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(54) Title: REMOVAL OF SKIN-DERIVED BODY SOILS

(57) Abstract: The present invention provides an enzymatic method for removing or releasing skin- derived body soils from textile.



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REMOVAL OF SKIN-DERIVED BODY SOILS**Reference to a Sequence Listing**

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

FIELD OF THE INVENTION

This invention generally relates to enzymatic removal of skin-derived body soils from
textile, in particular removal of "yellowing" from white textiles.

BACKGROUND

A primary source of so-called yellowing of fabric items such as bed linen, pillow cases,
cuffs and collars of shirt is due to accumulation and aging of body soil components. Here,
transfer to the textile matrix of sebum and skin residues are of key importance, see *J. Surf. Det.*
15 (1998), 3: 407-418; *J. Surf. Det.* (2001), 4: 35-41; and references therein. Accumulation of
oxidation labile compounds may upon aging render yellowish, oxidized and cross-linked
structures that are very hard to remove. Traditional detergency technology (such as surfactants,
polymers, chelators and classical hydrolytic enzymes like proteases and lipases) may affect the
degree of buildup of these soil components; whereas bleach technology under certain
20 conditions (e.g. hydrogen peroxide/peracid bleach systems at high pH and temperature) may
affect the aged and yellow body soil deposition. A number of more or less labor demanding
pretreatment procedures may also impact the yellowing problem, however, undesired abrasion,
discoloration or inconvenience are often associated with these procedures. It would therefore be
of great value to the field of laundry and fabric care to have a method for machine wash under
25 mild conditions (moderate pH and temperature) that would deliver superior benefits to the above
described yellowing phenomena.

The present invention provides enzymatic methods for degrading body soil structures on
textiles, and thus reducing yellowing and restoring whiteness.

SUMMARY

In a first aspect, the present invention provides a method for removing or releasing the
constituents of a skin-derived body soil deposition from textile, comprising contacting the body
soil deposition with an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC
1.13.11.

In an embodiment, the skin-derived body soil is sebum, preferably aged or oxidized sebum, comprising squalene cross linked through oxygen-bridging to sebum lipids, or proteins/peptides/carbohydrates derived from skin or textile.

5 In another embodiment, the textile is a white garment in direct skin contact, such as a cuff, collar, under garment, or a pillow case; preferably the textile is made of cotton.

In another embodiment, the oxidoreductase is a laccase, lipoxygenase, peroxidase and/or peroxygenase.

Other aspects and embodiments of the invention are apparent from the description and examples.

10

DETAILED DESCRIPTION

The inventors of the present invention have surprisingly found that a wash method including enzymes belonging to the family of oxidoreductases significantly restores loss of whiteness due to yellowing of laundry items, in particular regions of laundry items like collars, cuffs and pillow cases. The invention furthermore enables machine wash under mild conditions (moderate pH and temperature), while delivering new benefits like restoring whiteness, reducing risk of undesired abrasion and discoloration (fabric care), as well as added convenience to the consumer. Examples show that machine wash of aged (oxidized) real items of pillow cases that have lost whiteness due to yellowing are not significantly cleaned with respect to restoring whiteness in machine wash under mild conditions by conventional wash methods. In an additional example is shown that a wash method with specific oxidoreductases in machine wash under mild conditions provides surprising benefits with respect to restoring whiteness due to yellowing of fabric items with aged unsaturated sebum lipids. The benefits of the invention include improvement of whiteness, as measured by remission at 460 nanometer, of up to more than 8 units. The invention provides benefits for detergency, fabric care and home care in general where items in contact with skin and body soil lead to accumulation, oxidation, cross-linking and/or entrapment of undesired components to a surface structure.

20 The enzyme(s) used in the present invention are oxidoreductase enzymes from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11; based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), which describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided.

35 In the present context, whenever an oxidoreductase enzyme is mentioned that requires or benefits from the presence of acceptors (e.g. oxygen or hydrogen peroxide), enhancers, mediators and/or activators, such compounds should be considered to be included. Examples of enhancers and mediators are disclosed in EP 705327; WO 98/56899; EP 677102; EP

781328; and EP 707637. If desired a distinction could be made by defining an oxidoreductase enzyme system as the combination of the enzyme in question and its acceptor, and optionally also an enhancer and/or mediator for the enzyme in question.

5 Definitions

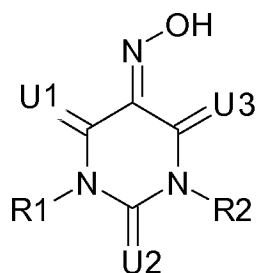
The term “mediator” means an enhancing agent which may be used with the enzymes having (laccase) oxidoreductase activity to improve the effect of the enzyme. “Enhancing agents” are also referred to as mediators, because they mediate, or enhance, the electron transfer between the oxidoreductase and the substrate.

10

MEDIATOR

The mediators according to the invention act as electron donors for the peroxidase. The mediator compounds improve the electron transfer between the peroxidase and the pulp to improve the bleaching effect of the methods of the invention. The mediators according to the

15 invention have the chemical structure:



wherein U1, U2 and U3 are identical or different, and are O, S or NOH; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, formyl, carbamoyl or sulfono radical, ester or salt of the sulfono radical, sulfamoyl, nitro, nitroso, amino, cyano, phenyl, benzyl, C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-carbonyl, carbonyl-C₁-C₄-alkyl.

20

In an embodiment, U1, U2 and U3 are identical or different, and are O or S; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, formyl, carbamoyl or sulfono radical, ester or salt of the sulfono radical, sulfamoyl, nitro, nitroso, amino, cyano, phenyl, benzyl, C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-carbonyl, carbonyl-C₁-C₄-alkyl.

25

In another embodiment, U1, U2 and U3 are O; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, formyl, carbamoyl or sulfono radical, ester or salt of the sulfono radical, sulfamoyl, nitro, nitroso, amino, cyano, phenyl, benzyl, C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-carbonyl, carbonyl-C₁-C₄-alkyl.

In another embodiment, U1, U2 and U3 are identical or different, and are O, S or NOH; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, methyl, ethyl, phenyl, benzyl, formyl, amino, cyano, nitroso, methoxy and/or ethoxy.

30

In another embodiment, U1, U2 and U3 are identical or different, and are O or S; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, methyl, ethyl, phenyl, benzyl, formyl, amino, cyano, nitroso, methoxy and/or ethoxy.

In another embodiment, U1, U2 and U3 are O; and R1 and R2 are identical or different, and are hydrogen, hydroxyl, methyl, ethyl, phenyl, benzyl, formyl, amino, cyano, nitroso, methoxy and/or ethoxy.

Preferred mediators are 1-methylvioluric acid, 1,3-dimethylvioluric acid, thiovioluric acid and violuric acid (alloxan-4,5-dioxime).

A particularly preferred mediator is alloxan-5-oxime (violuric acid) and/or its esters, ethers or salts.

According to the invention, the mediator may be present in a concentration in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, more preferably in the range of from 0.05 mM to 100 mM, and most preferably in the range of from 0.1 mM to 50 mM.

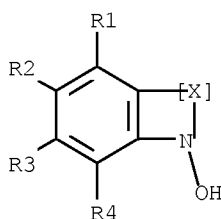
Examples of enhancers and mediators are disclosed in EP 705327; WO 98/56899; EP 677102; EP 781328; and EP 707637. If desired a distinction could be made by defining an oxidoreductase enzyme system (e.g. a laccase, or a peroxidase enzyme system) as the combination of the enzyme in question and its acceptor, and optionally also an enhancer and/or mediator for the enzyme in question.

Another preferred mediator is hydroxyl benzoate and hydroxyl benzotriazole.

ENHANCING AGENT

An enhancing agent may be used with the polypeptides having laccase activity to improve the effect of the methods of the invention. Enhancing agents are also referred to as mediators, because they mediate, or enhance, the electron transfer between the laccase and the substrate. Enhancing agents acting as electron donors for the polypeptides having laccase activity include both inorganic and organic compounds known in the art. Several examples of such enhancing agents are shown below.

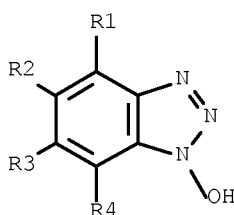
The enhancing agent may be selected from the group consisting of aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing the moiety >N-OH. In a preferred embodiment of the invention the enhancing agent is a compound of the general formula I:



wherein R^1 , R^2 , R^3 , R^4 are individually selected from the group consisting of hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C_{1-12} -alkyl, C_{1-6} -alkoxy, carbonyl(C_{1-12} -alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof, wherein the R^1 , R^2 , R^3 , R^4 may be substituted with
 5 R^5 , wherein R^5 represents hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C_{1-12} -alkyl, C_{1-6} -alkoxy, carbonyl(C_{1-12} -alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof;
 [X] represents a group selected from $(-N=N-)$, $(-N=CR^6-)_m$, $(-CR^6=N-)_m$, $(-CR^7=CR^8-)_m$, $(-CR^6=N-NR^7-)$, $(-N=N-CHR^6-)$, $(-N=CR^6-NR^7-)$, $(-N=CR^6-CHR^7-)$, $(-CR^6=N-CHR^7-)$, $(-CR^6=CR^7-NR^8-)$, and
 10 $(-CR^6=CR^7-CHR^8-)$, wherein R^6 , R^7 , and R^8 independently of each other are selected from H, OH, NH_2 , $COOH$, SO_3H , C_{1-6} -alkyl, NO_2 , CN, Cl, Br, F, CH_2OCH_3 , OCH_3 , and $COOCH_3$; and m is 1 or 2.

The term " C_{1-n} -alkyl" wherein n can be from 2 through 12, as used herein, represent a branched or straight alkyl group having from one to the specified number of carbon atoms.
 15 Typical C_{1-6} -alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, hexyl, iso-hexyl and the like.

In a more preferred embodiment of the invention, the enhancing agent is a compound of the general formula II:



wherein R^1 , R^2 , R^3 , R^4 are individually selected from the group consisting of hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C_{1-12} -alkyl, C_{1-6} -alkoxy, carbonyl(C_{1-12} -alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof, wherein the R^1 , R^2 , R^3 , R^4 may be substituted with
 20 R^5 , wherein R^5 represents hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C_{1-12} -alkyl, C_{1-6} -alkoxy, carbonyl(C_{1-12} -alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof.
 25

The enhancing agent may also be a salt or an ester of formula I or II.

Further preferred enhancing agents are oxoderivatives and N-hydroxy derivatives of heterocyclic compounds and oximes of oxo- and formyl-derivatives of heterocyclic compounds,
 30 said heterocyclic compounds including five-membered nitrogen-containing heterocycles, in particular pyrrol, pyrazole and imidazole and their hydrogenated counterparts (e.g. pyrrolidine) as well as triazoles, such as 1,2,4-triazole; six-membered nitrogen-containing heterocycles, in

particular mono-, di- and triazinanes (such as piperidine and piperazine), morpholine and their unsaturated counterparts (e.g. pyridine and pyrimidine); and condensed heterocycles containing the above heterocycles as substructures, e.g. indole, benzothiazole, quinoline and benzoazepine.

