[0030] Suitable nonionic detergent compounds which may be used include, in particular, the reaction products of compounds having an aliphatic hydrophobic group and a reactive hydrogen atom, for example, aliphatic alcohols, acids or amides, especially ethylene oxide either alone or with propylene oxide. Preferred nonionic detergent compounds are the condensation products of aliphatic C_8 to C_{18} primary or secondary linear or branched alcohols with ethylene oxide.

[0031] Most preferably the nonionic detergent compound is the alkyl ethoxylated non-ionic surfactant is a C_8 to C_{18} primary alcohol with an average ethoxylation of 7EO to 9EO units.

[0032] Preferably the surfactants used are saturated.

Protease

[0033] We have found that the protease performs well in salt water conditions. The protease can therefore be considered halotolerant. This means that it can function in a high salt environment.

[0034] We have also found that the protease can perform well at low temperature 20°C.

[0035] The protease outperforms commercial protease enzymes at both high temperature 40°C and low temperature 20°C salt water environments.

[0036] The protease is present at a level of from 0.0005 to 1 wt.%, preferably from 0.005 to 0.6 wt.%.

[0037] The protease enzyme has at least 90%, preferably 95%, or even 97% sequence identity to SEQ ID NO: 1. The protease enzyme most preferably may have 100% sequence identity to SEQ ID NO: 1.

Alkoxylated polyamine

[0038] When the detergent composition is in the form of a laundry composition, it is preferred that an alkoxylated polyamine is included.

[0039] Preferred levels of alkoxylated polyamine range from 0.1 to 8 wt.%, preferably from 0.2 to 6 wt.%, more preferably from 0.5 to 5 wt.%. Another preferred level is from 1 to 4 wt.%.

[0040] The alkoxylated polyamine may be linear or branched. It may be branched to the extent that it is a dendrimer. The alkoxylation may typically be ethoxylation or propoxylation, or a mixture of both. Where a nitrogen atom is alkoxylated, a preferred average degree of alkoxylation is from 10 to 30, preferably from 15 to 25.

[0041] A preferred material is alkoxylated polyethylenimine, most preferably ethoxylated polyethyleneimine, with an average degree of ethoxylation being from 10 to 30 preferably from 15 to 25, where a nitrogen atom is ethoxylated.

Soil release polymer

[0042] When the detergent composition is in the form of a laundry composition, it is preferred that a soil release polymer is included.

[0043] Preferred levels of soil release polymer range from 0.1 to 10 wt.%, preferably from 0.2 to 8 wt.%, more preferably from 0.25 to 7 wt.%, most preferably from 0.5 to 6 wt.%.

[0044] Suitable polyester based soil release polymers are described in WO 2014/029479 and WO 2016/005338.

Additional Enzymes

[0045] Additional enzymes, other than the specified protease may be present in the detergent composition. It is preferred that additional enzymes are present in the preferred laundry detergent composition.

[0046] If present, then the level of each enzyme in the laundry composition of the invention is from 0.0001 wt.% to 0.1 wt.%.

[0047] Levels of enzyme present in the composition preferably relate to the level of enzyme as pure protein.

[0048] Preferred further enzymes include those in the group consisting of: lipases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, mannanases, and/or additional proteases. Said preferred additional enzymes include a mixture of two or more of these enzymes.

[0049] Preferably the further enzyme is selected from: lipases, cellulases, alpha-amylases and/or additional protease.

[0050] Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422). Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202, WO 00/60063.

[0051] Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™, Lipex™ and Lipoclean ™ (Novozymes A/S).

[0052] The method of the invention may be carried out in the presence of phospholipase classified as EC 3.1.1.4 and/or EC 3.1.1.32. As used herein, the term phospholipase is an enzyme which has activity towards phospholipids.

[0053] Phospholipids, such as lecithin or phosphatidylcholine, consist of glycerol esterified with two fatty acids in an outer (sn-1) and the middle (sn-2) positions and esterified with phosphoric acid in the third position; the phosphoric acid, in turn, may be esterified to an amino-alcohol. Phospholipases are enzymes which participate in the hydrolysis of phospholipids. Several types of phospholipase activity can be distinguished, including phospholipases A_1 and A_2 which hydrolyze one fatty acyl group (in the sn-1 and sn-2 position, respectively) to form lysophospholipid; and lysophospholipase (or phospholipase B) which can hydrolyze the remaining fatty acyl group in lysophospholipid. Phospholipase C and phospholipase D (phosphodiesterases) release diacyl glycerol or phosphatidic acid respectively.

[0054] Protease enzymes hydrolyse bonds within peptides and proteins, in the laundry context this leads to enhanced removal of protein or peptide containing stains. Examples of suitable proteases families include aspartic proteases; cysteine proteases; glutamic proteases; aspargine peptide lyase; serine proteases and threonine proteases. Such protease families are described in the MEROPS peptidase database (http://merops.sanger.ac.uk/). Serine proteases are preferred. Subtilase type serine proteases are more preferred. The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501 -523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 subdivisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

[0055] Examples of subtilases are those derived from Bacillus such as Bacillus lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, Bacillus pumilus and Bacillus gibsonii described in; US7262042 and WO09/021867, and subtilisin lentus, subtilisin Novo, subtilisin Carlsberg, Bacillus licheniformis, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO 89/06279 and protease PD138 described in (WO 93/18140). Other useful proteases may

be those described in WO 92/175177, WO 01/016285, WO 02/026024 and WO 02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the Fusarium protease described in WO 89/06270, WO 94/25583 and WO 05/040372, and the chymotrypsin proteases derived from Cellumonas described in WO 05/052161 and WO 05/052146.

[0056] Most preferably the protease is a subtilisins (EC 3.4.21.62).

[0057] Examples of subtilases are those derived from Bacillus such as Bacillus lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, Bacillus pumilus and Bacillus gibsonii described in; US7262042 and WO09/021867, and subtilisin lentus, subtilisin Novo, subtilisin Carlsberg, Bacillus licheniformis, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO89/06279 and protease PD138 described in (WO93/18140). Preferably the subsilisin is derived from Bacillus, preferably Bacillus lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, Bacillus pumilus and Bacillus gibsonii as described in US 6,312,936 BI, US 5,679,630, US 4,760,025, US7,262,042 and WO 09/021867. Most preferably the subtilisin is derived from Bacillus gibsonii or Bacillus Lentus.

[0058] Suitable commercially available protease enzymes include those sold under the trade names names Alcalase®, Blaze®; DuralaseTm, DurazymTm, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Primase®, Polarzyme®, Kannase®, Liquanase®, Liquanase® Ultra, Ovozyme®, Coronase®, Coronase® Ultra, Neutrase®, Everlase® and Esperase® all could be sold as Ultra® or Evity® (Novozymes A/S).

[0059] The composition may use cutinase, classified in EC 3.1.1.74. The cutinase used according to the invention may be of any origin. Preferably cutinases are of microbial origin, in particular of bacterial, of fungal or of yeast origin.

[0060] Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more detail in GB 1,296,839, or the *Bacillus* sp. strains disclosed in WO 95/026397 or WO 00/060060. Commercially available amylases are Duramyl™, Termamyl™, Termamyl Ultra™, Natalase™, Stainzyme™, Amplify™, Fungamyl™ and BAN™ (Novozymes A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

[0061] Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Thielavia terrestris*, *Myceliophthora thermophila,* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757, WO 89/09259, WO 96/029397, and WO 98/012307. Commercially available cellulases include Celluzyme™, Carezyme™, Celluclean ™, Endolase™, Renozyme™ (Novozymes A/S), Clazinase™ and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation). Celluclean™ is preferred.

