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(54) **Title**: ENZYMATIC TEXTILE BLEACH-WHITENING METHODS

(57) **Abstract**: A method for bleach-whitening of synthetic textile material is provided comprising contacting said textile material with (a) an enzymatic textile bleaching composition comprising (i) a perhydrolase enzyme, (ii) an ester substrate for said perhydrolase enzyme, (iii) a hydrogen peroxide source, (iv) a surfactant and/or an emulsifier, (v) a peroxide stabilizer, (vi) a sequestering agent, (vii) a buffer which maintains a pH of about 6 to about 8, and (b) at least one fluorescent whitening agent, and, optionally, (c) at least one acid or disperse shading dye for shaded variations of white, for a length of time and under conditions suitable to permit measurable whitening of the textile material, thereby producing a bleached-whitened textile material.

Enzymatic textile bleach-whitening methods

The present invention relates to methods for the enzymatic bleach-whitening of synthetic textile material, in particular polyamide or polyamide-containing textile materials.

In the processing of textile materials, such as fibers, yarns or fabrics, a pretreatment or preparation step is typically required to properly prepare the textile materials for further use and in particular for the dyeing, printing and/or finishing stages typically required for commercial goods. These textile treatment steps remove impurities and color bodies, either naturally existing or those added by the spinning and weaving steps to the fibers and/or fabrics.

While textile treatments may include a number of varying treatments and stages, the most common include: de-sizing, the removal of sizing agents, such as starches, via enzymatic, alkali or oxidative soaking; scouring, the removal of greases, oils, waxes, pectic substances, protein and fats by contact with a solution of sodium hydroxide at temperatures near boiling; and bleaching, the removal and lightening of color bodies from textiles by commonly using oxidizing agents (such as hydrogen peroxide, hypochlorite, and chlorine dioxide), or by using reducing agents (such as, sulfur dioxide or dithionite salts).

Currently employed bleach-whitening processes of polyamide fibers are usually carried out in the presence of reducing agents like stabilized (buffered) sodium dithionite. Known disadvantages of these processes are related to ecology (odor, waste water etc.) and yellowing problems encountered in subsequent drying when the residual sulfur derivatives are not properly and thoroughly removed from the textile substrate.

An enzymatic bleach-whitening process which does not show the above indicated disadvantages and constitutes a new benchmark, as the white effects achieved are clearly improved in comparison with the whitening obtained in reduction bleach, would be desirable.

There is a need for an effective enzymatic textile bleach-whitening process which minimizes the adverse environmental impact and provides fabrics without yellowing problems when compared to conventional textile bleach-whitening processes.

It has been found that the problems indicated are largely solved by the enzymatic bleach-whitening method defined herein below.

The present invention accordingly relates to a method for bleach-whitening of synthetic textile material, in particular polyamide or polyamide-containing textile material, comprising contacting said textile material with
(a) an enzymatic textile bleaching composition comprising
(i) a perhydrolase enzyme,
(ii) an ester substrate for said perhydrolase enzyme,
(iii) a hydrogen peroxide source,
(iv) a surfactant and/or an emulsifier,
(v) a peroxide stabilizer,
(vi) a sequestering agent,
(vii) a buffer which maintains a pH of about 6 to about 8, and
(b) at least one fluorescent whitening agent, and, optionally,
(c) at least one acid or disperse shading dye for shaded variations of white,
for a length of time and under conditions suitable to permit measurable whitening of the textile material, thereby producing a bleached-whitened textile material.

The subject matter according to the present invention provides methods for bleaching and whitening of synthetic textile material, in particular polyamide or polyamide-containing textile materials, which are contacted with an enzymatic bleaching composition and a fluorescent whitening agent for a length of time and under conditions enabling a full white effect to be achieved. In another aspect, the invention is also suitable for the brightening of pastel shades.

The invention also provides dyed textiles produced from textiles that have been prepared according to the enzymatic bleaching-whitening methods described herein.

The enzymatic bleach-whitening process according to the present invention is environmentally friendly and provides synthetic textile material which does not exhibit yellowing problems encountered in subsequent drying when the residual sulfur derivatives are not properly and thoroughly removed as compared to the whitening obtained in conventional reduction bleach.

The enzymatic bleaching step of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, for example, *Molecular Cloning: A Laboratory Manual*, 2nd ed., (Sambrook et al., 1989); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Current Protocols in Molecular Biology* (F.M. Ausubel et al., eds., 1994); *PCR: The Polymerase Chain Reaction* (Mullis et al., eds., 1994); and *Gene Transfer and Expression: A Laboratory Manual* (Kriegler, 1990).

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

Singleton, et al., *Dictionary of Microbiology and Molecular Biology*, 2nd ed., John Wiley and Sons, New York (1994), and Hale & Markham, *The Harper Collins Dictionary of Biology*, Harper Perennial, New York (1991) provide one of skill in the art with a general dictionary of many of the biotechnology related terms used in this invention. Any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

Numeric ranges provided herein are inclusive of the numbers defining the range.
Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

The term “bleaching,” as used herein, means the process of treating a textile material for a sufficient length of time and under appropriate pH and temperature conditions to produce a lighter color in said textile material by removal, modification or masking of color-causing compounds in the textile material. Thus, “bleaching” refers to the treatment of a textile material to effect a brightening of the textile material.