5 Examples of preferred enhancing agent from these classes of compounds are pyridine aldoximes; N-hydroxypyrrolidinediones such as N-hydroxysuccinimide and N-hydroxyphthalimide; 3,4-dihydro-3-hydroxybenzo[1,2,3]triazine-4-one; formaldoxime trimer (N,N',N''-trihydroxy-1,3,5-triazinane); and violuric acid (1,3-diazinane-2,4,5,6-tetrone-5-oxime).

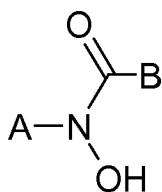
10 Still further enhancing agents which may be applied in the invention include oximes of oxo- and formyl-derivatives of aromatic compounds, such as benzoquinone dioxime and salicylaldoxime (2-hydroxybenzaldehyde oxime), and N-hydroxyamides and N-hydroxyanilides, such as N-hydroxyacetanilide.

Preferred enhancing agents are selected from the group consisting of 1-hydroxybenzotriazole; 1-hydroxybenzotriazole hydrate; 1-hydroxybenzotriazole sodium salt; 1-hydroxybenzotriazole potassium salt; 1-hydroxybenzotriazole lithium salt; 1-hydroxybenzotriazole ammonium salt; 1-hydroxybenzotriazole calcium salt; 1-hydroxybenzotriazole magnesium salt; and 1-hydroxybenzotriazole-6-sulphonic acid.

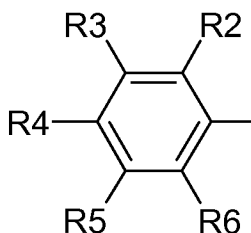
A particularly preferred enhancing agent is 1-hydroxybenzotriazole.

20 All the specifications of N-hydroxy compounds above are understood to include tautomeric forms such as N-oxides whenever relevant.

Another preferred group of enhancing agents comprises a -CO-NOH- group and has the general formula III:



in which A is:



25 and B is the same as A; or B is H or C₁₋₁₂-alkyl, said alkyl may contain hydroxy, ester or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R₂, R₃, R₄, R₅ and R₆ independently of each other are H, OH, NH₂, COOH, SO₃H, C₁₋₈-alkyl, acyl, NO₂, CN, Cl, Br, F, CF₃, NOH-CO-phenyl,

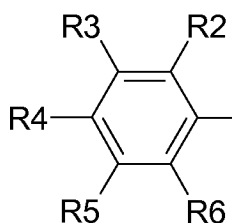
CO-NOH-phenyl, C₁₋₆-CO-NOH-A, CO-NOH-A, COR12, phenyl-CO-NOH-A, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8, R9, R10, R11 and R12 are C₁₋₁₂-alkyl or acyl.

R2, R3, R4, R5 and R6 of A are preferably H, OH, NH₂, COOH, SO₃H, C₁₋₃-alkyl, acyl, NO₂, CN, Cl, Br, F, CF₃, NOH-CO-phenyl, CO-NOH-phenyl, COR12, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8 and R9 are C₁₋₃-alkyl or acyl, and R10, R11 and R12 are C₁₋₃-alkyl; more preferably R2, R3, R4, R5 and R6 of A are H, OH, NH₂, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CF₃, CO-NOH-phenyl, COCH₃, OR7, NR8R9, or COOCH₃, wherein R7, R8 and R9 are CH₃ or COCH₃; even more preferably R2, R3, R4, R5 and R6 of A are H, OH, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CO-NOH-phenyl, OCH₃, COCH₃, or COOCH₃; and in particular R2, R3, R4, R5 and R6 of A are H, OH, COOH, SO₃H, CH₃, NO₂, CN, Cl, Br, CO-NOH-phenyl, or OCH₃.

R2, R3, R4, R5 and R6 of B are preferably H, OH, NH₂, COOH, SO₃H, C₁₋₃-alkyl, acyl, NO₂, CN, Cl, Br, F, CF₃, NOH-CO-phenyl, CO-NOH-phenyl, COR12, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8 and R9 are C₁₋₃-alkyl or acyl, and R10, R11 and R12 are C₁₋₃-alkyl; more preferably R2, R3, R4, R5 and R6 of B are H, OH, NH₂, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CF₃, CO-NOH-phenyl, COCH₃, OR7, NR8R9, or COOCH₃, wherein R7, R8 and R9 are CH₃ or COCH₃; even more preferably R2, R3, R4, R5 and R6 of B are H, OH, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CO-NOH-phenyl, OCH₃, COCH₃, or COOCH₃; and in particular R2, R3, R4, R5 and R6 of B are H, OH, COOH, SO₃H, CH₃, NO₂, CN, Cl, Br, CO-NOH-phenyl, or OCH₃.

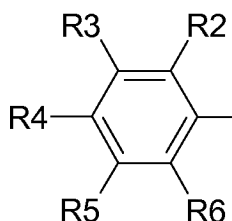
B is preferably H or C₁₋₃-alkyl, said alkyl may contain hydroxy, ester or ether groups; preferably said alkyl may contain ester or ether groups; more preferably said alkyl may contain ether groups.

In an embodiment, A and B independently of each other are:



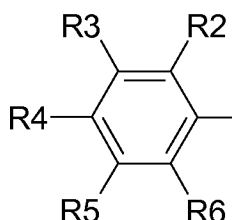
or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy, ester or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, NH₂, COOH, SO₃H, C₁₋₃-alkyl, acyl, NO₂, CN, Cl, Br, F, CF₃, NOH-CO-phenyl, CO-NOH-phenyl, COR12, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8 and R9 are C₁₋₃-alkyl or acyl, and R10, R11 and R12 are C₁₋₃-alkyl.

In another embodiment, A and B independently of each other are:



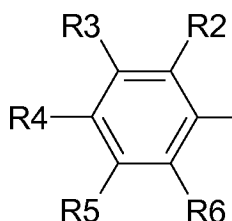
or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, NH₂, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CF₃, CO-NOH-phenyl, COCH₃, OR₇, NR₈R₉, or COOCH₃, wherein R₇, R₈ and R₉ are CH₃ or COCH₃.

In another embodiment, A and B independently of each other are:



or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, COOH, SO₃H, CH₃, acyl, NO₂, CN, Cl, Br, F, CO-NOH-phenyl, OCH₃, COCH₃, or COOCH₃.

In another embodiment, A and B independently of each other are:



or B is C₁₋₃-alkyl, said alkyl may contain ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, COOH, SO₃H, CH₃, NO₂, CN, Cl, Br, CO-NOH-phenyl, or OCH₃.

The terms "C_{1-n}-alkyl" wherein n can be from 2 through 12, as used herein, represent a branched or straight alkyl group having from one to the specified number of carbon atoms. Typical C₁₋₆-alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, hexyl, iso-hexyl and the like.

The term "acyl" as used herein refers to a monovalent substituent comprising a C₁₋₆-alkyl group linked through a carbonyl group; such as e.g. acetyl, propionyl, butyryl, isobutyryl, pivaloyl, valeryl, and the like.

In an embodiment, at least one of the substituents R2, R3, R4, R5 and R6 of A are H, preferably at least two of the substituents R2, R3, R4, R5 and R6 of A are H, more preferably at least three of the substituents R2, R3, R4, R5 and R6 of A are H, most preferably at least four of the substituents R2, R3, R4, R5 and R6 of A are H, in particular all of R2, R3, R4, R5 and R6 of A are H.

In another embodiment, at least one of the substituents R2, R3, R4, R5 and R6 of B are H, preferably at least two of the substituents R2, R3, R4, R5 and R6 of B are H, more preferably at least three of the substituents R2, R3, R4, R5 and R6 of B are H, most preferably at least four of the substituents R2, R3, R4, R5 and R6 of B are H, in particular all of R2, R3, R4, R5 and R6 of B are H.

In particular embodiments according to the invention, the enhancing agent is selected from the group consisting of

4-nitrobenzoic acid-N-hydroxyanilide;

4-methoxybenzoic acid-N-hydroxyanilide;

N,N'-dihydroxy-N,N'-diphenylterephthalamide;

decanoic acid-N-hydroxyanilide;

N-hydroxy-4-cyanoacetanilide;

N-hydroxy-4-acetylacetanilide;

N-hydroxy-4-hydroxyacetanilide;

N-hydroxy-3-(N'-hydroxyacetamide)acetanilide;

4-cyanobenzoic acid-N-hydroxyanilide;

N-hydroxy-4-nitroacetanilide;

N-hydroxyacetanilide;

N-hydroxy-N-phenyl-carbamic acid isopropyl ester;

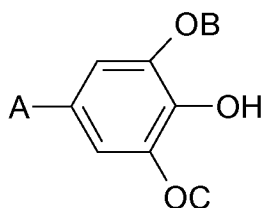
N-hydroxy-N-phenyl-carbamic acid methyl ester;

N-hydroxy-N-phenyl-carbamic acid phenyl ester;

N-hydroxy-N-phenyl-carbamic acid ethyl ester; and

N-hydroxy-N-(4-cyanophenyl)-carbamic acid methyl ester.

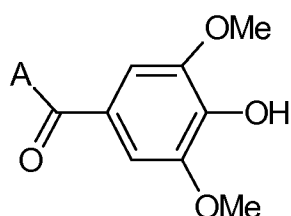
Another group of preferred enhancing agents is phenolic compounds (alkylsyringates) of the general formula IV:



wherein the letter A in said formula denotes be a group such as -D, -CH=CH-D, -CH=CH-CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D, in which D is selected from the group consisting of -CO-E, -SO₂-E, -N-XY, and -N⁺-XYZ, in which E may be -H, -OH, -R, or -OR, and X and Y and Z may be identical or different and selected from -H and -R; R being a C₁-C₁₆ alkyl, preferably a C₁-C₈ alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulpho or amino group; and B and C may be the same or different and selected from C_mH_{2m+1}, where m = 1, 2, 3, 4 or 5.

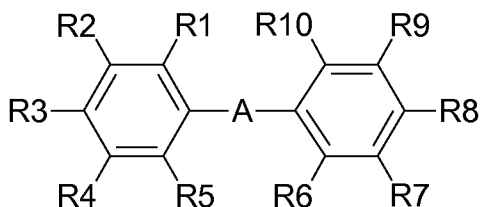
In the above mentioned general formula IV, A may be placed meta to the hydroxy group instead of being placed in the para-position as shown.

In particular embodiments of the invention, the enhancing agent is selected from the group having the general formula V:



in which A is a group such as -H, -OH, -CH₃, -OCH₃, -O(CH₂)_nCH₃, where n = 1, 2, 3, 4, 5, 6, 7 or 8.