[0062] Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus,* e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include Guardzyme™ and Novozym™ 51004 (Novozymes A/S).

[0063] Further enzymes suitable for use are discussed in WO 2009/087524, WO 2009/090576, WO 2009/107091, WO 2009/111258 and WO 2009/148983.

[0064] The aqueous solution used in the method preferably has an enzyme present. The enzyme is preferably present in the aqueous solution used in the method at a concentration in the range from 0.01 to 10ppm, preferably 0.05 to 1ppm.

Enzyme Stabilizers

[0065] Any enzyme present in the 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, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

Chelating Agent

[0066] Chelating agents may be present or absent from the detergent compositions.

[0067] Preferably the laundry detergent comprises phosphonic acid (or salt thereof) chelating agent at a level that is less than 0.1 wt.%, more preferably less than 0.01 wt.%, most preferably the composition is free from phosphonic acid (or salt thereof) chelating agent.

[0068] Example phosphonic acid (or salt thereof) chelating agents are: 1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP); Diethylenetriaminepenta(methylenephosphonic acid) (DTPMP); Hexamethylenediaminetetra(methylenephosphonic acid) (HDTMP); Aminotris(methylenephosphonic acid) (ATMP); Ethylenediaminetetra(methylenephosphonic acid) (EDTMP); Tetramethylenediaminetetra(methylenephosphonic acid) (TDTMP); and, Phosphonobutanetricarboxylic acid (PBTC).

Further materials

[0069] Further optional but preferred materials that may be included in the detergent compositions (preferably laundry detergent compositions) include fluorescent agent, perfume, shading dyes and polymers.

Fluorescent Agent

[0070] The composition preferably comprises a fluorescent agent (optical brightener). Fluorescent agents are well known and many such fluorescent agents are available commercially. Usually, these fluorescent agents are supplied and used in the form of their alkali metal salts, for example, the sodium salts.

[0071] The total amount of the fluorescent agent or agents used in the composition is generally from 0.0001 to 0.5 wt.%, preferably 0.005 to 2 wt.%, more preferably 0.01 to 0.1 wt.%. Preferred classes of fluorescer are: Di-styryl biphenyl compounds, e.g. Tinopal (Trade Mark) CBS-X, Di-amine stilbene di-sulphonic acid compounds, e.g. Tinopal DMS pure Xtra and Blankophor (Trade Mark) HRH, and Pyrazoline compounds, e.g. Blankophor SN.

[0072] Preferred fluorescers are fluorescers with CAS-No 3426-43-5; CAS-No 35632-99-6; CAS-No 24565-13-7; CAS-No 12224-16-7; CAS-No 13863-31-5; CAS-No 4193-55-9; CAS-No 16090-02-1; CAS-No 133-66-4; CAS-No 68444-86-0; CAS-No 27344-41-8.

[0073] Most preferred fluorescers are: sodium 2 (4-styryl-3-sulfophenyl)-2H-napthol[1,2-d]triazole, disodium 4,4'-bis{[(4-anilino-6-(N methyl-N-2 hydroxyethyl) amino 1,3,5-triazin-2-yl)]amino}stilbene-2-2' disulphonate, disodium 4,4'-bis{[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)]amino} stilbene-2-2' disulphonate, and disodium 4,4'-bis(2-sulphostyryl)biphenyl.

[0074] The aqueous solution used in the method has a fluorescer present. The fluorescer is present in the aqueous solution used in the method preferably in the range from 0.0001 g/l to 0.1 g/l, more preferably 0.001 to 0.02 g/l.

Perfume

[0075] The composition preferably comprises a perfume. Many suitable examples of perfumes are provided in the CTFA (Cosmetic, Toiletry and Fragrance Association) 1992 International Buyers Guide, published by CFTA Publications and OPD 1993 Chemicals Buyers Directory 80th Annual Edition, published by Schnell Publishing Co.

[0076] Preferably the perfume comprises at least one note (compound) from: alpha-isomethyl ionone, benzyl salicylate; citronellol; coumarin; hexyl cinnamal; linalool; pentanoic acid, 2-methyl-, ethyl ester; octanal; benzyl acetate; 1,6-octadien-3-ol, 3,7-dimethyl-, 3-acetate; cyclohexanol, 2-(1,1-dimethylethyl)-, 1-acetate; delta-damascone; beta-ionone; verdyl acetate; dodecanal; hexyl cinnamic aldehyde; cyclopentadecanolide; benzeneacetic acid, 2-phenylethyl ester; amyl salicylate; beta-caryophyllene; ethyl undecylenate; geranyl anthranilate; alpha-irone; beta-phenyl ethyl benzoate; alpa-santalol; cedrol; cedryl acetate; cedry formate; cyclohexyl salicyate; gamma-dodecalactone; and, beta phenylethyl phenyl acetate.

[0077] Useful components of the perfume include materials of both natural and synthetic origin. They include single compounds and mixtures. Specific examples of such components may be found in the current literature, e.g., in Fenaroli's Handbook of Flavour Ingredients, 1975, CRC Press; Synthetic Food Adjuncts, 1947 by M. B. Jacobs, edited by Van Nostrand; or Perfume and Flavour Chemicals by S. Arctander 1969, Montclair, N.J. (USA).

[0078] It is commonplace for a plurality of perfume components to be present in a formulation. In the compositions of the present invention it is envisaged that there will be four or more, preferably five or more, more preferably six or more or even seven or more different perfume components.

[0079] In perfume mixtures preferably 15 to 25 wt% are top notes. Top notes are defined by Poucher (Journal of the Society of Cosmetic Chemists 6(2):80 [1955]). Preferred top-notes are selected from citrus oils, linalool, linalyl acetate, lavender, dihydromyrcenol, rose oxide and cis-3-hexanol.

[0080] The International Fragrance Association has published a list of fragrance ingredients (perfumes) in 2011. (http://www.ifraorg.org/en-us/ingredients#.U7Z4hPldWzk)

[0081] The Research Institute for Fragrance Materials provides a database of perfumes (fragrances) with safety information.

[0082] Perfume top note may be used to cue the whiteness and brightness benefit of the invention.