The term “bleaching agent” as used herein encompasses any moiety that is capable of bleaching a textile. They may require the presence of a bleach activator. Bleaching may generally be performed using chemical bleaching agent(s) and/or enzymatically generated bleaching agent(s). Examples of suitable chemical bleaching agents useful in the processes

and methods described herein are sodium peroxide, sodium perborate, potassium permanganate, and peracids. In some aspects, H_2O_2 may be considered a chemical bleaching agent when it has been generated enzymatically in situ. A "chemical bleaching composition" contains one or more chemical bleaching agent(s).

An "enzymatic bleaching system" or "enzymatic bleaching composition" as understood in the context of the present invention contains one or more perhydrolase enzymes and one or more ester substrates capable of enzymatically generating a bleaching agent, and a hydrogen peroxide source, for production of a peracid bleaching agent. Such enzymatic bleaching systems are described, for example, in WO 2005/056782.

The term "synthetic textile material" comprises polyamides, polyurethanes, polyacrylics, polyesters, polyolefines, polylactide, and semi-synthetic material such as cellulose acetate.

The cited textile materials can be in a very wide range of forms of presentations, for example, in the form of fibers, yarns, fabrics, garments, knits, wovens and non-wovens.

The term "polyamide or polyamide-containing textile materials" comprises synthetic polyamide, for example, Nylon 6 (polycaproamide), Nylon 6,6 (polyhexamethylene adipamide), Nylon 7 (polyenanthamide), Nylon 6,12 (polyhexamethylene dodecamide), Nylon 11, Nylon 12 and copolyamides of Nylon 6,6 or Nylon 6, such as polymers made from hexamethylene diamine, ε-caprolactam and adipic acid and polymers prepared from adipic acid, hexamethylene diamine and isophthalic acid, or from adipic acid, hexamehylene diamine and 2-methyl-pentamethylene diamine or 2-ethyl-tetramethylene diamine. It also encompasses blends, for example, blends of polyamide/wool, polyamide/polyacrylonitril, polyamide/cellulose, polyamide/polyester, polyamide/cellulose acetate, polyamide/cellulose triacetate, polyamide/cellulose/wool and all blends indicated above blended with elastane fibers.

A "perhydrolase" refers to an enzyme that is capable of catalyzing a perhydrolysis reaction that results in the production of a sufficiently high amount of peracid suitable for use in an enzymatic textile bleaching composition according to the method described herein. Generally, a perhydrolase enzyme used in the methods described herein exhibits a high perhydrolysis to hydrolysis ratio. In some embodiments, the perhydrolase comprises, consists of, or consists essentially of the Mycobacterium smegmatis perhydrolase amino acid sequence set forth in

SEQ ID NO:1, or a variant or homolog thereof. In some embodiments, the perhydrolase enzyme comprises acyl transferase activity and catalyzes an aqueous acyl transfer reaction.

A “peracid” is an organic acid of the formula RC(=O)OOH, wherein R is an aliphatic, aromatic or araliphatic radical.

An “ester substrate” in reference to an enzymatic textile bleaching composition according to the invention described herein refers to a perhydrolase substrate that contains an ester linkage. Esters comprising aliphatic and/or aromatic carboxylic acids and alcohols may be utilized as substrates with perhydrolase enzymes. In some embodiments, the ester source is selected from the esters of one or more of the following acids: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. In some embodiments, the ester source is an acetate ester. In some embodiments, the ester source is selected from one or more of propylene glycol diacetate, ethylene glycol diacetate, glycerol triacetate, ethyl acetate, and glycerol tributyrate.

The term “perhydrolyzation” or “perhydrolyze” or “perhydrolysis” as used herein refer to a reaction wherein a peracid is generated from an ester substrate and a hydrogen peroxide source. The perhydrolyzation reaction is catalyzed with a perhydrolase, e.g., acyl transferase or aryl esterase, enzyme. In some embodiments, a peracid is produced by perhydrolysis of an ester substrate of the formula $RC(=O)OR^*$, where R and R^* are the same or different organic moieties, in the presence of hydrogen peroxide (H_2O_2). In one embodiment, $-OR^*$ is -OH. In one embodiment, $-OR^*$ is replaced by $-NH_2$. In some embodiments, a peracid is produced by perhydrolysis of a carboxylic acid or amide substrate.

The term ”peracid,” as used herein, refers to a molecule derived from a carboxylic acid ester which has been reacted with hydrogen peroxide to form a highly reactive product that is able to transfer one of its oxygen atoms. It is this ability to transfer oxygen atoms that enables a peracid, for example, peracetic acid, to function as a bleaching agent.

The phrase “perhydrolysis to hydrolysis ratio” refers to the ratio of the amount of enzymatically produced peracid to the amount of enzymatically produced acid by a perhydrolase enzyme

from an ester substrate under defined conditions and within a defined time. In some embodiments, the assays provided in WO 05/056782 are used to determine the amounts of peracid and acid produced by the enzyme.

As used herein, "effective amount of perhydrolase enzyme" refers to the quantity of perhydrolase enzyme necessary to achieve the enzymatic activity required in the processes or methods described herein. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular enzyme variant used, the pH used, the temperature used and the like, as well as the results desired (e.g., level of bleaching).