Yet another group of preferred enhancing agents are the compounds as described in general formula VI:



in which general formula A represents a single bond, or one of the following groups: (-CH₂-), (-CH=CH-), (-NR₁₁-), (-CH=N-), (-N=N-), (-CH=N-N=CH-), or (>C=O);

and in which general formula the substituent groups R₁-R₁₁, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, C₁₋₈-alkyl;

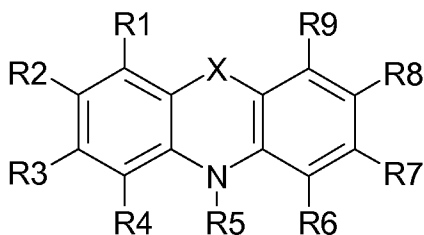
which carbamoyl, sulfamoyl, phenyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R₁₂; and which C₁₋₈-alkyl group may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R₁₂;

which substituent group R₁₂ represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and

salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, or C₁₋₈-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy or methyl;

and in which general formula R5 and R6 may together form a group -B-, in which B represents a single bond, one of the following groups (-CH₂-), (-CH=CH-), (-CH=N-); or B represents sulfur, or oxygen.

In particular embodiments of the invention, the enhancing agent is selected from the group having the general formula VII:



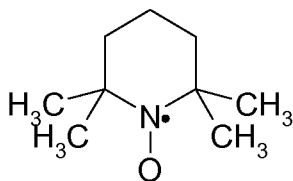
in which general formula X represents a single bond, oxygen, or sulphur;

and in which general formula the substituent groups R1-R9, which may be identical or different, independently represents any of the following side groups: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, C₁₋₈-alkyl;

which carbamoyl, sulfamoyl, phenyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R10; and which C₁₋₈-alkyl group may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R10;

which substituent group R10 represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, or C₁₋₈-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy or methyl.

A further preferred enhancing agent according to the invention is 2,2',6,6'-tetramethyl-piperidine-*N*-oxyl (TEMPO):



According to the invention, the enhancing agent may be present in a concentration in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, more

preferably in the range of from 0.1 mM to 100 mM, and most preferably in the range of from 0.1 mM to 50 mM.

Laccase

A laccase according to the invention is any laccase enzyme comprised by the enzyme classification EC 1.10.3.2 as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment derived therefrom exhibiting laccase activity, or a compound exhibiting a similar activity, such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5).

Preferred laccase enzymes are enzymes of microbial origin. The enzymes may be derived from plants, bacteria or fungi (including filamentous fungi and yeasts).

Suitable examples from fungi include a laccase derivable from a strain of *Aspergillus*, *Neurospora*, e.g., *N. crassa*, *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, e.g., *T. villosa* and *T. versicolor*, *Rhizoctonia*, e.g., *R. solani*, *Coprinopsis*, e.g., *C. cinerea*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, e.g., *P. condelleana*, *Panaeolus*, e.g., *P. papilionaceus*, *Myceliophthora*, e.g., *M. thermophila*, *Schytalidium*, e.g., *S. thermophilum*, *Polyporus*, e.g., *P. pinsitus*, *Phlebia*, e.g., *P. radiata* (WO 92/01046), or *Coriolus*, e.g., *C. hirsutus* (JP 2238885).

Suitable examples from bacteria include a laccase derivable from a strain of *Bacillus*.

A laccase derived from *Coprinopsis* or *Myceliophthora* is preferred; in particular a laccase derived from *Coprinopsis cinerea* or *Myceliophthora thermophila*. Preferably, the amino acid sequence of the laccase has at least 80% identity, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, and most preferably 100% identity to the *Myceliophthora thermophila* laccase shown as SEQ ID NO:1, or the *Coprinopsis cinerea* laccase shown as SEQ ID NO:2.

The laccase enzyme may furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase, in a culture medium under conditions permitting the expression of the laccase enzyme, and recovering the laccase from the culture.

In further particular embodiments of the method and use of the invention, the laccase and/or compound exhibiting laccase activity is used in an amount of 0.005-50 ppm (mg/l), or 0.01-40, 0.02-30, 0.03-25, 0.04-20, 0.05-15, 0.05-10, 0.05-5, 0.05-1, 0.05-0.8, 0.05-0.6, or 0.1-0.5 ppm. The amount of enzyme refers to mg of enzyme protein.

Laccase Activity

Laccase activity may be determined from the oxidation of syringaldazine under aerobic conditions. The violet colour produced is measured at 530 nm. The analytical conditions are 19 mM syringaldazine, 23 mM Tris/maleate buffer, pH 7.5, 30°C, 1 min. reaction time. One laccase unit (LAMU) is the amount of enzyme that catalyses the conversion of 1.0 mmole syringaldazine per minute at these conditions.

Lipoxygenase

The lipoxygenase (may be referred to as LOX) of the invention is a lipoxygenase, classified as EC 1.13.11.12, which is an enzyme that catalyzes the oxygenation of polyunsaturated fatty acids, especially *cis,cis*-1,4-dienes, e.g., linoleic acid and produces a hydroperoxide. But also other substrates may be oxidized, e.g., monounsaturated fatty acids.

Microbial lipoxygenases can be derived from, e.g., *Saccharomyces cerevisiae*, *Thermoactinomyces vulgaris*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Thermomyces lanuginosus*, *Pyricularia oryzae*, and strains of *Geotrichum*. The preparation of a lipoxygenase derived from *Gaeumannomyces graminis* (SEQ ID NO: 4) is described in Examples 3-4 of WO 02/20730. The expression in *Aspergillus oryzae* of a lipoxygenase derived from *Magnaporthe salvinii* (SEQ ID NO: 5) is described in Example 2 of WO 02/086114, and this enzyme can be purified using standard methods, e.g. as described in Example 4 of WO 02/20730.

Lipoxygenase may also be extracted from plant seeds, such as soybean, pea, chickpea, and kidney bean. Alternatively, lipoxygenase may be obtained from mammalian cells, e.g. rabbit reticulocytes.

The lipoxygenase of the present invention is preferably recombinantly produced, and comprises or consists of an amino acid sequence having at least 70% identity, preferably at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 19.

In an embodiment, the lipoxygenase comprises or consists of the amino acid sequence of SEQ ID NO: 3; or a fragment thereof having lipoxygenase activity; preferably the lipoxygenase comprises or consists of the mature polypeptide of SEQ ID NO: 3.

Preferably, amino acid changes are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

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

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline, and alpha-methyl serine) may be substituted for amino acid residues of a wild-type polypeptide. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for amino acid residues. "Unnatural amino acids" have been modified after protein synthesis, and/or have a chemical structure in their side chain(s) different from that of the standard amino acids. Unnatural amino acids can be chemically synthesized, and preferably, are commercially available, and include pipercolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

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

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

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

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

The total number of amino acid substitutions, deletions and/or insertions of the lipoyxygenase of SEQ ID NO: 19 is at most 10, preferably at most 9, more preferably at most 8, more preferably at most 7, more preferably at most 6, more preferably at most 5, more preferably at most 4, even more preferably at most 3, most preferably at most 2, and even most preferably at most 1.

The concentration of lipoyxygenase is typically 0.05 mg/ml to 50 mg/ml, preferably 0.05 mg/ml to 10 mg/ml, more preferably 0.1 mg/ml to 10 mg/ml, and most preferably 0.1 mg/ml to 5 mg/ml.

Lipoyxygenase activity

Lipoyxygenase activity may be determined spectrophotometrically at 25°C by monitoring the formation of hydroperoxides. For the standard analysis, 10 micro liters enzyme is added to a 1 ml quartz cuvette containing 980 micro liter 25 mM sodium phosphate buffer (pH 7.0) and 10 micro liter of substrate solution (10 mM linoleic acid dispersed with 0.2% (v/v) Tween20). The enzyme is typically diluted sufficiently to ensure a turn-over of maximally 10% of the added substrate within the first minute. The absorbance at 234 nm is followed and the rate is estimated from the linear part of the curve. The *cis-trans*-conjugated hydro(pero)xy fatty acids are assumed to have a molecular extinction coefficient of 23,000 M⁻¹cm⁻¹.

Oxygen

The oxygen required by the laccase and/or lipoxygenase may be oxygen from the atmosphere or an oxygen precursor for in situ production of oxygen. In many industrial applications, oxygen from the atmosphere will usually be present in sufficient quantity. If more
 5 O₂ is needed, additional oxygen may be added, e.g. as pressurized atmospheric air or as pure pressurized O₂.

Peroxidase

A peroxidase according to the invention is a peroxidase enzyme comprised by the
 10 enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity.

Preferably, the peroxidase according to the invention is producible by plants (e.g., horseradish peroxidase (see SEQ ID NO: 7), soybean peroxidase (see SEQ ID NO: 8), or palm tree peroxidase (see SEQ ID NO: 9)) or microorganisms such as fungi or bacteria. In an
 15 embodiment, the amino acid sequence of the peroxidase at least 80% identity, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, and most preferably 100% identity to the soybean peroxidase shown as SEQ ID NO: 8, the horseradish peroxidase shown as SEQ ID NO: 7, or the palm tree peroxidase shown as SEQ ID NO: 9.

Some preferred fungi include strains belonging to the subdivision *Deuteromycotina*, class
 20 *Hyphomycetes*, e.g., *Fusarium*, *Humicola*, *Trichoderma*, *Myrothecium*, *Verticillium*, *Arthomyces*, *Caldariomyces*, *Ulocladium*, *Embellisia*, *Cladosporium* or *Dreschlera*, in particular *Fusarium oxysporum* (DSM 2672), *Humicola insolens*, *Trichoderma reesei*, *Myrothecium verrucaria* (IFO 6113), *Verticillium alboatrum*, *Verticillium dahliae*, *Arthomyces ramosus* (FERM P-7754), *Caldariomyces fumago*, *Ulocladium chartarum*, *Embellisia alli* or *Dreschlera halodes*.

Other preferred fungi include strains belonging to the subdivision *Basidiomycotina*, class
 25 *Basidiomycetes*, e.g., *Coprinopsis*, *Phanerochaete*, *Coriolus* or *Trametes*, in particular *Coprinopsis cinerea* f. *microsporus* (IFO 8371), *Coprinopsis macrorhizus*, *Phanerochaete chrysosporium* (e.g. NA-12) or *Trametes* (previously called *Polyporus*), e.g., *T. versicolor* (e.g. PR4 28-A).

Further preferred fungi include strains belonging to the subdivision *Zygomycotina*, class
 30 *Mycoraceae*, e.g., *Rhizopus* or *Mucor*, in particular *Mucor hiemalis*.

Some preferred bacteria include strains of the order *Actinomycetales*, e.g. *Streptomyces spheroides* (ATTC 23965), *Streptomyces thermoviolaceus* (IFO 12382) or *Streptoverticillum verticillium* ssp. *verticillium*.

Other preferred bacteria include *Rhodobacter sphaeroides*, *Rhodomonas palustri*,
 35 *Streptococcus lactis*, *Pseudomonas putrefaciens* (ATCC 15958), *Pseudomonas fluorescens*

(NRRL B-11) and *Bacillus* strains, e.g. *Bacillus pumilus* (ATCC 12905) and *Bacillus stearothermophilus*.

Further preferred bacteria include strains belonging to *Myxococcus*, e.g., *M. virescens*.