[0083] Some or all of the perfume may be encapsulated, typical perfume components which it is advantageous to encapsulate, include those with a relatively low boiling point, preferably those with a boiling point of less than 300, preferably 100-250 Celsius. It is also advantageous to encapsulate perfume components which have a low CLog P (ie. those which will have a greater tendency to be partitioned into water), preferably with a CLog P of less than 3.0. These materials, of relatively low boiling point and relatively low CLog P have been called the "delayed blooming" perfume ingredients and include one or more of the following materials: allyl caproate, amyl acetate, amyl propionate, anisic aldehyde, anisole, benzaldehyde, benzyl acetate, benzyl acetone, benzyl alcohol, benzyl formate, benzyl iso valerate,

benzyl propionate, beta gamma hexenol, camphor gum, laevo-carvone, d-carvone, cinnamic alcohol, cinamyl formate, cis-jasmone, cis-3-hexenyl acetate, cuminic alcohol, cyclal c, dimethyl benzyl carbinol, dimethyl benzyl carbinol acetate, ethyl acetate, ethyl aceto acetate, ethyl amyl ketone, ethyl benzoate, ethyl butyrate, ethyl hexyl ketone, ethyl phenyl acetate, eucalyptol, eugenol, fenchyl acetate, flor acetate (tricyclo decenyl acetate), frutene (tricyclco decenyl propionate), geraniol, hexenol, hexenyl acetate, hexyl acetate, hexyl formate, hydratropic alcohol, hydroxycitronellal, indone, isoamyl alcohol, iso menthone, isopulegyl acetate, isoquinolone, ligustral, linalool, linalool oxide, linalyl formate, menthone, menthyl acetphenone, methyl amyl ketone, methyl anthranilate, methyl benzoate, methyl benyl acetate, methyl eugenol, methyl heptenone, methyl heptine carbonate, methyl heptyl ketone, methyl hexyl ketone, methyl phenyl carbinyl acetate, methyl salicylate, methyl-n-methyl anthranilate, nerol, octalactone, octyl alcohol, p-cresol, p-cresol methyl ether, p-methoxy acetophenone, p-methyl acetophenone, phenoxy ethanol, phenyl acetaldehyde, phenyl ethyl acetate, phenyl ethyl alcohol, phenyl ethyl dimethyl carbinol, prenyl acetate, propyl bornate, pulegone, rose oxide, safrole, 4-terpinenol, alpha-terpinenol, and /or viridine. It is commonplace for a plurality of perfume components to be present in a formulation. In the compositions of the present invention it is envisaged that there will be four or more, preferably five or more, more preferably six or more or even seven or more different perfume components from the list given of delayed blooming perfumes given above present in the perfume.

[0084] Another group of perfumes with which the present invention can be applied are the so-called 'aromatherapy' materials. These include many components also used in perfumery, including components of essential oils such as Clary Sage, Eucalyptus, Geranium, Lavender, Mace Extract, Neroli, Nutmeg, Spearmint, Sweet Violet Leaf and Valerian.

[0085] It is preferred that the laundry treatment composition does not contain a peroxygen bleach, e.g., sodium per-carbonate, sodium perborate, and peracid.

Shading Dye

[0086] Preferably when the composition is a laundry detergent composition, then it comprises a shading dye. Preferably the shading dye is present at from 0.0001 to 0.1 wt.% of the composition.

[0087] Dyes are described in Color Chemistry Synthesis, Properties and Applications of Organic Dyes and Pigments, (H Zollinger, Wiley VCH, Zurich, 2003) and, Industrial Dyes Chemistry, Properties Applications. (K Hunger (ed), Wiley-VCH Weinheim 2003).

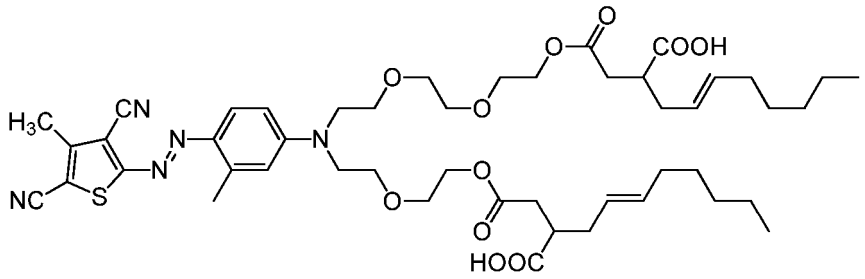
[0088] Shading Dyes for use in laundry compositions preferably have an extinction coefficient at the maximum absorption in the visible range (400 to 700nm) of greater than 5000 L mol^{-1} cm^{-1}, preferably greater than 10000 L mol^{-1} cm^{-1}. The dyes are blue or violet in colour.

[0089] Preferred shading dye chromophores are azo, azine, anthraquinone, and triphenylmethane.

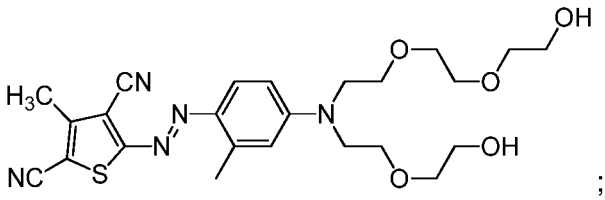
[0090] Azo, anthraquinone, phthalocyanine and triphenylmethane dyes preferably carry a net anionic charged or are uncharged. Azine preferably carry a net anionic or cationic charge. Blue or violet shading dyes deposit to fabric during the wash or rinse step of the washing process providing a visible hue to the fabric. In this regard the dye gives a blue or violet colour to a white cloth with a hue angle of 240 to 345, more preferably 250 to 320, most preferably 250 to 280. The white cloth used in this test is bleached non-mercerised woven cotton sheeting.

[0091] Shading dyes are discussed in WO 2005/003274, WO 2006/032327(Unilever), WO 2006/032397(Unilever), WO 2006/045275(Unilever), WO 2006/027086(Unilever), WO 2008/017570(Unilever), WO 2008/141880 (Unilever), WO 2009/132870(Unilever), WO 2009/141173 (Unilever), WO 2010/099997(Unilever), WO 2010/102861 (Unilever), WO 2010/148624(Unilever), WO 2008/087497 (P&G), WO 2011/011799 (P&G), WO 2012/054820 (P&G), WO 2013/142495 (P&G) and WO 2013/151970 (P&G).

[0092] Mono-azo dyes preferably contain a heterocyclic ring and are most preferably thiophene dyes. The mono-azo dyes are preferably alkoxylated and are preferably uncharged or anionically charged at pH=7. Alkoxylated thiophene dyes are discussed in WO/2013/142495 and WO/2008/087497. Preferred examples of thiophene dyes are shown below:

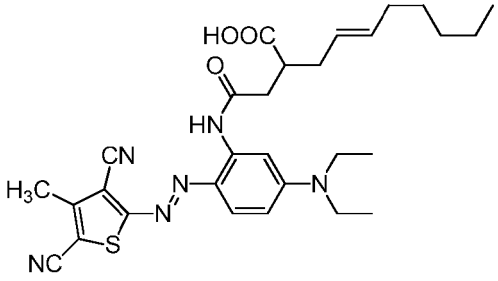


;



;

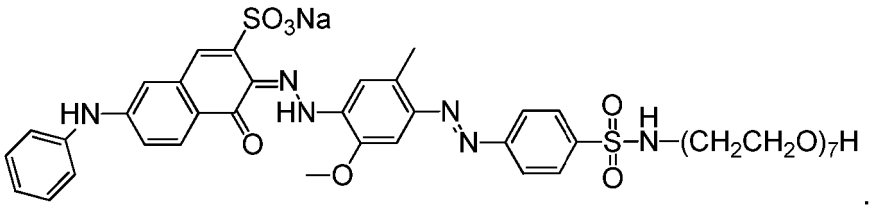
and,



.

[0093] Bis-azo dyes are preferably sulphonated bis-azo dyes. Preferred examples of sulphonated bis-azo compounds are direct violet 7, direct violet 9, direct violet 11, direct violet 26, direct violet 31, direct violet 35, direct violet 40, direct violet 41, direct violet 51, Direct Violet 66, direct violet 99 and alkoxylated versions thereof. Alkoxylated bis-azo dyes are discussed in WO2012/054058 and WO2010/151906.