As used herein, the term “transferase” refers to an enzyme that catalyzes the transfer of a functional group from one substrate to another substrate. For example, an acyl transferase may transfer an acyl group from an ester substrate to a hydrogen peroxide substrate to form a peracid.

As used herein, the term “acyl” refers to an organic group with the general formula RCO-, derived from an organic acid by removal of the -OH group. Typically, acyl group names end with the suffix “-oyl,” e.g., ethanoyl chloride, CH_3CO-Cl, is the acyl chloride formed from ethanoic acid, CH_3CO-OH.

As used herein, the term “acylation” refers to a chemical transformation in which one of the substituents of a molecule is substituted by an acyl group, or the process of introduction of an acyl group into a molecule.

As used herein, “oxidizing chemical” refers to a chemical that has the capability of bleaching a textile. The oxidizing chemical is present at an amount, pH and temperature suitable for bleaching. The term includes, but is not limited to hydrogen peroxide and peracids.

As used herein, the terms “purified” and “isolated” refer to the removal of contaminants from a sample and/or to a material (e.g., a protein, nucleic acid, cell, etc.) that is removed from at least one component with which it is naturally associated. For example, these terms may refer to a material which is substantially or essentially free from components which normally accompany it as found in its native state, such as, for example, an intact biological system.

As used herein, the term “polynucleotide” refers to a polymeric form of nucleotides of any length and any three-dimensional structure and single- or multi-stranded (e.g., single-stranded, double-stranded, triple-helical, etc.), which contain deoxyribonucleotides, ribonucleotides, and/or analogs or modified forms of deoxyribonucleotides or ribonucleotides, including modified nucleotides or bases or their analogs. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the polynucleotides applied applied within the context of the present invention encode a particular amino acid sequence. Any type of modified nucleotide or nucleotide analog may be used, so long as the polynucleotide retains the desired functionality under conditions of use, including modifications that increase nuclease resistance (e.g., deoxy, 2'-O--Me, phosphorothioates, etc.). Labels may also be incorporated for purposes of detection or capture, for example, radioactive or nonradioactive labels or anchors, e.g., biotin. The term polynucleotide also includes peptide nucleic acids (PNA). Polynucleotides may be naturally occurring or non-naturally occurring. The terms “polynucleotide” and “nucleic acid” and “oligonucleotide” are used herein interchangeably. Polynucleotides of the invention may contain RNA, DNA, or both, and/or modified forms and/or analogs thereof. A sequence of nucleotides may be interrupted by non-nucleotide components. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S (“thioate”), P(S)S (“dithioate”), (O)NR_2 (“amidate”), P(O)R, P(O)OR’, CO or CH_2 (“formacetal”), in which each R or R’ is independently H or substituted or unsubstituted alkyl (C_1-C_{20}) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. Polynucleotides may be linear or circular or comprise a combination of linear and circular portions. Suitable polynucleotides are described in WO 2005/056782.

As used herein, “polypeptide” refers to any composition comprised of amino acids and recognized as a protein by those of skill in the art. The conventional one-letter or three-letter code for amino acid residues is used herein. The terms “polypeptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation

with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

The terms “analogous sequence”, “homologous protein”, “wild-type or native proteins”, “wild-type sequence”, “native sequence“, “naturally-occuring sequence”, “wild-type gene”, “related proteins”, “derivative proteins” and “variant proteins”, as used herein are familiar to those skilled in the art and are described in more detail in WO 2005/056782 on pages 12, 13 and 50 to 52, which are herein incorporated by reference. In some embodiments, homologous proteins are engineered to produce enzymes with desired activity(ies).

Several methods are known in the art that are suitable for generating variants of the enzymes described herein, including but not limited to site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches.

The degree of homology between sequences may be determined using any suitable method known in the art. For example, PILEUP is a useful program to determine sequence homology levels. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. Another example of a useful algorithm is the BLAST algorithm. Useful methods and programs are referred to in WO 2005/056782 on pages 59 and 60, which are herein incorporated by reference.

The terms “substantially similar” and “substantially identical” as generally used in the context of a polynucleotide or polypeptide sequence compared to a reference (i.e., wild-type) sequence as well as methods to determine sequence identity are described in more detail in WO 2005/056782 on pages 61 and 62, which are herein incorporated by reference.

“Surfactant” refers to a substance that reduces surface tension of a liquid.

“Emulsifier” refers to a substance that promotes the suspension of one liquid in another.

"Sequestering agent" refers to a substance capable of reacting with metallic ions by forming a water-soluble complex in which the metal is held in a non-ionizable form.

The terms "size" or "sizing" refer to compounds used in the textile industry to improve weaving performance by increasing the abrasion resistance and strength of the yarn. Size is usually made of, for example, starch or starch-like compounds.

The terms "desize" or "desizing," as used herein, refer to the process of eliminating size, generally starch, from textiles usually prior to applying special finishes, dyes or bleaches.

"Desizing enzyme(s)" as used herein refer to enzymes that are used to enzymatically remove the size. Exemplary enzymes are amylases, cellulases and mannanases.