The peroxidase may furthermore be one which is producible by a method comprising
5 cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said peroxidase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the peroxidase, in a culture medium under conditions permitting the expression of the peroxidase and recovering the peroxidase from the culture.

10 Particularly, a recombinantly produced peroxidase is a peroxidase derived from a *Coprinus* sp. (also referred to as *Coprinopsis* sp.), in particular *C. macrorhizus* or *C. cinereus* (see e.g. SEQ ID NO: 6).

In the context of this invention, compounds possessing peroxidase activity comprise peroxidase enzymes and peroxidase active fragments derived from cytochromes, haemoglobin
15 or peroxidase enzymes.

Peroxidase Activity

One peroxidase unit (POXU) is the amount of enzyme which catalyzes the conversion of one μ mole hydrogen peroxide per minute at 30°C in a mixture containing:

20 0.1 M phosphate buffer, pH 7.0;

0.88 mM hydrogen peroxide; and

1.67 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS).

The reaction is continued for 60 seconds (15 seconds after mixing) while the change in absorbance at 418 nm is measure. The absorbance should be in the range of 0.15 to 0.30. In
25 the calculation of peroxidase activity is used an absorption coefficient of oxidized ABTS of 36 $\text{mM}^{-1} \text{cm}^{-1}$ and a stoichiometry of one μ mole H_2O_2 converted per two μ mole ABTS oxidized.

Peroxygenase

The term "peroxygenase" means an enzyme exhibiting "unspecific peroxygenase" activity
30 according to EC 1.11.2.1, that catalyzes insertion of an oxygen atom from H_2O_2 into a variety of substrates, such as nitrobenzodioxole.

The peroxygenase of the present invention is preferably recombinantly produced, and comprises or consists of an amino acid sequence having at least 70% identity, preferably at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity to the amino acid sequence
35 shown as any of SEQ ID NO: 10 to SEQ ID NO: 29.

In a preferred embodiment, the peroxygenase comprises an amino acid sequence represented by the motif: E-H-D-[G,A]-S-[L,I]-S-R.

In yet another embodiment, the peroxygenase of the first aspect comprises or consists of the amino acid sequence shown as any of SEQ ID NO: 10 to SEQ ID NO: 29; or a fragment thereof having peroxygenase activity.

Preferably, amino acid changes are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

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

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline, and alpha-methyl serine) may be substituted for amino acid residues of a wild-type polypeptide. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for amino acid residues. "Unnatural amino acids" have been modified after protein synthesis, and/or have a chemical structure in their side chain(s) different from that of the standard amino acids. Unnatural amino acids can be chemically synthesized, and preferably, are commercially available, and include pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

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

Essential amino acids in the parent polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine

mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (*i.e.*, peroxygenase activity) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by
5 physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identities of essential amino acids can also be inferred from analysis of
10 identities with polypeptides that are related to a polypeptide according to the invention.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO
15 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, Lowman *et al.*, 1991, *Biochem.* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

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

The total number of amino acid substitutions, deletions and/or insertions of the peroxygenase shown as any of SEQ ID NO: 10 to SEQ ID NO: 29; is at most 10, preferably at most 9, more preferably at most 8, more preferably at most 7, more preferably at most 6, more preferably at most 5, more preferably at most 4, even more preferably at most 3, most
30 preferably at most 2, and even most preferably at most 1.

The concentration of peroxygenase is typically 0.05 mg/ml to 50 mg/ml, preferably 0.05 mg/ml to 10 mg/ml, more preferably 0.1 mg/ml to 10 mg/ml, and most preferably 0.1 mg/ml to 5 mg/ml.

The concentration of peroxygenase is typically 0.05 mg/L to 50 mg/L, preferably 0.05
35 mg/L to 10 mg/L, more preferably 0.1 mg/L to 10 mg/L, and most preferably 0.1 mg/L to 5 mg/L.

Peroxygenase activity

For purposes of the present invention, peroxygenase activity is determined according to the procedure described in M. Poraj-Kobielska, M. Kinne, R. Ullrich, K. Scheibner, M. Hofrichter, "A spectrophotometric assay for the detection of fungal peroxygenases", *Analytical Biochemistry* 5 (2012), vol. 421, issue 1, pp. 327–329.

The peroxygenase of the present invention has at least 20%, preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 100% of the peroxygenase activity of the peroxygenase shown as 10 any of SEQ ID NO: 10 to SEQ ID NO: 29.

Hydrogen peroxide

The hydrogen peroxide required by the peroxidase and/or peroxygenase may be provided as an aqueous solution of hydrogen peroxide or a hydrogen peroxide precursor for in situ 15 production of hydrogen peroxide. Any solid entity which liberates upon dissolution a peroxide, which is useable by peroxidase or peroxygenase, can serve as a source of hydrogen peroxide. Compounds which yield hydrogen peroxide upon dissolution in water or an appropriate aqueous based medium include but are not limited to metal peroxides, percarbonates, persulphates, perphosphates, peroxyacids, alkylperoxides, acylperoxides, peroxyesters, urea peroxide, 20 perborates, tert-butyl hydroperoxide, cumene peroxide, lipid hydroperoxide and peroxycarboxylic acids or salts thereof.

Another source of hydrogen peroxide is a hydrogen peroxide generating enzyme system, such as an oxidase together with a substrate for the oxidase. Examples of combinations of oxidase and substrate comprise, but are not limited to, amino acid oxidase (see e.g. US 25 6,248,575) and a suitable amino acid, glucose oxidase (see e.g. WO 95/29996) and glucose, lactate oxidase and lactate, galactose oxidase (see e.g. WO 00/50606) and galactose, and aldose oxidase (see e.g. WO 99/31990) and a suitable aldose. By studying EC 1.1.3._, EC 1.2.3._, EC 1.4.3._, and EC 1.5.3._ or similar classes (under the International Union of Biochemistry), other examples of such combinations of oxidases and substrates are easily 30 recognized by one skilled in the art.

Alternative oxidants which may be applied for peroxidases or peroxygenases may be oxygen combined with a suitable hydrogen donor like ascorbic acid, dehydroascorbic acid, dihydroxyfumaric acid or cysteine. An example of such oxygen hydrogen donor system is described by Pasta *et al.*, *Biotechnology & Bioengineering*, (1999) vol. 62, issue 4, pp. 489-493.

35 Hydrogen peroxide or a source of hydrogen peroxide may be added at the beginning of or during the method of the invention, e.g. as one or more separate additions of hydrogen

peroxide; or continuously as fed-batch addition. Typical amounts of hydrogen peroxide correspond to levels of from 0.001 mM to 25 mM, preferably to levels of from 0.005 mM to 5 mM, and particularly to levels of from 0.01 to 1 mM or 0.02 to 2 mM hydrogen peroxide. Hydrogen peroxide may also be used in an amount corresponding to levels of from 0.1 mM to 25 mM, preferably to levels of from 0.5 mM to 15 mM, more preferably to levels of from 1 mM to 10 mM, preferably to levels of from 2 mM to 8 mM hydrogen peroxide, most preferably to levels of from 0.3 mM to 8 mM hydrogen peroxide.

Skin-derived body soil

In the context of the present invention, skin-derived body soils include cutaneous (epidermal) and subcutaneous cell components and secretions, in particular sebum and other secretions transported to the skin surface from subcutaneous structures.

Sebum

Sebaceous glands secrete the oily, waxy substance called sebum that is made of triglyceride oils, wax, squalene, and metabolites of fat-producing cells. In the glands, sebum is produced within specialized cells and is released as these cells burst. Sebaceous glands can usually be found in hair-covered areas, where they are connected to hair follicles. But sebum is also secreted from non-haired areas (glabrous skin), where it traverses ducts that terminate in sweat pores on the surface of the skin.

The composition of sebum in humans is approximately 25% wax monoesters, 41% triglycerides, 16% free fatty acids, and 12% squalene.

Yellowing

Yellow stains (yellowing) on textile occur when sebum and other skin-derived body soils are not washed out of clothing and form a yellow stain during the drying process.

Gradual yellowing of textile in use is related to the accumulation and oxidation of sebum. Unsaturated compounds in sebum, unsaturated fatty acids and their esters, form dark yellow colored oxidation products, which is also referred to as "yellowing" of the textile. This effect is most visible on white textile items, such as white garments in direct skin contact, like cuffs, collars, under garments, and pillow cases.

The stain composed of sebum-constituents may also capture/immobilize colored or black particles from the environment, and thus lead to greying or color-casting of the textile, in particular in areas of the textile, which are exposed to skin-derived body soils like sebum.

Measuring removal or release of body soilMini Launder-O-Meter (LOM) Model Wash System

The mini Launder-O-Meter (LOM) is based on a medium scale model wash system called a launder-O-meter that can be applied to test up to 20 different wash conditions simultaneously.

- 5 A LOM is basically a large temperature controlled water bath with 20 closed metal beakers rotating inside it. In contrast, the mini LOM is a down scaled version of this set up using 50 mL plastic sealed tubes. Each beaker or tube constitutes one small washing machine and during an experiment, each will contain a solution of a specific detergent/enzyme system to be tested along with the soiled and unsoiled fabrics it is tested on. Mechanical stress is achieved by the
- 10 beakers being rotated in the water bath and by including metal balls in the beaker.

Wash performance Δ REM

- Wash performance can be expressed as a delta remission value (Δ REM). After washing and rinsing the swatches were spread out flat and allowed to air dry at room temperature
- 15 overnight. All washes are evaluated the day after the wash. Light reflectance evaluations of the swatches were done using a Macbeth Color Eye 7000 reflectance spectrophotometer with very small aperture. The measurements were made without UV in the incident light and remission at 460 nm was extracted. Measurements were made on unwashed and washed swatches. A selection of up to 12 swatches of the same type was measured prior to washing. This value was
- 20 recorded as the "before wash" value. Swatches were placed in triplicate per beaker. These three replicates were placed on top of each other for measurement. Remission values for individual swatches were calculated by subtracting the remission value of this averaged unwashed swatch from the remission value of the averaged washed swatches.

- Calculating the enzyme effect (Δ REM) is done by taking the measurements from washed
- 25 swatches with enzymes and subtract with the measurements from washed without enzyme for each stain.

Wash performance Δ L, Δ a and Δ b

- Wash performance can be expressed as Lab color vector (Δ L). After washing and rinsing
- 30 the swatches were spread out flat and allowed to air dry at room temperature overnight. All washes are evaluated the day after the wash. To evaluate the specific nature of the cleaning, the CIE L^* , a^* and b^* values were also recorded by the Macbeth Color Eye 7000 reflectance spectrophotometer during the measurement. The Lab color measurements taken using the Color Eye 7000 are calculated from the CIE (*Commission internationale de l'éclairage*) XYZ
- 35 color space co-ordinates. Lab is the abbreviation used to describe the CIE 1976 L^* , a^* , b^* color space, where L is lightness and a, and b are color dimensions.

ΔL denotes the change in L^* when taken the measurements from swatches washed with the enzyme of the invention and subtract with the measurements from swatches washed without enzyme for each stain.