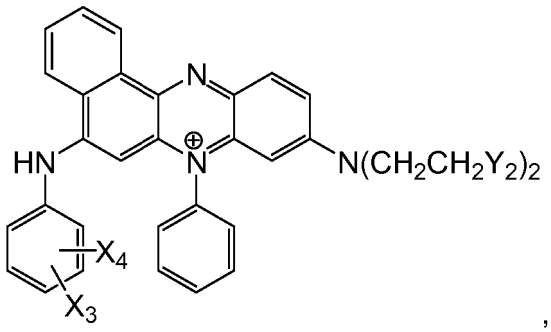
[0094] An example of an alkoxylated bis-azo dye is :



.

[0095] Thiophene dyes are available from Milliken under the tradenames of Liquitint Violet DD and Liquitint Violet ION.

[0096] Azine dye are preferably selected from sulphonated phenazine dyes and cationic phenazine dyes. Preferred examples are acid blue 98, acid violet 50, dye with CAS-No 72749-80-5, acid blue 59, and the phenazine dye selected from:



,

wherein:

X_3 is selected from: -H; -F; $-CH_3$; $-C_2H_5$; $-OCH_3$; and, $-OC_2H_5$;
X_4 is selected from: -H; $-CH_3$; $-C_2H_5$; $-OCH_3$; and, $-OC_2H_5$;
Y_2 is selected from: -OH; $-OCH_2CH_2OH$; $-CH(OH)CH_2OH$; $-OC(O)CH_3$; and, $C(O)OCH_3$.

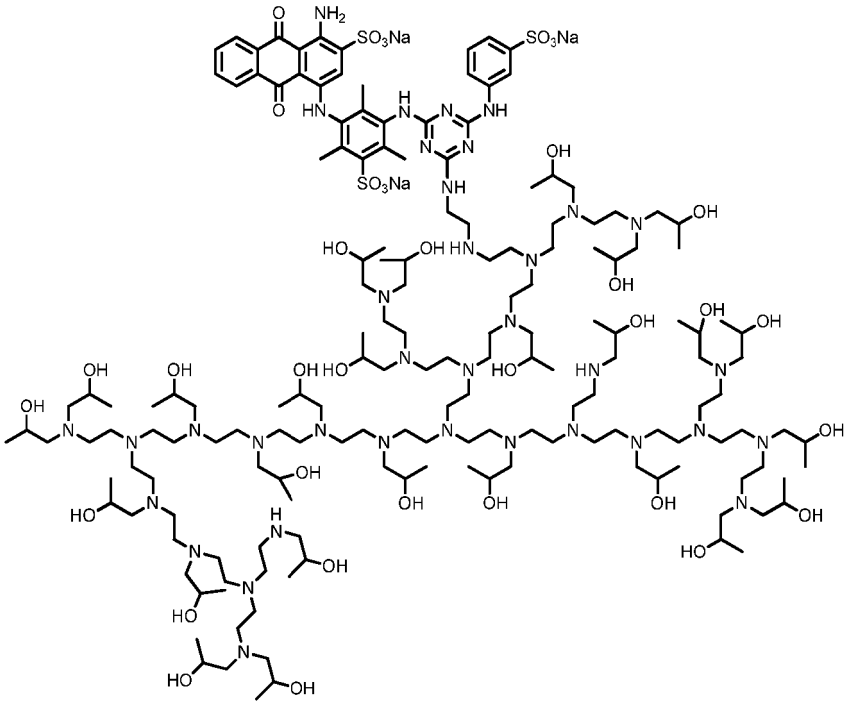
[0097] The shading dye is present is present in the composition in range from 0.0001 to 0.5 wt %, preferably 0.001 to 0.1 wt%. Depending upon the nature of the shading dye there are preferred ranges depending upon the efficacy of the

shading dye which is dependent on class and particular efficacy within any particular class. As stated above the shading dye is a blue or violet shading dye.

[0098] A mixture of shading dyes may be used.

[0099] The shading dye is most preferably a reactive blue anthraquinone dye covalently linked to an alkoxylated polyethyleneimine. The alkoxylation is preferably selected from ethoxylation and propoxylation, most preferably propoxylation. Preferably 80 to 95 mol% of the N-H groups in the polyethylene imine are replaced with iso-propyl alcohol groups by propoxylation. Preferably the polyethylene imine before reaction with the dye and the propoxylation has a molecular weight of 600 to 1800.

[0100] An example structure of a preferred reactive anthraquinone covalently attached to a propoxylated polyethylene imine is:



(Structure I).

Polymers

[0101] The composition may comprise one or more further polymers. Examples are carboxymethylcellulose, poly (ethylene glycol), poly(vinyl alcohol), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

Sequence Listing: SEQ ID NO: 1

[0102]