The term "scouring," as used herein, means to remove impurities, for example, much of the impurities (e.g., pectins, proteins, wax, etc.) naturally found in textile materials. In addition to the natural impurities, scouring can remove, in some embodiments, residual materials introduced by manufacturing processes, such as spinning, coning or slashing lubricants. In some embodiments, bleaching may be employed to remove impurities from textiles.

The term "bioscouring enzyme(s)" refers to an enzyme(s) capable of removing at least a portion of the impurities found in textile materials.

The term "pectinase" denotes a pectinase enzyme defined according to the art where pectinases are a group of enzymes that cleave glycosidic linkages of pectic substances mainly poly(1,4-alpha-D-galacturonide) and its derivatives (see Sakai et al. (1993) Advances in Applied Microbiology 39:213-294). Preferably, a pectinase useful herein is a pectinase enzyme which catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid also called polygalacturonic acid by transelimination, such as the enzyme class polygalacturonate lyase (EC 4.2.2.2) (PGL), also known as poly(1,4-alpha-D-galacturonide) lyase, also known as pectate lyase.

The term "pectic substances" denotes pectate, polygalacturonic acid and pectin which may be esterified to a higher or lower degree.

The term “cutinase,” as used herein, refers to as a plant, bacterial or fungal derived enzyme used in textile processing. Cutinases are lipolytic enzymes capable of hydrolyzing the substrate cutin. Cutinases can breakdown fatty acid esters and other oil-based compositions need to be removed in the processing (e.g., the scouring) of textiles. “Cutinase” means an enzyme that has significant plant cutin hydrolysis activity. Specifically, a cutinase will have hydrolytic activity on the biopolyester polymer cutin found on the leaves of plants. Suitable cutinases may be isolated from many different plant, fungal and bacterial sources.

The term “α-amylase,” as used herein, refers to an enzyme that cleaves the α (1-4)glycosidic linkages of amylose to yield maltose molecules (disaccharides of α-glucose). Amylases are digestive enzymes found in saliva and are also produced by many plants. Amylases break down long-chain carbohydrates (such as starch) into smaller units. An “oxidative stable” α-amylase is an α-amylase that is resistive to degradation by oxidative means, when compared to non-oxidative stable α-amylase, especially when compared to the non-oxidative stable α-amylase form which the oxidative stable α-amylase was derived.

The term “protease” means a protein or polypeptide domain of a protein or polypeptide derived from a microorganism, e.g. a fungus, bacterium, or from a plant or animal, and that has the ability to catalyze cleavage of peptide bonds at one or more of various positions of a protein carbohydrate backbone.

“Catalase” as used herein refers to an enzyme (i.e., a polypeptide having catalytic activity) that catalyzes the decomposition of hydrogen peroxide to water and oxygen.

“Batch process” or “batchwise process” or “discontinuous process” refers to processing of textiles as lots or batches in which the whole of each batch is subjected to a process or one stage of a process at a time.

“Exhaust process” refers to a batch process in which pretreatment chemicals and/or an enzymatic pretreatment composition and dye are added simultaneously or sequentially in a single textile treatment bath.

“Liquor ratio” refers to the ratio of the weight of liquor (liquid) employed in a textile treatment process to the weight of the textile treated.

"A," "an" and "the" include plural references unless the context clearly dictates otherwise.

The enzymatic textile bleach-whitening methods described herein are particularly carried out as a discontinuous process (one-bath two-steps exhaustion method) but can also be carried out as a semi-continuous process like pad-batch or pad-roll.

The enzymatic bleaching composition used in accordance with the enzymatic textile bleach-whitening method of the present invention contain a perhydrolase enzyme, an ester substrate for the perhydrolase enzyme suitable for production of a peracid upon catalytic reaction of the perhydrolase enzyme on the substrate in the presence of a hydrogen peroxide source and/or hydrogen peroxide, a surfactant and/or an emulsifier, a peroxide stabilizer, a sequestering agent, and a buffer which maintains a pH of about 6 to about 8 during a textile bleaching process. The enzymatic bleaching composition may, optionally, further contain a bioscouring agent or enzyme and/or a desizing agent or enzyme.

In the following the components applied in accordance with the method of the present invention along with information on the quantities of these components are described in more detail. Parts (ppm) are parts by weight, unless noted otherwise.

Perhydrolase Enzyme

One or more perhydrolase enzymes may be used in the compositions according to the methods for enzymatic textile bleach-whitening as described herein.

In some embodiments, the perhydrolase enzyme is naturally-occurring (i.e., a perhydrolase enzyme encoded by a genome of a cell). In some embodiments, the perhydrolase enzyme comprises, consists of, or consists essentially of an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% identical to the amino acid sequence of a naturally-occurring perhydrolase enzyme.

In some embodiments, the perhydrolase enzyme is a naturally-occurring M. smegmatis perhydrolase enzyme. In some embodiments, the perhydrolase enzyme comprises, consists of, or consists essentially of the amino acid sequence set forth in SEQ ID NO:1 or a variant or

homologue thereof. In some embodiments, the perhydrolase enzyme comprises, consists of, or consists essentially of an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% identical to the amino acid sequence set forth in SEQ ID NO:1.