5 Δa denotes the change in a^* when taken the measurements from swatches washed with the enzyme of the invention and subtract with the measurements from swatches washed without enzyme for each stain.

Δb denotes the change in b^* when taken the measurements from swatches washed with the enzyme of the invention and subtract with the measurements from swatches washed without enzyme for each stain.

10 Reference is made to János Schanda (2007). Colorimetry. Wiley-Interscience. p. 61. ISBN 978-0-470-04904-4.

Textile

The term "textile" means any textile material including yarns, yarn intermediates, fibers, 15 non-woven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles). The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts, yarns, and towelling. The textile may be cellulose based such as natural cellulose, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulose (e.g. originating from wood 20 pulp) including viscose/rayon, ramie, cellulose acetate fibers (tricell), lyocell or blends thereof. The textile or fabric may also be non-cellulose based such as natural polyamides including wool, camel, cashmere, mohair, rabbit and silk or synthetic polymers such as nylon, aramid, polyester, acrylic, polypropylene and spandex/elastane, or blends thereof as well as blends of cellulose based and non-cellulose based fibers. Examples of blends are blends of cotton and/or 25 rayon/viscose with one or more companion material such as wool, synthetic fiber (e.g. polyamide fiber, acrylic fiber, polyester fiber, polyvinyl alcohol fiber, polyvinyl chloride fiber, polyurethane fiber, polyurea fiber, aramid fiber), and/or cellulose-containing fiber (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fiber, lyocell). Fabric may be conventional washable laundry, for example stained household laundry. When the term fabric or 30 garment is used it is intended to include the broader term textiles as well.

Detergent composition

In one embodiment, the invention is directed to detergent compositions comprising an oxidoreductase enzyme, as described above, in combination with one or more additional 35 cleaning composition components. The choice of additional components is within the skill of the

artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below.

The choice of components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

In one embodiment of the present invention, the oxidoreductase enzyme may be added to a detergent composition in an amount corresponding to 0.001-200 mg of protein, such as 0.005-100 mg of protein, preferably 0.01-50 mg of protein, more preferably 0.05-20 mg of protein, even more preferably 0.1-10 mg of protein per liter of wash liquor.

The enzyme(s) of the detergent composition of the invention 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, for example, WO 92/19709 and WO 92/19708.

A polypeptide of the present invention may also be incorporated in the detergent formulations disclosed in WO 97/07202, which is hereby incorporated by reference.

Surfactants

The detergent composition may comprise one or more surfactants, which may be anionic and/or cationic and/or non-ionic and/or semi-polar and/or zwitterionic, or a mixture thereof. In a particular embodiment, the detergent composition includes a mixture of one or more nonionic surfactants and one or more anionic surfactants. The surfactant(s) is typically present at a level of from about 0.1% to 60% by weight, such as about 1% to about 40%, or about 3% to about 20%, or about 3% to about 10%. The surfactant(s) is chosen based on the desired cleaning application, and includes any conventional surfactant(s) known in the art. Any surfactant known in the art for use in detergents may be utilized.

When included therein the detergent will usually contain from about 1% to about 40% by weight, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 20% to about 25% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS),

primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or soap, and combinations thereof.

When included therein the detergent will usually contain from about 0.1% to about 10% by weight of a cationic surfactant. Non-limiting examples of cationic surfactants include alkyltrimethylammonium bromide (CTAB), dimethyldistearylammonium chloride (DSDMAC), and alkylbenzyltrimethylammonium, alkyl quaternary ammonium compounds, alkoxyated quaternary ammonium (AQA) compounds, and combinations thereof.

When included therein the detergent will usually contain from about 0.2% to about 40% by weight of a non-ionic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, or from about 8% to about 12%. Non-limiting examples of non-ionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxyated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxyated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxy alkyl fatty acid amides, or *N*-acyl *N*-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamide, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof.

When included therein the detergent will usually contain from about 0.1% to about 20% by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyltrimethylamineoxide, *N*-(coco alkyl)-*N,N*-dimethylamine oxide and *N*-(tallow-alkyl)-*N,N*-bis(2-hydroxyethyl)amine oxide, fatty acid alkanolamides and ethoxylated fatty acid alkanolamides, and combinations thereof.

When included therein the detergent will usually contain from about 0.1% to about 10% by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaine, alkyltrimethylbetaine, sulfobetaine, and combinations thereof.

Hydrotropes

A hydrotrope is a compound that solubilises hydrophobic compounds in aqueous solutions (or oppositely, polar substances in a non-polar environment). Typically, hydrotropes have both

hydrophilic and a hydrophobic character (so-called amphiphilic properties as known from surfactants); however the molecular structure of hydrotropes generally do not favor spontaneous self-aggregation, see e.g. review by Hodgdon and Kaler (2007), Current Opinion in Colloid & Interface Science 12: 121-128. Hydrotropes do not display a critical concentration
5 above which self-aggregation occurs as found for surfactants and lipids forming micellar, lamellar or other well defined meso-phases. Instead, many hydrotropes show a continuous-type aggregation process where the sizes of aggregates grow as concentration increases. However, many hydrotropes alter the phase behavior, stability, and colloidal properties of systems containing substances of polar and non-polar character, including mixtures of water, oil,
10 surfactants, and polymers. Hydrotropes are classically used across industries from pharma, personal care, food, to technical applications. Use of hydrotropes in detergent compositions allow for example more concentrated formulations of surfactants (as in the process of compacting liquid detergents by removing water) without inducing undesired phenomena such as phase separation or high viscosity.

15 The detergent may contain 0-5% by weight, such as about 0.5 to about 5%, or about 3% to about 5%, of a hydrotrope. Any hydrotrope known in the art for use in detergents may be utilized. Non-limiting examples of hydrotropes include sodium benzene sulfonate, sodium p-toluene sulfonate (STS), sodium xylene sulfonate (SXS), sodium cumene sulfonate (SCS), sodium cymene sulfonate, amine oxides, alcohols and polyglycolethers, sodium
20 hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

Builders and Co-Builders

The detergent composition may contain about 0-65% by weight, such as about 5% to about
25 50% of a detergent builder or co-builder, or a mixture thereof. In a dish wash detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in laundry detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP
30 or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (*e.g.*, SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as iminodiethanol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethanol), and carboxymethyl inulin (CMI), and combinations thereof.

The detergent composition may also contain 0-50% by weight, such as about 5% to about
35 30%, of a detergent co-builder, or a mixture thereof. The detergent composition may include include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-

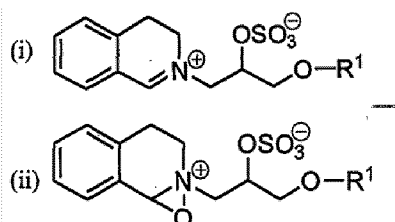
limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2''-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-*N,N'*-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-*N,N*-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTPMPA or DTPMPA), *N*-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-*N*-monoacetic acid (ASMA), aspartic acid-*N,N*-diacetic acid (ASDA), aspartic acid-*N*-monopropionic acid (ASMP), iminodisuccinic acid (IDA), *N*-(2-sulfomethyl)-aspartic acid (SMAS), *N*-(2-sulfoethyl)-aspartic acid (SEAS), *N*-(2-sulfomethyl)-glutamic acid (SMGL), *N*-(2-sulfoethyl)-glutamic acid (SEGL), *N*-methyliminodiacetic acid (MIDA), α -alanine-*N,N*-diacetic acid (α -ALDA), serine-*N,N*-diacetic acid (SEDA), isoserine-*N,N*-diacetic acid (ISDA), phenylalanine-*N,N*-diacetic acid (PHDA), anthranilic acid-*N,N*-diacetic acid (ANDA), sulfanilic acid-*N,N*-diacetic acid (SLDA), taurine-*N,N*-diacetic acid (TUDA) and sulfomethyl-*N,N*-diacetic acid (SMDA), *N*-(2-hydroxyethyl)-ethylenediamine-*N,N'*, *N'*-triacetate (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053.

Bleaching Systems

The detergent may contain 0-50% of a bleaching system. Any bleaching system known in the art for use in laundry detergents may be utilized. Suitable bleaching system components include bleaching catalysts, photobleaches, bleach activators, sources of hydrogen peroxide such as sodium percarbonate and sodium perborates, preformed peracids and mixtures thereof. Suitable preformed peracids include, but are not limited to, peroxydicarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone (R), and mixtures thereof. Non-limiting examples of bleaching systems include peroxide-based bleaching systems, which may comprise, for example, an inorganic salt, including alkali metal salts such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulfate, perphosphate, persilicate salts, in combination with a peracid-forming bleach activator. The term bleach activator is meant herein as a compound which reacts with peroxygen bleach like hydrogen peroxide to form a peracid. The peracid thus formed constitutes the activated bleach. Suitable bleach activators to be used herein include those belonging to the class of esters amides, imides or anhydrides. Suitable examples are tetracetylene diamine

(TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene sulfonate (ISONOBS), diperoxy dodecanoic acid, 4-(dodecanoyloxy)benzenesulfonate (LOBS), 4-(decanoyloxy)benzenesulfonate, 4-(decanoyloxy)benzoate (DOBS), 4-(nonanoyloxy)benzenesulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that it is environmental friendly as it eventually degrades into citric acid and alcohol. Furthermore acetyl triethyl citrate and triacetin has a good hydrolytical stability in the product upon storage and it is an efficient bleach activator. Finally ATC provides a good building capacity to the laundry additive.

Alternatively, the bleaching system may comprise peroxyacids of, for example, the amide, imide, or sulfone type. The bleaching system may also comprise peracids such as 6-(phthalimido)peroxyhexanoic acid (PAP). The bleaching system may also include a bleach catalyst. In some embodiments the bleach component may be an organic catalyst selected from the group consisting of organic catalysts having the following formulae:



and mixtures thereof; wherein each R^1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R^1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R^1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylonyl, 2-hexyldecyl, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, iso-nonyl, iso-decyl, iso-tridecyl and iso-pentadecyl. Other exemplary bleaching systems are described, e.g. in WO 2007/087258, WO 2007/087244, WO 2007/087259 and WO 2007/087242. Suitable photobleaches may for example be sulfonated zinc phthalocyanine.

Polymers

The detergent may contain 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1% of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC),

poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers, hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-N-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated.

Fabric hueing agents

The detergent compositions of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO 2005/03274, WO 2005/03275, WO 2005/03276 and EP 1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO 2007/087243.

Additional enzymes

The enzyme(s) comprised in the detergent composition include one or more enzymes such as a protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, and/or xylanase.

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g., the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having color care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Proteases: Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metalloprotease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g., of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235, and 274.

Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Esperase™, and Kannase™ (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

Lipases and Cutinases: Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples include lipase from *Thermomyces*, e.g., from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described in EP 258 068 and EP 305 216, cutinase from *Humicola*, e.g. *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. stearotherophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

5 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, WO 97/07202, WO 00/060063, WO2007/087508 and WO 2009/109500.