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        35                  40                  45
Asn Gly Thr Ser Gly Ser Ala Ala Tyr Ser Tyr Asn Cys Ala Asp Gly
    50                  55                  60
Thr Asn Tyr Thr Asp His Lys Tyr Ile Asn Gly Ala Tyr Ser Pro Leu
65                  70                  75                  80
Asn Asp Ala His Tyr Phe Gly Asn Val Val Phe Asp Met Tyr Lys Glu
                85                  90                  95
Trp Met Asn Thr Ser Pro Leu Thr Phe Gln Leu Thr Met Arg Val His
            100                 105                 110
Tyr Asp Thr Asp Tyr Glu Asn Ala Phe Trp Asn Gly Ser Ser Met Thr
        115                 120                 125
Phe Gly Asp Gly Lys Asn Thr Phe Tyr Pro Leu Val Asp Ile Asn Val
    130                 135                 140
Ser Ala His Glu Val Ser His Gly Phe Thr Glu Gln Asn Ser Gly Leu
145                 150                 155                 160
Val Tyr Gln Asn Met Ser Gly Gly Ile Asn Glu Ala Phe Ser Asp Ile
                165                 170                 175
Ala Gly Glu Ala Ala Glu Tyr Tyr Leu Arg Gly Asn Val Asp Trp Val
            180                 185                 190
Val Gly Ser Asp Ile Phe Lys Ser Glu Gly Gly Leu Arg Tyr Phe Asp
        195                 200                 205
Gln Pro Ser Lys Asp Gly Arg Ser Ile Asp His Ala Ser Gln Tyr Tyr
    210                 215                 220
Asp Gly Leu Asn Val His Leu Ser Ser Gly Val Tyr Asn Arg Ala Phe
225                 230                 235                 240
Tyr Leu Leu Ala Asn Lys Ser Gly Trp Asp Val Arg Lys Gly Phe Glu
                245                 250                 255
Ile Phe Thr Val Ala Asn Gln Leu Tyr Trp Thr Ala Asn Ser Thr Phe
            260                 265                 270
Asp Ala Gly Ala Cys Gly Val Ala Lys Ala Ala Ala Asp Met Gly Tyr
        275                 280                 285
Val Val Ala Asp Val Glu Asp Ala Phe Asn Thr Val Gly Val Asn Gly
    290                 295                 300
Ser Cys Gly Ser Thr Pro Pro Thr Gly Asn Val Leu Thr Lys Gly Thr
305                 310                 315                 320
Pro Ile Ala Asn Leu Ser Gly Asn Gln Ser Ser Glu Ser Phe Tyr Thr
                325                 330                 335
Phe Thr Val Asp Ser Ala Ser Ser Ala Thr Val Ser Met Ser Gly Gly
            340                 345                 350
Ser Gly Asp Ala Asp Leu Tyr Val Lys Ser Gly Ser Lys Pro Thr Thr
        355                 360                 365
Ser Ser Tyr Asp Cys Arg Pro Tyr Arg Ala Gly Asn Asn Glu Gln Cys
    370                 375                 380
Ser Val Ser Ala Gln Pro Gly Ile Thr Tyr His Val Leu Leu Arg Gly
385                 390                 395                 400
Tyr Ser Asn Tyr Ser Gly Leu Thr Leu Arg Leu Asp
                405                 410
```

Examples

[0103] The invention will be demonstrated by the following non-limiting examples.

Isolation and cultivation of a protease-producing microorganism

[0104] The protease-producing microorganism strains were isolated from marine sediment samples collected from Jiaozhou Bay (China Yellow Sea, 36°09'N, 120°32'E) by selective screening on skim milk agar plates containing (g/L seawater): tryptone 5, yeast extract 2, skim milk powder 40, agar powder 16. The plates were incubated at 20°C for 48-72 h to obtain bacterial colonies. The colonies with a clear hydrolysis circle of casein in milk were evaluated as protease producers. The proteolytic LA-05 strain exhibiting the larger hydrolysis circle was selected for further experiments. The seed and fermentation medium used for LA-05 strain culture consisted of 5 g tryptone, 2 g yeast extract and 1 L seawater, pH 7.0. The media were autoclaved at 121°C for 20 min. At first, the isolated LA-05 strain was inoculated with 2% (v/v) seed culture and cultivated on a rotary shaker incubator at 20°C and 150 rpm for 12 h. Then the culture was transferred to 2-L conical flasks containing 400 ml of fermentation medium and incubated for 78 h at 15-30°C and 150 rpm.

[0105] To obtain the maximum enzyme yield, the kinetics of growth and enzyme production were measured every 6 h during the incubation period (78 h). The cell density was monitored by measuring the absorbance at 600 nm. The cell-free supernatant was recovered by centrifugation at 12000 rpm for 20 min at 4°C, and then used as crude enzyme preparation to determine protease activity.

Strain identification and phylogenetic analysis

[0106] Analytical profiling index (API) strip tests and 16S rRNA gene sequencing were carried out for the genus identification of LA-05 strain. API strips were used to investigate the physiological and biochemical characteristics of strain LA-05 according to the manufacturer's instructions.

[0107] The 16S rRNA sequence was amplified by PCR using forward primer (27F, 5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer (1492R, 5'-TACGGYTACCTTGTTACGACTT-3'). The genomic DNA of strain LA-05 was purified by TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China) and then used as the template for PCR amplification including 30 cycles (the cycling parameters: denaturation at 94°C for 50 s, primer annealing at 58°C for 50 s, extension at 72°C for 100 s).

[0108] The amplified product was cloned in pMD18-T vector (Takara, Dalian, China), and the recombinant plasmid pMD-16S was constructed. Then, the recombinant plasmid was transformed into the competent cells of *Escherichia coli* DH5a. LB broth media containing ampicillin (60μg/ml) was applied to culture recombinant clones of *E. coli* DH5a. The DNA fragment of 16S rRNA ligated into the recombinant plasmid was confirmed by commercial DNA sequencing (Sangon Biotech Co., Ltd., Shanghai, China). The multiple sequence alignment was performed using Identify program through EzTaxon database. Type culture strains with pairwise similarity above 97% were selected and subjected to phylogenetic and molecular evolutionary analyses with Molecular Evolutionary Genetics Analysis (MEGA) software (version 7.0.9) by the neighbor-joining method. Statistical evaluation of the tree topology was calculated by bootstrap analysis with1000 replications.

Protease purification

[0109] All purification steps were performed at 4°C unless stated otherwise.

Concentration by ultrafiltration

[0110] Four hundred milliliter of 30-h old culture was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was filtrated through 0.22 μm filters to remove bacteria cells and medium debris thoroughly, and recovered as the crude protease preparation. The prepared supernatant was concentrated by Millipore's Amicon Ultra-4 centrifugal filter devices with 3KDa molecular weight cut-off. After washing three times with ultrapure water, the buffer of crude protease was replaced by 50 mM Tris-HCl buffer containing 0.1 M NaCl (pH 8.0).

Purification

[0111] Two milliliter of the concentrated solution was loaded onto a gel filtration column (HiLoad 16/600 Superdex 200 pg, GE Healthcare) which was equilibrated and eluted with50 mM Tris-HCl buffer containing 0.1 M NaCl (pH 8.0). The column was eluted with 180 ml buffer at a flow rate of 1 ml/min until the optical density of eluent at 280 nm was zero. Elution fractions of 3 ml each were collected and protease activity was analyzed. Fractions showing protease activity were pooled and then were applied to a HiTrap Q FF (1 ml) ion exchange column equilibrated with 50 mM Tris-HCl buffer containing 0.1 M NaCl (pH 8.0). Subsequently, the column was rinsed with the same buffer and bound proteins were eluted with a linear gradient of NaCl in the range of 0.1-1 M at a flow rate of 1 ml/min. Elution fractions of 1 ml each