The amino acid sequence of M. smegmatis perhydrolase is shown below:
MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLAQQLGADFEVIEEGLSARTTNID
DPTDPRLNGASYLPSCLATHLPLDLVIIMLGTNDTKAYFRRTPLDIALGMSVLVTQVLTSAGG
VGTTYPAPKVLVVSPPPLAPMPHPWFQLIFEGGEQKTTELARVYSALASFMKVPFFDAGSVI
STDGVDGIHFTEANNRDLGVALAEQVRSLL (SEQ ID NO:1).

The corresponding polynucleotide sequence encoding M. smegmatis perhydrolase is:
5'-ATGGCCAAGCGAATTCTGTGTTTCGGTGATTCCCTGACCTGGGGCTGGGTCC
CCGTCGAAGACGGGGCACCCACCGAGCGGTTCGCCCCCGACGTGCGCTGGACCGGTG
TGCTGGCCCAGCAGCTCGGAGCGGACTTCGAGGTGATCGAGGAGGGACTGAGCGCGC
GCACCACCAACATCGACGACCCCACCGATCCGCGGCTCAACGGCGCGAGCTACCTGC
CGTCGTGCCTCGCGACGCACCTGCCGCTCGACCTGGTGATCATCATGCTGGGCACCAA
CGACACCAAGGCCTACTTCCGGCGCACCCCGCTCGACATCGCGCTGGGCATGTCGGT
GCTCGTCACGCAGGTGCTCACCAGCGCGGGCGGCGTCGGCACCACGTACCCGGCACC
CAAGGTGCTGGTGGTCTCGCCGCCACCGCTGGCGCCCATGCCGCACCCCTGGTTCCA
GTTGATCTTCGAGGGCGGCGAGCAGAAGACCACTGAGCTCGCCCGCGTGTACAGCGC
GCTCGCGTCGTTCATGAAGGTGCCGTTCTTCGACGCGGGTTCGGTGATCAGCACCGAC
GGCGTCGACGGAATCCACTTCACCGAGGCCAACAATCGCGATCTCGGGGTGGCCCTC
GCGGAACAGGTGCGGAGCCTGCTGTAA-3' (SEQ ID NO:2).

In some embodiments, the perhydrolase enzyme comprises one or more substitutions at one or more amino acid positions equivalent to position(s) in the M. smegmatis perhydrolase amino acid sequence set forth in SEQ ID NO:1. In some embodiments, the perhydrolase enzyme comprises any one or any combination of substitutions of amino acids selected from M1, K3, R4, I5, L6, C7, D10, S11, L12, T13, W14, W16, G15, V17, P18, V19, D21, G22, A23, P24, T25, E26, R27, F28, A29, P30, D31, V32, R33, W34, T35, G36, L38, Q40, Q41, D45, L42, G43, A44, F46, E47, V48, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, I60, D61, D62, P63, T64, D65, P66, R67, L68, N69, G70, A71, S72, Y73, S76, C77, L78, A79, T80, L82, P83, L84, D85, L86, V87, N94, D95, T96, K97, Y99F100, R101, R102, P104, L105, D106, I107, A108,

L109, G110, M111, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P146, P148, W149, F150, I153, F154, I194, and F196.

In some embodiments, the perhydrolase enzyme comprises one or more of the following substitutions at one or more amino acid positions equivalent to position(s) in the M. smegmatis perhydrolase amino acid sequence set forth in SEQ ID NO:1: L12C, Q, or G; T25S, G, or P; L53H, Q, G, or S; S54V, L A, P, T, or R; A55G or T; R67T, Q, N, G, E, L, or F; K97R; V125S, G, R, A, or P; F154Y; F196G.

In some embodiments, the perhydrolase enzyme is the S54V variant of SEQ ID NO:1.

In some embodiments, the perhydrolase enzyme comprises a combination of amino acid substitutions at amino acid positions equivalent to amino acid positions in the M. smegmatis perhydrolase amino acid sequence set forth in SEQ ID NO:1: L12I S54V; L12M S54T; L12T S54V; L12Q T25S S54V; L53H S54V; S54P V125R; S54V V125G; S54V F196G; S54V K97R V125G; or A55G R67T K97R V125G.

In some embodiments, the perhydrolase enzyme comprises a perhydrolysis to hydrolysis ratio of at least 1. In some embodiments, the perhydrolase enzyme comprises a perhydrolysis to hydrolysis ratio greater than 1.

In some embodiments, the perhydrolase enzyme is provided in the enzymatic textile bleaching composition used according to the textile bleach-whitening method of the present invention at a concentration of about 0.5 to about 2.5 ppm, about 1.5 to about 2.0 ppm, for example, about 1.7 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Ester Substrate

The enzymatic bleaching compositions used in accordance with the method described herein include an ester which serves as a substrate for the perhydrolase enzyme for production of a peracid in the presence of hydrogen peroxide. In some embodiments, the ester substrate is an ester of an aliphatic and/or aromatic carboxylic acid. In some embodiments, the ester substrate is an ester of one or more of the following: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. In some embodiments, glycerol triacetate, glycerol tributyrate, and other esters serve as acyl donors for peracid formation. In some embodiments, the ester substrate is selected from propylene glycol diacetate, ethylene glycol diacetate, glycerol triacetate, ethyl acetate, and glycerol tributyrate. In some embodiments, the ester substrate is propylene glycol diacetate, ethylene glycol diacetate, or ethyl acetate. In one embodiment, the ester substrate is propylene glycol diacetate.