10 Preferred commercially available lipase enzymes include LipolaseTM, Lipolase UltraTM, and LipexTM; LecitaseTM, LipolexTM, LipocleanTM, LipoprimeTM (Novozymes A/S). Other commercially available lipases include Lumafast (Genencor Int Inc); Lipomax (Gist-Brocades/Genencor Int Inc) and *Bacillus* sp lipase from Solvay.

Amylases: Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for
15 example, α -amylases obtained from *Bacillus*, e.g., a special strain of *Bacillus licheniformis*, described in more detail in GB 1,296,839.

Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208,
20 209, 243, 264, 304, 305, 391, 408, and 444.

Commercially available amylases are DuramylTM, TermamylTM, FungamylTM and BANTM (Novozymes A/S), RapidaseTM and PurastarTM (from Genencor International Inc.).

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of
25 these enzymes. A detergent additive of the invention, *i.e.*, a separate additive or a combined additive, can be formulated, for example, as a granulate, liquid, slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

Non-dusting granulates may be produced, e.g. as disclosed in US 4,106,991 and 4,661,452
30 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids.
35 Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a

polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

5 Adjunct materials

Any detergent components known in the art for use in laundry detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, 10 CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish inhibitors, and wicking agents, either alone or in combination. Any ingredient known in the art for use in laundry detergents may be utilized. The choice of such ingredients is well within the skill of 15 the artisan.

Dispersants - The detergent compositions of the present invention can also contain dispersants. In particular powdered detergents may comprise dispersants. Suitable water-soluble organic materials include 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 20 more than two carbon atoms. Suitable dispersants are for example described in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc.

Dye Transfer Inhibiting Agents - The detergent compositions of the present invention may also 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 25 and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels 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 composition.

Fluorescent whitening agent - The detergent compositions of the present invention will 30 preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of 35 diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent

whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2-anilino-4-(*N*-methyl-*N*-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2*H*-naphtho[1,2-*d*][1,2,3]triazol-2-yl)-2-[(*E*)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenylstyryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins.

Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

Soil release polymers - The detergent compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalate based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers are amphiphilic alkoxyated grease cleaning polymers comprising a core structure and a plurality of alkoxyate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose derivatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

Anti-redeposition agents - The detergent compositions of the present invention may also include one or more anti-redeposition agents such as carboxymethylcellulose (CMC), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyoxyethylene and/or polyethyleneglycol (PEG), homopolymers of acrylic acid, copolymers of acrylic acid and maleic acid, and ethoxylated polyethyleneimines. The cellulose based polymers described under soil release polymers above may also function as anti-redeposition agents.

Other suitable adjunct materials include, but are not limited to, anti-shrink agents, anti-wrinkling agents, bactericides, binders, carriers, dyes, enzyme stabilizers, fabric softeners, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

Formulation of detergent products

The detergent composition of the invention may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

Pouches can be configured as single or multi compartments. It can be of any form, shape and material which is suitable for holding the composition, e.g. without allowing release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivatives thereof are selected polyacrylates, and water soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blended compositions comprising hydrolytically degradable and water soluble polymer blends such as polylactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by MonoSol LLC, Indiana, USA) plus plasticisers like glycerol, ethylene glycerol, propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water soluble film. The compartment for liquid components can be different in composition than compartments containing solids.

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets. Thereby negative storage interaction

between components can be avoided. Different dissolution profiles of each of the compartments can also give rise to delayed dissolution of selected components in the wash solution.

A liquid or gel detergent, which is not unit dosed, may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel. An aqueous liquid or gel detergent may contain from 0-30% organic solvent. A liquid or gel detergent may be non-aqueous.

10 Methods and Uses

In a first aspect, the present invention provides a method for removing or releasing the constituents of a skin-derived body soil deposition from textile, comprising contacting the body soil deposition with an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11.

15 In an embodiment, the skin-derived body soil comprises squalene; preferably the skin-derived body soil is sebum, preferably aged or oxidized sebum. More preferably, the constituents of the sebum include squalene cross linked through oxygen-bridging to sebum lipids, or proteins/peptides/carbohydrates derived from skin or textile.

Thus, the textile to be treated with the method of the invention comprises a skin-derived body soil deposition (stain), such as a sebum derived or sebum containing stain.

In an embodiment, when measuring the color of the textile surface, the CIE Lab color vector of the deposition is changed by more than 3 units after treatment with the method of the invention. Preferably, the yellow color of the deposition is reduced, thus reducing yellowing, more preferably the b-value of the CIE Lab color of the deposition is reduced. The L-value of the CIE Lab color of the deposition may also be increased, thus reducing greying.

In an embodiment, the textile is a white garment in direct skin contact, such as a cuff, collar, under garment, or a pillow case; preferably, the textile is made of cotton fabric.

In an embodiment, the enzyme is selected from the group consisting of peroxygenase, laccase, peroxidase and lipoxygenase. Preferably, the enzyme is a peroxygenase.

30 In another aspect, the invention provides an aqueous detergent composition, comprising water, a surfactant; an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11; and a white textile item with a sebum derived or sebum containing stain.

The invention also provides for use of the methods and compositions mentioned above for removing or releasing the constituents of a skin-derived body soil deposition from textile. For example, the invention provides for use of an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11 for removing or releasing the constituents of a skin-derived

body soil deposition from textile. Preferably, as mentioned above, the skin-derived body soil deposition is a sebum derived or sebum containing stain (deposition).

5 The methods according to the invention may be carried out at a temperature between 0 and 70 degrees Celsius, preferably between 5 and 60 degrees Celsius, more preferably between 5 and 50 degrees Celsius, even more preferably between 5 and 40 degrees Celsius, even more preferably between 5 and 35 degrees Celsius, most preferably between 5 and 30 degrees Celsius, and in particular between 10 and 30 degrees Celsius.

10 The methods of the invention may employ a treatment time of from 5 minutes to 120 minutes, preferably from 5 minutes to 90 minutes, more preferably from 5 minutes to 60 minutes, more preferably from 5 minutes to 45 minutes, more preferably from 5 minutes to 30 minutes, most preferably from 5 minutes to 20 minutes, and in particular from 5 minutes to 15 minutes.

15 The oxidoreductase enzymes for use in the methods and compositions of the invention may be formulated by well-known methods in the art. Generally, the enzymes could be considered a (detergent) additive, as described above, and formulated as such.

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

The invention is further summarized in the below paragraphs:

20 1. A method for removing or releasing the constituents of a skin-derived body soil deposition from textile, comprising contacting the body soil deposition with an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11.

25 2. The method of paragraph 1, wherein the skin-derived body soil is sebum, preferably aged or oxidized sebum.

30 3. The method of paragraph 2, wherein the constituents of the sebum include squalene cross linked through oxygen-bridging to sebum lipids, or proteins/peptides/carbohydrates derived from skin or textile.

4. The method of any of paragraphs 1-3, wherein the Lab color vector of the deposition is changed by at least 3 units.

35 5. The method of any of paragraphs 1-4, wherein the yellow color of the deposition is reduced, preferably the b-value of the CIE Lab color of the deposition is reduced, more preferably the b-value is reduced at least 1, 2, or 3 units.

6. The method of paragraph 5, wherein the L-value of the Lab color of the deposition is increased.
- 5 7. The method of any of paragraphs 1-6, wherein the textile is made of cotton fabric.
8. The method of any of paragraphs 1-7, wherein the textile is a white garment in direct skin contact, such as a cuff, collar, under garment, or a pillow case.
- 10 9. The method of any of paragraphs 1-8, wherein the enzyme is selected from the group consisting of peroxygenase, laccase, peroxidase and lipxygenase.
10. The method of any of paragraphs 1-8, wherein the enzyme is a peroxygenase.
- 15 11. The method of any of paragraphs 1-10, wherein the pH is 7 to 10, preferably 7 to 9.
12. The method of any of paragraphs 1-11, wherein the temperature is 10-40°C, preferably 15-30°C
- 20 13. An aqueous detergent composition, comprising water, a surfactant; an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11; and a white textile item with a sebum derived or sebum containing stain.
14. Use of an oxidoreductase enzyme from EC 1.10.3, EC 1.11.1, EC 1.11.2, or EC 1.13.11 for
25 removing or releasing the constituents of a skin-derived body soil deposition from textile.
15. The use according to paragraph 14, wherein the skin-derived body soil deposition is a sebum derived or sebum containing stain.
- 30 The invention is also summarized in the following paragraphs:
1. A detergent composition comprising a surfactant and an oxidoreductase enzyme, which oxidoreductase enzyme is selected from the group consisting of:
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a
35 similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5)

- b. a lipoxxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxxygenase activity;
 - c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.
2. A detergent composition according to paragraph 1, wherein the composition further comprises: hydrotropes, builders, co-builders, a bleaching systems, polymers, fabric hueing agents, mediators and/or enzymes.
3. A detergent composition according to any of the preceding paragraphs, wherein the composition comprises a mediator.
4. A detergent composition according to any of the preceding paragraphs, wherein the mediator is selected from the group consisting of: alkyl syringates, N-hydroxy types of mediators and hydroxyl benzotiazole.
5. A detergent composition according to any of the preceding paragraphs, wherein the composition comprises
 - a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5); and
 - b. a mediator.
6. A detergent composition according to any of the preceding paragraphs, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.
7. A detergent composition according to paragraphs 1 and 5-6, wherein the laccase enzyme comprises SEQ ID NO: 1.
8. A detergent composition according to any of paragraphs 1-4, wherein the composition comprises
 - a. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - b. a mediator.
9. A detergent composition according to any of paragraphs 1-4 and 8, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

10. A detergent composition according to any of paragraphs 1-4 and 8-9, wherein the peroxidase comprises SEQ ID NO: 6.
11. A detergent composition according to any of paragraphs 1-4, wherein the composition comprises
- 5 a. a peroxygenase comprised by the enzyme classification EC 1.11.2.1; and
- b. a mediator.
12. A detergent composition according to any of paragraphs 1-4 and 11, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.
- 10 13. A detergent composition according to any of paragraphs 1-4 and 11-12, wherein wherein the peroxidase comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 12 or SEQ ID NO: 24.
14. A detergent composition according to any of the preceding paragraphs, wherein the concentration of the oxidoreductase enzyme is in the range of 0,001-5 mg enzyme per
- 15 gram detergent composition, in the range of 0,005-3 mg enzyme per gram detergent composition, in the range of 0,008-2 mg enzyme per gram detergent composition, in the range of 0,01-0,5 mg enzyme per gram detergent composition or in the range of 0,02-0,3 mg enzyme per gram detergent composition.
15. A detergent composition according to any of the preceding paragraphs, wherein the
- 20 concentration of the mediator is in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, in the range of from 0.05 mM to 100 mM or in the range of from 0.1 mM to 50 mM.
16. The detergent composition according to any of the preceding paragraphs, wherein the detergent is a bar, a homogenous tablet, a tablet having two or more layers, a pouch
- 25 having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.
17. A method for removing or releasing the constituents of a skin-derived body soil deposition from a textile having a skin-derived body soil deposition, which method comprises exposing the textile to an aqueous solution of an oxidoreductase enzyme,
- 30 which oxidoreductase enzyme is selected from the group consisting of:
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5)
- 35 b. a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity; and

- c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
- d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.