were collected and protease activity was analyzed. Fractions containing protease activity were pooled and stored at -80°C for further studies.

Determination of protease activity

[0112] The protein concentration was determined with a BCA Protein Assay Kit (Sangon Biotech, Shanghai, China) using bovine serum albumin (BSA) as a reference. Protease activity was determined according to the modified method (Lagzian and Asoodeh, 2012). Briefly, 0.25 ml aliquot of purified protease was incubated with 0.75 ml 50 mM Tris-HCl buffer (pH 8.0) containing 1% (w/v) casein at 45°C for 10 min. The reaction was terminated by adding 0.5 mL of 20 % (w/v) trichloroacetic acid (TCA). The mixture was blended with a lab-dancer and placed at room temperature for 20 min. Subsequently, the precipitate was removed by centrifugation at 12,000 rpm for 20 min and the absorbance of supernatant was measured at 280 nm. One unit (U) of protease activity was defined as the amount of enzyme that hydrolyzed casein to release 1 μg tyrosine per minute under experimental conditions. Protease activity units were calculated using tyrosine (0-100 μg/ml) as standard.

Electrophoresis, mass spectrometry, zymography and isoelectric focusing

Molecular mass

[0113] The molecular mass of purified protease was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using (5%, w/v) stacking gel and (12%, w/v) resolving gel according to standard protocols with Bio-Rad Mini-PROTEIN equipment (Farhadian et al., 2015). The gel was stained with Coomassie Brilliant Blue R-250 and destained with methanol-acetic acid-water (5/1/4, v/v/v). The relative molecular weight of purified enzyme was estimated by comparing its mobility to standard protein marker.

[0114] Meanwhile, the accurate molecular mass of purified protease was analyzed, in a linear positive mode, by the method of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) using Flash-Detector™ instrument (Bruker microflex™ LRF, Bruker Daltonics, USA). The data was collected by FlexControl 3.4 and analyzed with FlexAnalysis 3.4 software.

Zymography

[0115] Zymography was carried out to visualize the enzyme activity with a modified method (Machado et al., 2016). Briefly, samples were mixed with β-mercaptoethanol-free gel loading buffer and loaded to the electrophoresis gel without heating. Electrophoresis was performed in ice water. Next, the gel was immersed in 50 mM Tris-HCl buffer (pH 8.0) containing 2.5% Triton X-100 at 4°C and shook gently for 1 h to remove SDS. The gel was rinsed twice with 50 mM Tris-HCl buffer (pH 8.0) at 4°C for 20min in order to extract residual Triton X-100, and then incubated with 1% (w/v) casein in 50 mM Tris-HCl buffer (pH 8.0) at 25°C for 1 h. The gel was soaked in 20% (w/v) trichloroacetic acid (TCA) to terminate the protease reaction, stained with Coomassie Brilliant Blue R-250 for 2 h, and destained with methanol-acetic acid-water (5/1/4, v/v/v) over night to reveal protease hydrolysis bands.

Isoelectric focusing

[0116] The isoelectric focusing of purified protease was performed on gel strips with immobilized pH gradients (IPG) from 3 to 10 (Bio-Rad, USA) using multiphor II electrophoresis system (GE Healthcare). Briefly, the purified protease was desalted and rinsed with 1% glycine buffer by ultrafiltration. Pre-electrophoresis started at 700 V for 20 min at 15°C after fixing the IPG strip. Then, the sample and IEF standards were subjected to electrophoresis at 2000 V for 90 min at 15°C. Finally, the band was fixed by TCA buffer treatment for 30min, stained with Coomassie Blue R-250 and destained with methanol-acetic acid-water mixture. The p/ value of purified protease was assessed by ImageQuant TL Version 7.0 software.

N-terminal amino acid sequence determination

[0117] The protease purified by SDS-PAGE was transferred to a polyvinylidene difluoride (PVDF) membrane in CAPS buffer according to Matsudaira's protocol (Matsudaira, 1987). The PVDF membrane was slightly stained with Coomassie Brilliant Blue R-250 and the band containing enzyme was excised. Subsequently, the N-terminal amino acid sequence was analyzed by the automated Edman degradation method using a PPSQ-21A protein sequencer (SHIMADZU). The first 20 amino acid residues were aligned with those in the UniProtKB/Swiss-Prot database and Protein Data Bank proteins database using the BLAST homology search (NCBI, USA).

Potential protease-coding gene identification and three-dimensional structure modeling

[0118] The strip containing protease obtained by denatured SDS-PAGE was excised carefully and proteins were analyzed by tandem mass spectrometry (MALDI MS/MS) as described in published protocol (Marchand et al., 2009). All acquired spectra of samples were processed using TOF/TOF Explorer™ Software (AB SCIEX) in a default mode. The identification of peptide mass fingerprint (PMF) was searched using GPS Explorer (V3.6) with the search engine MASCOT (2.3) against the NCBI database (Non-redundant protein sequences). Proteins with protein score confidence intervals (C.I.) above 95% were considered confident identifications.

[0119] The candidate proteins were selected based on the MASCOT search results, proteolytic activity and secretion mechanism. In order to amplify the encoding sequence of protease by PCR, the multiple sequence alignment was performed on the encoding sequence of candidates by DNAMAN software. One pair of primers, 5'-ATGAAC-CAACAACGTCAACTAAGCTG -3' and 5'-CGGGTCAATCTAAACGCAACG-3', was designed in accordance with conserved regions of the upstream and downstream coding sequences. The coding sequence was amplified and cloned in pMD18-T vector using an *Escherichia coli* DH5a as the host strain for Sanger sequencing. Eventually, the obtained nucleotide sequence was translated to amino acid sequence, a mature protease with 321 amino acid residues. Trace metals in purified protease was determined by flame atomic absorption spectrometry.

Wash studies in Mini-bottles

[0120] Cotton swatches stained with blood/milk/ink on cotton E116 (Centre for Testmaterials - Netherlands) together with cotton ballast were used for the mini-bottle washes. Stains were applied in triplicate within wash bottles. Using water prepared to FH26, containing 1g/L laundry formulation (two types, labelled F1 and F2), protease (control benchmark or halotolerant) was added to a concentration of 5mg/L in a total volume of 100mL. This enzyme level equates to a level of 0.5wt.% of protease in the formulation. The control benchmark used was the leading commercial protease material (Carnival Evity ex. Novozymes). To replicate salty water conditions, 2% final salt concentration (NaCl) was also used.

[0121] Washes were carried out at 20°C and 40°C, with shaking at 250rpm for 1h. The washing at 20°C shows the benefit of the invention even at low temperature conditions.

[0122] Following washing, the stains were separated from the wash liqueur and rinsed 2x in a beaker containing 1L of FH26 water, before leaving to dry overnight. After drying, the stain plates were digitally scanned and their deltaE measured. This value is used to express cleaning effect and is defined as the colour difference between a white cloth and that of the stained cloth after being washed.

[0123] Mathematically, the definition of deltaE is:

$$\text{deltaE} = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

wherein ΔL is a measure of the difference in darkness between the washed and white cloth; Δa and Δb are measures for the difference in redness and yellowness respectively between both cloths. From this equation, it is clear that the lower the value of deltaE, the whiter the cloth will be. With regard to this colour measurement technique, reference is made to Commission International de l'Eclairage (CIE); Recommendation on Uniform Colour Spaces, colour difference equations, psychometric colour terms, supplement no. 2 to CIE Publication, no. 15, Colormetry, Bureau Central de la CIE, Paris 1978.