In some embodiments, the ester substrate, for example, propylene glycol diacetate, is provided at a concentration of about 2000 to about 4000 ppm, about 2500 to about 3500 ppm, about 2800 ppm to about 3200 ppm, or about 3000 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Hydrogen Peroxide Source

The enzymatic bleaching compositions used in accordance with the method described herein include a hydrogen peroxide source. Hydrogen peroxide can be either added directly in batch, or generated continuously “in situ” by chemical, electro-chemical, and/or enzymatic means.

In some embodiments, the hydrogen peroxide source is hydrogen peroxide. In some embodiments, the hydrogen peroxide source is a solid compound that generates hydrogen peroxide spontaneously upon addition to water. Such compounds include adducts of hydrogen peroxide with various inorganic or organic compounds, of which the most widely employed is sodium carbonate perhydrate, also referred to as sodium percarbonate.

Inorganic perhydrate salts are one embodiment of hydrogen peroxide source. Examples of inorganic perhydrate salts include perborate, percarbonate, perphosphate, persulfate and persilicate salts. The inorganic perhydrate salts are normally the alkali metal salts.

Other hydrogen peroxide adducts useful in the compositions used in accordance with the method described herein include adducts of hydrogen peroxide with zeolites, or urea hydrogen peroxide.

The hydrogen peroxide source compounds may be included as the crystalline and/or substantially pure solid without additional protection. For certain perhydrate salts however, the preferred executions of such granular compositions utilize a coated form of the material which provides better storage stability for the perhydrate salt in the granular product. Suitable coatings comprise inorganic salts such as alkali metal silicate, carbonate or borate salts or mixtures thereof, or organic materials such as waxes, oils, or fatty soaps.

In some embodiments, the hydrogen peroxide source is an enzymatic hydrogen peroxide generation system. In one embodiment, the enzymatic hydrogen peroxide generation system comprises an oxidase and its substrate. Suitable oxidase enzymes include, but are not limited to: glucose oxidase, sorbitol oxidase, hexose oxidase, choline oxidase, alcohol oxidase, glycerol oxidase, cholesterol oxidase, pyranose oxidase, carboxyalcohol oxidase, L-amino acid oxidase, glycine oxidase, pyruvate oxidase, glutamate oxidase, sarcosine oxidase, lysine oxidase, lactate oxidase, vanillyl oxidase, glycolate oxidase, galactose oxidase, uricase, oxalate oxidase, and xanthine oxidase.

The following equation provides an example of a coupled system for enzymatic production of hydrogen peroxide.

$$\text{Glucose} + H_2O \xrightarrow[\text{Glucose oxidase}]{} \text{gluconic acid} + H_2O_2$$

$$+$$

$$H_2O_2 + \text{ester substrate} \xrightarrow[\text{Perhydrolase}]{} \text{alcohol} + \text{peracid}$$

It is not intended that the present invention be limited to any specific enzyme, as any enzyme that generates H_2O_2 with a suitable substrate may be used in the present invention. For

example, lactate oxidases from Lactobacillus species which are known to create H_2O_2 from lactic acid and oxygen may be used. One advantage of the enzymatic generation of acid (e.g., gluconic acid in the above example) is that this reduces the pH of a basic solution to the pH range in which a peracid is most effective in bleaching (i.e., at or below the pKa). Other enzymes (e.g., alcohol oxidase, ethylene glycol oxidase, glycerol oxidase, amino acid oxidase, etc.) that can generate hydrogen peroxide also may be used with ester substrates in combination with the perhydrolase enzymes of the present invention to generate peracids.

In some embodiments, the hydrogen peroxide generating oxidase is a carbohydrate oxidase.

Hydrogen peroxide may also be generated electrochemically, for example using a fuel cell fed oxygen and hydrogen gas.

In some embodiments, the hydrogen peroxide source is hydrogen peroxide provided at a concentration of about 1000 to about 3200 ppm, about 1500 to about 2800 ppm, about 2000 ppm to about 2200 ppm, or about 2100 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Surfactants and Emulsifier

The enzymatic textile bleaching compositions used in accordance with the present method contain one or more, i.e., at least one surfactant and/or at least one emulsifier. Surfactants suitable for use in practicing the present invention include, without limitation, nonionic (see, e.g., U.S. Pat. No. 4,565,647, which is herein incorporated by reference); anionic; cationic; and zwitterionic surfactants (see, e.g., U.S. Pat. No. 3,929,678 which is herein incorporated by reference). Anionic surfactants include, without limitation, linear alkylbenzenesulfonate, α-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid, and soap. Non-ionic surfactants include, without limitation, fatty alcohol ethoxylate, isotridecanol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, and N-acyl N-alkyl derivatives of glucosamine (“glucamides”).

In some embodiments, the surfactant and/or emulsifier comprises a non-ionic surfactant. In one embodiment, the non-ionic surfactant is a fatty alcohol ethoxylate. In one embodiment, the non-ionic surfactant is isotridecanol ethoxylate. In one embodiment, the non-ionic surfactant is a fatty alcohol ethoxylate and isotridecanol ethoxylate.

In one embodiment, the composition used in accordance with the present method comprises a surfactant and an emulsifier.