- 5 18. A method according to paragraph 17, wherein the skin-derived body soil is sebum, preferably aged or oxidized sebum.
19. A method according to any of the preceding method paragraphs, wherein the constituents of the sebum include squalene cross linked through oxygen-bridging to sebum lipids, or proteins/peptides/carbohydrates derived from skin or textile.
- 10 20. A method according to any of the preceding method paragraphs, wherein Lab color vector (ΔL) of the deposition is changed by at least 0.5 units when using mini-LOM assay.
21. A method according to any of the preceding method paragraphs, wherein Lab color vector (ΔL) of the deposition is changed by at least 0.7 units, by at least 0.9 units, by at least 1.1 units, by at least 1.3 units, by at least 1.5 units, by at least 2.0 units, by at least 2.5 units, by at least 3.0 units, by at least 3.5 units, by at least 4.0 units or by at least 5.0 units.
- 15 22. A method according to any of the preceding method paragraphs, wherein the yellow color of the deposition is reduced.
23. A method according to any of the preceding method paragraphs, wherein the b-value of the CIE Lab color of the deposition is reduced when using the mini-LOM assay.
- 20 24. A method according to any of the preceding method paragraphs, wherein Δb is at least -1
25. A method according to any of the preceding method paragraphs, wherein Δb is at least -1.5, at least -2.0, at least -2.5, at least -3.0, at least -3.5, at least -4.0, at least -4.5 or at least -5.0.
- 25 26. A method according to any of the preceding method paragraphs, wherein ΔREM is at least 1 when using the mini-LOM assay.
27. A method according to any of the preceding method paragraphs, wherein ΔREM is at least 1.5, ΔREM is at least 2.0, ΔREM is at least 2.5, ΔREM is at least 3.0, ΔREM is at least 3.5, ΔREM is at least 4.0, ΔREM is at least 4.5, ΔREM is at least 5.0, ΔREM is at least 5.5, ΔREM is at least 6.0, ΔREM is at least 6.5, ΔREM is at least 7.0, ΔREM is at least 7.5, ΔREM is at least 8.0 or ΔREM is at least 8.5.
- 30 28. A method according to any of the preceding method paragraphs, wherein the textile is made of cotton fabric.

29. A method according to any of the preceding method paragraphs, wherein the textile is a white garment in direct skin contact, such as a cuff, collar, under garment, or a pillow case.
30. A method according to any of the preceding method paragraphs, wherein the pH is 7 to 10, preferably 7 to 9.
31. A method according to any of the preceding method paragraphs, wherein the temperature is 10-40°C, preferably 15-30°C.
32. A method according to any of the preceding method paragraphs, wherein the textile is further exposed to a mediator.
33. A method according to any of the preceding method paragraphs, wherein the mediator is selected from the group consisting of: alkyl syringates, N-hydroxy types of mediators and hydroxyl benzotiazole.
34. A method according to any of the preceding method paragraphs, wherein the textile is exposed to
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5); and
 - b. a mediator.
35. A method according to any of the preceding method paragraphs, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.
36. A method composition according to any of the preceding method paragraphs, wherein the laccase enzyme comprises SEQ ID NO: 1.
37. A method according to any of paragraphs 17-33, wherein the textile is exposed to
- a. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - b. a mediator.
38. A method according to any of paragraphs 17-33 and 37, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.
39. A method composition according to any of paragraphs 17-33 and 37-38, wherein the peroxidase comprises SEQ ID NO: 6.
40. A method according to any of paragraphs 17-33, wherein the textile is exposed to
- a. a peroxygenase comprised by the enzyme classification EC 1.11.2.1; and

b. a mediator.

41. A method according to any of paragraphs 17-33 and 40, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

42. A method according to any of paragraphs 17-33 and 40-41, wherein wherein the peroxidase comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 12 or SEQ ID NO: 24.

43. A method according to any of the preceding method paragraphs, wherein the concentration of the oxidoreductase enzyme is in the range of 0,001-5 mg enzyme per gram detergent composition, in the range of 0,005-3 mg enzyme per gram detergent composition, in the range of 0,008-2 mg enzyme per gram detergent composition, in the range of 0,01-0,5 mg enzyme per gram detergent composition or in the range of 0,02-0,3 mg enzyme per gram detergent composition.

44. A method according to any of the preceding method paragraphs, wherein the concentration of the oxidoreductase enzyme is 2 mg/l.

45. A method according to any of the preceding method paragraphs, wherein the concentration of the mediator is in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, in the range of from 0.05 mM to 100 mM or in the range of from 0.1 mM to 50 mM.

46. A method according to any of the preceding method paragraphs, wherein the concentration of the mediator is 0.1 mM.

47. Use of an oxidoreductase enzyme for removing or releasing the constituents of a skin-derived body soil deposition from a textile having a skin-derived body soil deposition, wherein the oxidoreductase enzyme is selected from the group consisting of:

a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5)

b. a lipxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipxygenase activity;

c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and

d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.

48. Use according to paragraph 47, wherein the skin-derived body soil is sebum, preferably aged or oxidized sebum.

49. Use according to any of the preceding use paragraphs, wherein the constituents of the sebum include squalene cross linked through oxygen-bridging to sebum lipids, or proteins/peptides/carbohydrates derived from skin or textile.
50. Use according to any of the preceding use paragraphs, wherein Lab color vector (ΔL) of the deposition is changed by at least 0.5 units when using the mini-LOM assay.
51. Use according to any of the preceding use paragraphs, wherein Lab color vector (ΔL) of the deposition is changed by at least 0.7 units, by at least 0.9 units, by at least 1.1 units, by at least 1.3 units, by at least 1.5 units, by at least 2.0 units, by at least 2.5 units, by at least 3.0 units, by at least 3.5 units, by at least 4.0 units or by at least 5.0 units.
52. Use according to any of the preceding use paragraphs, wherein the yellow color of the deposition is reduced.
53. Use according to any of the preceding use paragraphs, wherein the b-value of the CIE Lab color of the deposition is reduced.
54. Use according to any of the preceding use paragraphs, wherein Δb is at least -1 when using the mini-LOM assay.
55. Use according to any of the preceding use paragraphs, wherein Δb is at least -1.5, at least -2.0, at least -2.5, at least -3.0, at least -3.5, at least -4.0, at least -4.5 or at least -5.0.
56. Use according to any of the preceding use paragraphs, wherein ΔREM is at least 1 when using the mini-LOM assay.
57. Use according to any of the preceding use paragraphs, wherein ΔREM is at least 1.5, ΔREM is at least 2.0, ΔREM is at least 2.5, ΔREM is at least 3.0, ΔREM is at least 3.5, ΔREM is at least 4.0, ΔREM is at least 4.5, ΔREM is at least 5.0, ΔREM is at least 5.5, ΔREM is at least 6.0, ΔREM is at least 6.5, ΔREM is at least 7.0, ΔREM is at least 7.5, ΔREM is at least 8.0 or ΔREM is at least 8.5.
58. Use according to any of the preceding use paragraphs, wherein the textile is made of cotton fabric.
59. Use according to any of the preceding use paragraphs, wherein the textile is a white garment in direct skin contact, such as a cuff, collar, under garment, or a pillow case.
60. Use according to any of the preceding use paragraphs, wherein the pH is 7 to 10, preferably 7 to 9.
61. Use according to any of the preceding use paragraphs, wherein the temperature is 10-40°C, preferably 15-30°C.
62. Use according to any of the preceding use paragraphs, wherein the textile is further exposed to a mediator.

63. Use according to any of the preceding use paragraphs, wherein the mediator is selected from the group consisting of: selected from the group consisting of: alkyl syringates, N-hydroxy types of mediators and hydroxyl benzotiazole.

64. Use according to any of the preceding use paragraphs, wherein the textile is exposed to

- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5); and
- b. a mediator.

65. Use according to any of the preceding use paragraphs, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

66. Use composition according to any of the preceding use paragraphs, wherein the laccase enzyme comprises SEQ ID NO: 1.

67. Use according to any of paragraphs 47-63, wherein the textile is exposed to

- a. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
- b. a mediator.

68. Use according to any of paragraphs 47-63 and 67, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

69. Use according to any of paragraphs 47-63 and 67-68, wherein the peroxidase comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 12 or SEQ ID NO: 24.

70. Use according to any of paragraphs 47-63, wherein the textile is exposed to

- a. a peroxygenase comprised by the enzyme classification EC 1.11.2.1; and
- b. a mediator.

71. Use according to any of paragraphs 47-63 and 70, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

72. Use according to any of paragraphs 47-63 and 70-71, wherein wherein the peroxidase comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 12 or SEQ ID NO: 24.

73. Use according to any of the preceding use paragraphs, wherein the concentration of the oxidoreductase enzyme is in the range of 0,001-5 mg enzyme per gram detergent composition, in the range of 0,005-3 mg enzyme per gram detergent composition, in the range of 0,008-2 mg enzyme per gram detergent composition, in the range of 0,01-0,5 mg enzyme per gram detergent composition or in the range of 0,02-0,3 mg enzyme per gram detergent composition.

74. Use according to any of the preceding use paragraphs, wherein the concentration of the oxidoreductase enzyme is 2 mg/l.
75. Use according to any of the preceding use paragraphs, wherein the concentration of the mediator is in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, in the range of from 0.05 mM to 100 mM or in the range of from 0.1 mM to 50 mM.
76. Use according to any of the preceding use paragraphs, wherein the concentration of the mediator is 0.1 mM.
77. An aqueous detergent composition, comprising water, a surfactant; an oxidoreductase enzyme, and a white textile item with a sebum derived or sebum containing stain, where said oxidoreductase enzyme is selected from the group consisting of:
- a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5);
 - a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity;
 - a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - a peroxygenase comprised by the enzyme classification EC 1.11.2.1.

EXAMPLES

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

The enzymes used in the following Examples have the amino acid sequences shown as:

Peroxidase: SEQ ID NO: 6,

Laccase: SEQ ID NO: 1,

Peroxygenase 1: SEQ ID NO: 11,

Peroxygenase 2: SEQ ID NO: 17,

Peroxygenase 3: SEQ ID NO: 12, and

Peroxygenase 4: SEQ ID NO: 24.

EXAMPLE 1

Chi and Obendorf, "Aging of oily soils on textile materials: A literature review", *Journal of Surfactants & Detergents* (1998), 1, p. 407, proposes that the yellowing phenomenon is linked

to the oxidation of residual unsaturated fatty residues and squalene found in sebum. Thus, we have selected squalene, a triterpene precursor to cholesterol found in w/w concentration of approximate 12-20% in sebum as one model compound for the study of yellowing on textile. To represent a simple fatty acid present in sebum, we have chosen linoleic acid. Free fatty acids

5 comprise 30% w/w of sebum, and of these, linoleic acid is the only one with two unsaturated carbon bonds. Investigations were carried out using squalene, linoleic acid and as an alternative monounsaturated free fatty acid, oleic acid.