[0124] Herein the cleaning effect is expressed in the form of a stain removal index (SRI):

$$\text{SRI} = 100 - \text{deltaE}$$

[0125] The higher the SRI the cleaner the cloth, SRI = 100 (white).

Formulations used

[0126]

Formulation 1 (F1)	(wt. %)
Demin water	to 100
Nonionic surfactant (25-7)	4.365

(continued)

Formulation 1 (F1)	(wt. %)
Tinopal 5BMGX	0.200
Acusol WR	0.700
TEA	8.820
EU LAS acid	5.820
Glycerol	2.000
Prifac 5908	0.860
Dequest 2010	1.500
EU SLES	4.365
BIT - Proxel	0.040
Citric acid	1.000

Formulation 2 (F2)	(wt. %)
Demin water	to 100%
Tinopal CBS-X	0.090
NaOH	0.457
TEA	1.500
Citric Acid	0.400
LAS acid (Indiatuba)	4.500
EPEI	2.325
SLES 3EO (Texapon N70 LST)	13.500
EPEI	0.775
BIT	0.0200
MIT	0.0095
Soladona LA059 - 2012 used	0.9800
NaCl	1.5000
CAPB	1.5000

[0127] The two tested enzymes were added to formulations 1 and 2 to give an effective enzyme level in the formulation of 0.5 wt.%. The proteases were:-

Protease 1 (comparison) = Carnival Evity, a commercially available enzyme from Novozymes
Protease 2 (according to the invention) = a protease with 100% accordance to SEQ ID NO:1

[0128] The results are shown in table 1

20°C						
	F1	**F1 + Protease 1**	**F1 + Protease 2**	**F2**	**F2 + Protease 1**	**F2 + Protease 2**
SRI	59.90	67.54	70.26	54.88	57.99	72.57
std dev	0.84	1.29	1.82	0.95	0.25	0.57

(continued)

40°C						
	F1	F1 + Protease 1	F1 + Protease 2	F2	F2 + Protease 1	F2 + Protease 2
SRI	54.13	67.05	69.20	52.39	57.45	63.75
std dev	1.42	0.48	0.90	0.68	1.14	1.01

[0129] The higher the SRI (stain removal index), then better the cleaning.

[0130] The results of wash studies show that as expected, both proteases give a cleaning benefit over the formulation only control in all wash settings (both 20°C and 40°C, as well as in different formulations). The wash study at 20°C shows the added benefit of improved cleaning even in low temperature washing conditions (20°C).

[0131] In FH26 water containing 2% salt (NaCl) the performance benefits of the halotolerant protease are clearly apparent. At both 20°C and 40°C the haloterant protease outperforms the control commercial protease both in formulations 1 and 2. The cleaning benefit (above the benchmark protease 1) due to the halotolerant protease is even more noticeable in laundry formulation 2 which does not contain the chelating agent which is present in formulation 1. Interestingly the performance benefit is vastly improved at a 20°C wash temperature, which further serves to highlight the potential applicability of this technology to improve cleaning of stains in sea water conditions.

SEQUENCE LISTING

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        35                  40                  45
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Thr Asn Tyr Thr Asp His Lys Tyr Ile Asn Gly Ala Tyr Ser Pro Leu
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Phe Gly Asp Gly Lys Asn Thr Phe Tyr Pro Leu Val Asp Ile Asn Val
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Ser Ala His Glu Val Ser His Gly Phe Thr Glu Gln Asn Ser Gly Leu
145                 150                 155                 160
Val Tyr Gln Asn Met Ser Gly Gly Ile Asn Glu Ala Phe Ser Asp Ile
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Ala Gly Glu Ala Ala Glu Tyr Tyr Leu Arg Gly Asn Val Asp Trp Val
            180                 185                 190
Val Gly Ser Asp Ile Phe Lys Ser Glu Gly Gly Leu Arg Tyr Phe Asp
        195                 200                 205
Gln Pro Ser Lys Asp Gly Arg Ser Ile Asp His Ala Ser Gln Tyr Tyr
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Asp Gly Leu Asn Val His Leu Ser Ser Gly Val Tyr Asn Arg Ala Phe
225                 230                 235                 240
Tyr Leu Leu Ala Asn Lys Ser Gly Trp Asp Val Arg Lys Gly Phe Glu
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Ile Phe Thr Val Ala Asn Gln Leu Tyr Trp Thr Ala Asn Ser Thr Phe
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Asp Ala Gly Ala Cys Gly Val Ala Lys Ala Ala Ala Asp Met Gly Tyr
        275                 280                 285
Val Val Ala Asp Val Glu Asp Ala Phe Asn Thr Val Gly Val Asn Gly
    290                 295                 300
Ser Cys Gly Ser Thr Pro Pro Thr Gly Asn Val Leu Thr Lys Gly Thr
305                 310                 315                 320
Pro Ile Ala Asn Leu Ser Gly Asn Gln Ser Ser Glu Ser Phe Tyr Thr
                325                 330                 335
Phe Thr Val Asp Ser Ala Ser Ser Ala Thr Val Ser Met Ser Gly Gly
            340                 345                 350

Ser Gly Asp Ala Asp Leu Tyr Val Lys Ser Gly Ser Lys Pro Thr Thr
        355                 360                 365
Ser Ser Tyr Asp Cys Arg Pro Tyr Arg Ala Gly Asn Asn Glu Gln Cys
    370                 375                 380
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385                 390                 395                 400
Tyr Ser Asn Tyr Ser Gly Leu Thr Leu Arg Leu Asp
                405                 410
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Claims

1. A detergent composition comprising:

   (i) from 1 to 60 wt.%, preferably from 2 to 50 wt.%, more preferably from 4 to 50 wt.% of surfactant;
   (ii) from 0.0005 to 1 wt.%, preferably from 0.005 to 0.6 wt.% of a protease enzyme having at least 90% sequence identity to SEQ ID NO: 1.

2. A detergent composition according to claim 1 wherein the protease enzyme has at least 95%, more preferably 97% sequence identity to SEQ ID NO: 1.

3. A detergent composition according to claim 1 or 2 where the wherein the protease enzyme has 100% sequence identity to SEQ ID NO: 1.

4. A detergent composition according to any preceding claim wherein the detergent composition is a laundry detergent composition.

5. A laundry detergent composition according to claim 4 wherein the laundry detergent composition is a liquid or a powder, preferably a liquid detergent.

6. A laundry detergent composition according to claim 4 or claim 5 wherein the laundry detergent composition comprises anionic and/or nonionic surfactant, preferably comprising both anionic and nonionic surfactant.

7. A laundry detergent composition according to any one of claims 4 to 6 wherein the laundry detergent composition comprises an alkoxylated polyamine, preferably at a level of from 0.1 to 8 wt.%, more preferably from 0.2 to 6 wt.%, most preferably from 0.5 to 5 wt.%.

8. A laundry detergent composition according to any one of claims 4 to 7 wherein the laundry detergent composition comprises a soil release polymer, preferably a polyester based soil released polymer.

9. A laundry detergent composition according to any one of claims 4 to 8 wherein the level of phosphonic acid (or salt thereof) chelating agent is less than 0.1 wt.%, preferably 0.01 wt.%, more preferably the composition is free from phosphonic acid (or salt thereof) chelating agent.

10. A detergent composition according to any preceding claim, additionally comprising a further enzyme selected from the group consisting of: lipases, cellulases, alpha-amylases, peroxidases/oxidases, pectate lyases, mannanases, and/or additional proteases.

11. A method of improving enzymatic cleaning in water having a sodium chloride content of from 0.1 to 4%, preferably from 0.25 to 3 wt.% at 20°C, said method comprising incorporation of a protease enzyme having at least 90% sequence identity to SEQ ID NO: 1 into a detergent composition comprising from 1 to 60 wt.% of a surfactant; and subsequent treatment of a substrate, preferably textiles, with said composition.