A surfactant may be present at a concentration of about 300 ppm to about 4800 ppm, about 600 ppm to about 3600 ppm, or about 300 ppm to about 1200 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

In some embodiments, the enzymatic bleaching composition contains isotridecanol ethoxylate at a concentration of about 300 ppm to about 3600 ppm, about 600 ppm to about 3000 ppm, or about 900 ppm to about 2400 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Peroxide Stabilizer

The enzymatic bleaching compositions used in accordance with the method described herein contain a peroxide stabilizer. Examples of peroxide stabilizers include, but are not limited to, sodium silicate, sodium carbonate, acrylic polymers, magnesium salts, and phosphonic acid. In one embodiment, the peroxide stabilizer is phosphonic acid.

A peroxide stabilizer may be present in the enzymatic textile bleaching composition at a concentration of about 60 ppm to about 600 ppm, about 60 ppm to about 1200 ppm, or about 120 ppm to about 960 ppm based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Sequestering Agent

The enzymatic bleaching compositions used in accordance with the method described herein include a sequestering agent. Examples of sequestering agents include, but are not limited to, amino carboxylates, amino phosphonates, polyfunctionally-substituted aromatic chelating

agents, polyhydroxy-carboxylic acids, aminopolycarboxylic acids, polyphosphonates, and polyacrylic acids, and mixtures thereof.

Amino carboxylates useful as sequestering agents include ethylenediaminetetracetates, N-hydroxyethylethylenediaminetriacetates, nitrilotriacetates, ethylenediamine tetraproprionates, and triethylenetetraaminehexacetates.

Polyfunctionally-substituted aromatic sequestering agents are also useful in the compositions herein (see U.S. Pat. No. 3,812,044). Preferred compounds of this type in acid form are dihydroxydisulfobenzenes such as 1,2-dihydroxy-3,5-disulfobenzenediethylenetriamine-pentaacetates, and ethanoldiglycines, alkali metal, ammonium, and substituted ammonium salts therein and mixtures thereof.

Amino phosphonates are also suitable for use as sequestering agents in the compositions of the invention when at least low levels of total phosphorus are permitted.

A biodegradable sequestering agent suitable for use herein is ethylenediamine disuccinate ("EDDS"), especially the [S,S] isomer as described in U.S. Pat. No. 4,704,233.

In one embodiment, the sequestering agent is polyacrylic acid.

A sequestering agent may be present in the enzymatic textile bleaching composition at a concentration of about 60 ppm to about 1800 ppm, about 300 ppm to about 1200 ppm, or about 180 ppm to about 1200 ppm based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Bleaching Processor

Appropriately, at least one surfactant and/or emulsifier, at least one peroxide stabilizer and at least one sequestering agent are applied as a combination product containing each of at least one surfactant and/or emulsifier, at least one peroxide stabilizer and at least one sequestering agent. The said combination product is designated bleaching processor and is commercially available, for example, CLARITE® LTC (product of Huntsman).

A surfactant may be present at a concentration of about 5% to about 40%, about 20% to about 30%, or about 5% to about 10%, based on the total weight of the bleaching processor.

A peroxide stabilizer may be present in the bleaching processor at a concentration of about 1% to about 5%, about 1% to about 10%, or about 2% to about 8%, based on the total weight of the bleaching processor.

A sequestering agent may be present in the bleaching processor at a concentration of about 1% to about 15%, about 5% to about 10%, or about 3% to about 10%, based on the total weight of the bleaching processor.

In some embodiments, the bleaching processor contains isotridecanol ethoxylate at a concentration of about 5% to about 30%, about 10% to about 25%, or about 15% to about 20%, based on the total weight of the bleaching processor.

The bleaching processor is suitably provided as an aqueous composition comprising the above indicated components.

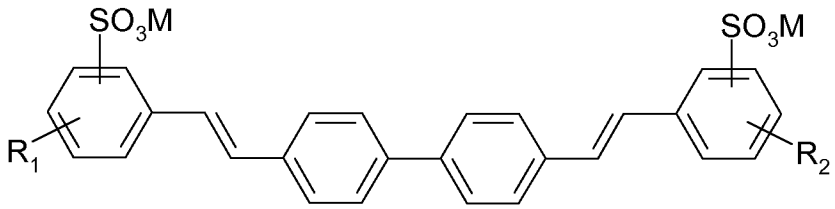
Buffer

The enzymatic bleaching composition contains a buffer that is capable of maintaining the pH of the composition at a pH of about 6 to about 8. In one embodiment, the buffer is a carbonate buffer, pH 8.

Fluorescent Whitening Agent

Suitable fluorescent whitening agents used in accordance with the method described herein are, for example, distyryl biphenyl derivatives as described in U.S. Pat. No. 3,984,399, U.S. Pat. No. 5,969,204 and U.S. Pat. No. 6,096,919 which are herein incorporated by reference.