Effect of bleach system on yellowing

10 The following mini-laundrometer (mini-LOM) wash was carried out to illustrate the current state of the art.

Real items were collected from a consumer study. Pillow cases which had become yellow after use over time were used instead of the model compounds to illustrate the effects of current technology on real life yellowed items. During wash the items were exposed to the detergent

15 solution below including a commercial TAED based bleaching system, dosed at 5 times the normal concentration.

Simple bleach system: In another wash, yellowed items were exposed to the detergent solution below including a simplified bleaching system dosed at 5 times the normal concentration (50 mM hydrogen peroxide and 10 mM peracetic acid) containing only the active

20 ingredients of the above commercial bleaching system was also introduced.

Detergent with no bleach system: The wash liquid (aqueous detergent solution) was prepared by adding 3.33 g/l in water of a concentrated detergent composition containing 7.2% LAS, 6.6% AEO Biosoft N25-7 (NI), 4.2% AEOS (SLES), 6% MPG, 3% ethanol, 3% TEA, 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 1.2% sodium hydroxide, 2% sodium citrate,

25 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (all percentages are w/w). Controlled water hardness was added to achieve 15°dH and the pH is maintained using 500 mM HEPES buffer, pH 7.5.

Wash Liquid 40 mL, Temperature 30°C, Water hardness 15°dH 4:1:7.5, Wash time 20 min. Each beaker contained three real-life item (cotton) swatches of 2 cm in diameter.

30 Table 1. Wash effects of current bleaching technologies evaluated at typical liquid detergent pH. Delta values are based on subtracting the wash effects of the detergent alone.

Bleach system	pH	REM460 After wash	Delta REM460*
None	7.1	66.3	0.0

Simple	6.8	67.0	0.7
Commercial TAED	7.5	67.3	1.3

Even at five times the dose of both a commercial bleach system and a simplified model bleach system, there are no significant wash effects of the bleach system, compared to the detergent alone.

5

Enzymatic effects on yellowing

In order to demonstrate the enzymatic effects, the following wash was performed.

Wash Liquid 40 mL, Temperature 30°C, Water hardness 15°dH 4:1:7.5 (Ca:Mg:HCO₃), Wash time 20 min. Each beaker contained three cotton swatches of 2 cm in diameter, and enzyme
10 according to Table 2, and 5 metal balls of 6 mm diameter.

Initial washes were performed in 500 mM HEPES buffer pH 7.5. The swatches were then removed from each beaker, rinsed under running water, and a “rinse” wash in fresh wash liquid (detergent solution) was performed. This was conducted by replacing the swatches in the individual beakers and adding wash liquid as described above with water hardness 15°dH, and
15 washing for a further 20 min.

Aged squalene swatches were prepared in a manner adapted from Spangler *et al.*, “A detergency test based on rapid ageing of unremoved sebum”, *J. American oil chemists' society* (1967), vol 44, pp. 728-732.

20 Table 2. Wash effects of oxidoreductase enzymes on aged squalene swatches. Delta values are based on subtracting the wash effects of the detergent alone.

Treatment	Enzyme	REM460 after wash	Delta REM460	b	Delta b
Blank	None	48.3	0	24.9	0.0
10 mg/l enzyme	Peroxidase	53.6	5.3	21.6	-3.3
	Peroxygenase 3	50.1	1.9	23.6	-1.3
	Peroxygenase 1	52.2	3.9	22.4	-2.5
	Peroxygenase 2	53.0	4.7	22.0	-2.9
	Peroxygenase 4	54.6	6.3	21.0	-3.9
	Laccase	51.1	2.9	23.0	-1.9
2 mg/l enzyme +	Peroxidase	52.1	3.8	22.6	-2.4

0.1 mM violuric acid	Peroxygenase 3	50.4	2.1	23.7	-1.3
	Peroxygenase 1	53.9	5.6	21.4	-3.5
	Peroxygenase 2	54.5	6.2	20.9	-4.0
	Peroxygenase 4	50.3	2.0	23.7	-1.2
	Laccase	55.1	6.8	20.8	-4.2
2 mg/l enzyme + 0.1 mM methyl syringate	Peroxidase	54.6	6.3	21.0	-3.9
	Peroxygenase 3	54.8	6.5	20.9	-4.0
	Peroxygenase 1	50.7	2.4	23.5	-1.5
	Peroxygenase 2	54.9	6.6	20.8	-4.1
	Peroxygenase 4	54.7	6.4	20.9	-4.0
	Laccase	54.1	5.8	21.4	-3.5
2 mg/l enzyme + 0.1 mM hydroxyl benzoate	Peroxidase	55.5	7.2	20.5	-4.4
	Peroxygenase 3	51.5	3.2	23.0	-1.9
	Peroxygenase 1	53.0	4.7	22.1	-2.8
	Peroxygenase 2	56.7	8.4	19.9	-5.0
	Peroxygenase 4	55.3	7.0	20.8	-4.1
	Laccase	54.2	6.0	21.3	-3.6

The data in Table 2 shows impressive enzymatic effects, with an increase of remission (Δ REM) at 460 nm of up to more than 8 units. The b value of the CIE Lab measurements is reduced up to 5 units, showing a decreased yellow color of the swatches.

5

Table 3. All L, a, values

Treatment	Enzyme	L after wash	Delta L	a after wash	Delta a
Blank	None	88.2	0.0	-0.1	0.0
10 mg/l enzyme	Peroxidase	89.4	1.3	-0.5	-0.4
	Peroxygenase 3	88.6	0.4	0.0	0.1
	Peroxygenase 1	89.1	0.9	-0.4	-0.3
	Peroxygenase 2	89.4	1.2	-0.2	-0.1
	Peroxygenase 4	89.7	1.6	-0.6	-0.5
	Laccase	88.8	0.7	0.0	0.1
2 mg/l enzyme +	Peroxidase	89.1	1.0	-0.2	-0.1

0.1 mM violuric acid	Peroxygenase 3	88.7	0.6	-0.1	0.0
	Peroxygenase 1	89.5	1.4	-0.5	-0.4
	Peroxygenase 2	89.6	1.4	-0.6	-0.4
	Peroxygenase 4	88.7	0.5	-0.1	0.1
	Laccase	89.8	1.7	-0.7	-0.6
2 mg/l enzyme + 0.1 mM methyl syringate	Peroxidase	89.7	1.5	-0.7	-0.6
	Peroxygenase 3	89.7	1.6	-0.8	-0.7
	Peroxygenase 1	88.8	0.6	-0.2	-0.1
	Peroxygenase 2	89.7	1.6	-0.7	-0.6
	Peroxygenase 4	89.7	1.5	-0.8	-0.7
	Laccase	89.6	1.5	-0.5	-0.4
2 mg/l enzyme + 0.1 mM hydroxyl benzoate	Peroxidase	90.0	1.8	-0.7	-0.6
	Peroxygenase 3	89.1	0.9	-0.1	0.0
	Peroxygenase 1	89.4	1.3	-0.4	-0.3
	Peroxygenase 2	90.3	2.1	-0.8	-0.7
	Peroxygenase 4	90.0	1.8	-0.7	-0.6
	Laccase	89.7	1.5	-0.6	-0.4

Descriptions of yellowing in the literature

a) Effect of fiber substrates on appearance and removal of aged oily soils. Yong-Seung Chi and S. Kay Obendorf, J. Surfact. Deterg. Vol 4, No 1 p. 35-41.

5

b) Ageing of oily soils on textile materials: chemical changes upon oxidation and interaction of aged oils with textile fibers. J. Surfact. Deterg. Vol. 1, No. 3, 371-380 (1998).

c) A detergency test based on rapid ageing of unremoved sebum. W.G Spangler, R.C. Roga and H.D. Cross III, J. American oil chemists' society, vol 44, 728-732 (1967).

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d) Ageing of oily soils on textile materials: A literature review. Yong-Seung Chi and S. Kay Obendorf, J. Surfact. Deterg. Vol. 1, No. 3, 407-418 (1998).

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CLAIMS

1. A method for removing or releasing the constituents of a skin-derived body soil deposition from a textile having a skin-derived body soil deposition, which method
5 comprises exposing the textile to an aqueous solution of an oxidoreductase enzyme, which oxidoreductase enzyme is selected from the group consisting of:
 - a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol
10 oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5);
 - b. a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity; and
 - c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting
15 peroxidase activity; and
 - d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.
2. A method according to claim 1, wherein the skin-derived body soil is sebum, preferably aged or oxidized sebum.
3. A method according to any of the preceding claims, wherein Lab color vector (ΔL) of the
20 deposition is changed by at least 0.5 units when using mini-LOM assay.
4. A method according to any of the preceding claims, wherein the yellow color of the deposition is reduced.
5. A method according to any of the preceding method claims, wherein Δb is at least -1
6. A method according to any of the preceding method claims, wherein the pH is 7 to 10,
25 preferably 7 to 9.
7. A method according to any of the preceding claims, wherein the temperature is 10-40°C, preferably 15-30°C.
8. A method according to any of the preceding claims, wherein the textile is further exposed to a mediator.
- 30 9. A method according to any of the preceding claims, wherein the mediator is selected from the group consisting of: alkyl syringates, N-hydroxy types of mediators and hydroxyl benzotiazole.
10. A method according to any of claims 1-8, wherein the mediator is selected from the group consisting of violuric acid, methyl syringate and hydroxyl benzoate.

11. Use of an oxidoreductase enzyme for removing or releasing the constituents of a skin-derived body soil deposition from a textile having a skin-derived body soil deposition, wherein the oxidoreductase enzyme is selected from the group consisting of:
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5);
 - b. a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity;
 - c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.
12. Use according to claim 11, wherein the skin-derived body soil is sebum, preferably aged or oxidized sebum.
13. Use according to any of claims 11-12, wherein the textile is further exposed to a mediator.
14. An aqueous detergent composition, comprising water, a surfactant; an oxidoreductase enzyme, and a white textile item with a sebum derived or sebum containing stain, where said oxidoreductase enzyme is selected from the group consisting of:
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5);
 - b. a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity;
 - c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
 - d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.
15. A detergent composition comprising a surfactant and an oxidoreductase enzyme, which oxidoreductase enzyme is selected from the group consisting of:
- a. a laccase enzyme comprised by the enzyme classification EC 1.10.3.2 or any fragment derived therefrom exhibiting laccase activity or a compound exhibiting a similar activity such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5);

5

- b. a lipoxygenase, classified as EC 1.13.11.12, or any fragment thereof exhibiting lipoxygenase activity;
- c. a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, EC 1.11.1.14 or EC 2.11.1.16, or any fragment derived therefrom, exhibiting peroxidase activity; and
- d. a peroxygenase comprised by the enzyme classification EC 1.11.2.1.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/076369

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C11D3/386
 ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, Sequence Search

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Further documents are listed in the continuation of Box C.



See patent family annex.

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

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"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

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"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

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/076369

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International application No

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