12. A method according to claim 11, wherein the protease enzyme has at least 95%, even more preferably 97% sequence identity to SEQ ID NO: 1; most preferably the protease enzyme has 100% sequence identity to SEQ ID NO: 1.

13. A method according to claim 11 or claim 12, wherein the composition that treats the substrate is a composition according to any one of claims 4 to 10.

14. Use of a protease enzyme having at least 90%, preferably, 95%, more preferably 97%, most preferably 100%, sequence identity to SEQ ID NO: 1 to improve enzymatic cleaning in water having a sodium chloride content of from 0.1 to 4%, at a temperature of from 15°C to 45°C.

Patentansprüche

1. Reinigungsmittelzusammensetzung, umfassend:

   (i) 1 bis 60 Gew.-%, bevorzugt 2 bis 50 Gew.-%, bevorzugter 4 bis 50 Gew.-%, Tensid;
   (ii) 0,0005 bis 1 Gew.-%, bevorzugt 0,005 bis 0,6 Gew.-%, eines Proteaseenzyms mit mindestens 90% Sequenzidentität zu SEQ ID NO: 1.

2. Reinigungsmittelzusammensetzung nach Anspruch 1, wobei das Proteaseenzym mindestens 95%, bevorzugter 97% Sequenzidentität zu SEQ ID NO: 1 aufweist.

3. Reinigungsmittelzusammensetzung nach Anspruch 1 oder 2, wobei das Proteaseenzym 100% Sequenzidentität zu SEQ ID NO: 1 aufweist.

4. Reinigungsmittelzusammensetzung nach irgendeinem vorhergehenden Anspruch, wobei die Reinigungsmittelzusammensetzung eine Waschmittelzusammensetzung ist.

5. Waschmittelzusammensetzung nach Anspruch 4, wobei die Waschmittelzusammensetzung eine Flüssigkeit oder ein Pulver ist, bevorzugt ein flüssiges Waschmittel.

6. Waschmittelzusammensetzung nach Anspruch 4 oder Anspruch 5, wobei die Waschmittelzusammensetzung anionisches und/oder nichtionisches Tensid umfasst, wobei es bevorzugt sowohl anionisches als auch nichtionisches Tensid umfasst.

7. Waschmittelzusammensetzung nach irgendeinem der Ansprüche 4 bis 6, wobei die Waschmittelzusammensetzung ein alkoxyliertes Polyamin umfasst, bevorzugt in einer Menge von 0,1 bis 8 Gew.-%, bevorzugter von 0,2 bis 6 Gew.-%, am meisten bevorzugt von 0,5 bis 5 Gew.-%.

8. Waschmittelzusammensetzung nach irgendeinem der Ansprüche 4 bis 7, wobei die Waschmittelzusammensetzung ein Soil-release-Polymer umfasst, bevorzugt ein Soil-release-Polymer auf Polyesterbasis.

9. Waschmittelzusammensetzung nach irgendeinem der Ansprüche 4 bis 8, wobei der Gehalt an Phosphonsäure (oder Salz davon)-Chelatbildner weniger als 0,1 Gew.-%, bevorzugt 0,01 Gew.-%, beträgt, wobei die Zusammensetzung bevorzugter frei von Phosphonsäure (oder Salz davon)-Chelatbildner ist.

10. Reinigungsmittelzusammensetzung nach irgendeinem vorhergehenden Anspruch, zusätzlich umfassend ein weiteres Enzym ausgewählt aus der Gruppe bestehend aus: Lipasen, Cellulasen, alpha-Amylasen, Peroxidasen/ Oxidasen, Pektatlyasen, Mannanasen und/oder zusätzlichen Proteasen.

11. Verfahren zur Verbesserung der enzymatischen Reinigung in Wasser mit einem Natriumchloridgehalt von 0,1 bis 4 Gew.-%, bevorzugt von 0,25 bis 3 Gew.-%, bei 20 °C, wobei das Verfahren die Aufnahme eines Proteaseenzyms mit mindestens 90% Sequenzidentität zu SEQ ID NO: 1 in eine Reinigungsmittelzusammensetzung, umfassend 1 bis 60 Gew.-% eines Tensids; und die anschließende Behandlung eines Substrats, bevorzugt Textilien, mit der Zusammensetzung umfasst.

12. Verfahren nach Anspruch 11, wobei das Proteaseenzym mindestens 95%, noch bevorzugter 97% Sequenzidentität zu SEQ ID NO: 1 aufweist; wobei das Proteaseenzym am meisten bevorzugt eine 100% Sequenzidentität zu SEQ ID NO: 1 aufweist.

13. Verfahren nach Anspruch 11 oder Anspruch 12, wobei die Zusammensetzung, die das Substrat behandelt, eine Zusammensetzung nach irgendeinem der Ansprüche 4 bis 10 ist.

14. Verwendung eines Proteaseenzyms mit mindestens 90%, bevorzugt 95%, bevorzugter 97%, am meisten bevorzugt 100% Sequenzidentität zu SEQ ID NO: 1 zur Verbesserung der enzymatischen Reinigung in Wasser mit einem Natriumchloridgehalt von 0,1 bis 4% bei einer Temperatur von 15 °C bis 45 °C.

Revendications

1. Composition de détergent comprenant :

   (i) de 1 à 60 % en masse, de préférence de 2 à 50 % en masse, encore mieux de 4 à 50 % en masse de tensioactif ;
   (ii) de 0,0005 à 1 % en masse, de préférence de 0,005 à 0,6 % en masse d'une enzyme protéase ayant au moins 90 % d'identité de séquence par rapport à SEQ ID NO:1.

2. Composition de détergent selon la revendication 1, dans laquelle l'enzyme protéase présente au moins 95 %, encore mieux 97 % d'identité de séquence par rapport à SEQ ID NO:1.

3. Composition de détergent selon la revendication 1 ou 2, dans laquelle l'enzyme protéase présente une identité de séquence de 100 % par rapport à SEQ ID NO:1.

4. Composition de détergent selon l'une quelconque des revendications précédentes, dans laquelle la composition de détergent est une composition de détergent de lessive.

5. Composition de détergent de lessive selon la revendication 4, dans laquelle la composition de détergent de lessive est un liquide ou une poudre, de préférence un détergent liquide.

6. Composition de détergent selon la revendication 4 ou revendication 5, dans laquelle la composition de détergent de lessive comprend un tensioactif anionique et/ou non ionique, comprenant de préférence à la fois un tensioactif anionique et non ionique.

7. Composition de détergent de lessive selon l'une quelconque des revendications 4 à 6, dans laquelle la composition

de détergent de lessive comprend une polyamine alcoxylée, de préférence à une teneur de 0,1 à 8 % en masse, encore mieux de 0,2 à 6 % en masse, bien mieux encore de 0,5 à 5 % en masse.

8. Composition de détergent de lessive selon l'une quelconque des revendications 4 à 7, dans laquelle la composition de détergent de lessive comprend un polymère de libération des salissures, de préférence un polymère de libération des salissures à base de polyester.

9. Composition de détergent de lessive selon l'une quelconque des revendications 4 à 8, dans laquelle la teneur en agent chélatant d'acide phosphonique (ou sel de celui-ci) est inférieure à 0,1 % en masse, de préférence 0,01 % en masse, la composition est encore mieux exempte d'agent chélatant d'acide phosphonique (ou sel de celui-ci).

10. Composition de détergent selon l'une quelconque des revendications précédentes, comprenant de plus une autre enzyme choisie dans le groupe consistant en : lipases, cellulases, alpha-amylases, peroxydases/oxydases, pectate lyases, mannanases, et/ou protéases supplémentaires.

11. Procédé d'amélioration de nettoyage enzymatique dans de l'eau ayant une teneur en chlorure de sodium de 0,1 à 4 %, de préférence de 0,25 à 3 % en masse à 20°C, ledit procédé comprenant l'incorporation d'une enzyme protéase ayant une identité de séquence d'au moins 90 % par rapport à SEQ ID NO:1 dans une composition de détergent comprenant de 1 à 60 % en masse d'un tensioactif ; et un traitement subséquent d'un substrat, de préférence de textiles, avec ladite composition.

12. Procédé selon la revendication 11, dans lequel l'enzyme protéase présente une identité de séquence d'au moins 95 %, encore mieux 97 % par rapport à SEQ ID NO:1 ; l'enzyme protéase présente bien mieux encore une identité de séquence de 100 % par rapport à SEQ ID NO:1.

13. Procédé selon la revendication 11 ou revendication 12, dans lequel la composition qui traite le substrat est une composition selon l'une quelconque des revendications 4 à 10.

14. Utilisation d'une enzyme protéase ayant une identité de séquence d'au moins 90 %, de préférence 95 %, encore mieux 97 %, bien mieux encore 100 % par rapport à SEQ ID NO:1 pour améliorer le nettoyage enzymatique dans de l'eau ayant une teneur en chlorure de sodium de 0,1 à 4 %, à une température de 15°C à 45°C.

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