In one embodiment the distyryl biphenyl derivatives indicated correspond to compounds of the formula



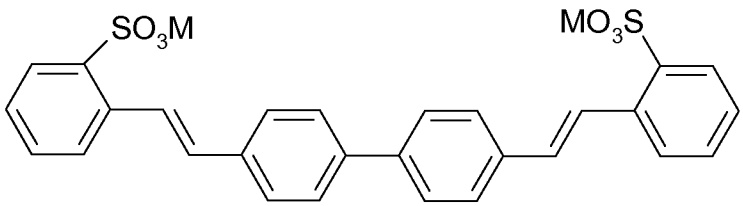
(1),

in which

M is hydrogen, lithium, sodium, potassium, ammonium or ammonium that is mono-, di-, tri- or tetra-substituted by C_1-C_6alkyl or C_2-C_6hydroxyalkyl which may be interrupted by an oxygen atom or a mixture thereof, and

R_1 and R_2 independently of one another are hydrogen, C_1-C_6alkyl, C_1-C_6alkoxy or halogen.

In another embodiment the compound of the formula (1) corresponds to the compound of the formula (1a)



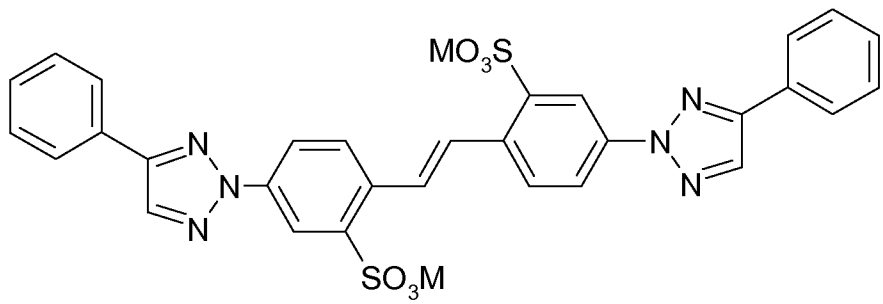
(1a),

in which

M is defined as given above.

Further suitable fluorescent whitening agents are, for example, triazolyl stilbene disulfonic acid derivatives as described in U.S. Pat. No. 3,485,831,U.S. Pat. No. 3,453,268 and U.S. Pat. No. 3,994,834 which are herein incorporated by reference.

In one embodiment the triazolyl stilbene disulfonic acid derivatives indicated correspond to the compound of the formula



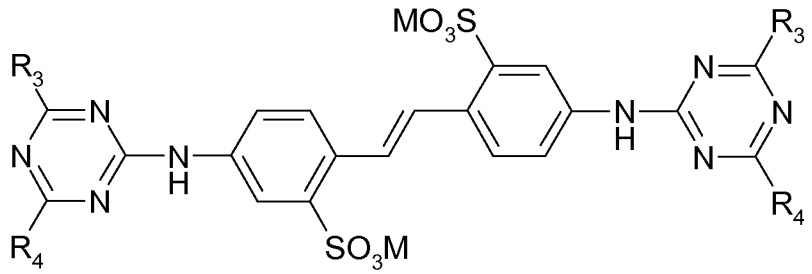
(2),

in which

M is defined as given above.

Other fluorescent whitening agents which come into consideration for practising the present invention are, for example,

diamino stilbene derivatives of the formula



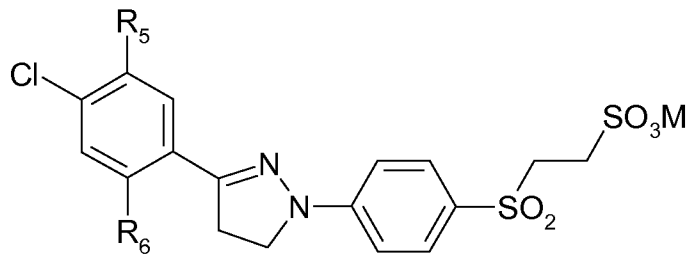
(3),

in which

M is is defined as given above, and

R_3 and R_4 independently of one another are C_1-C_4alkoxy, C_1-C_4alkylthio, substituted or unsubstituted amino or an N-heterocycle which may contain further heteroatoms;

diphenyl pyrazoline derivatives of the formula



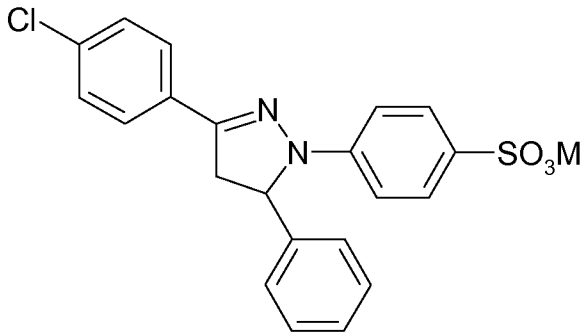
(4),

in which

M is defined as given above, and

R_5 and R_6 independently of one another are hydrogen, C_1-C_4alkyl, C_1-C_4alkoxy or halogen;

diphenyl pyrazoline derivatives of the formula

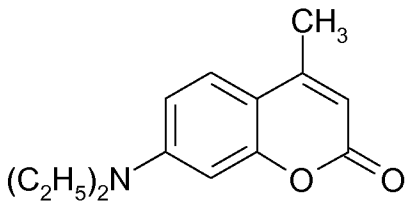


(5),

in which

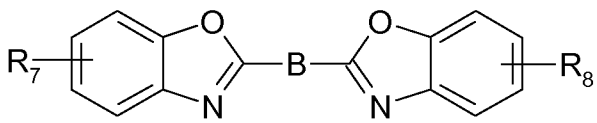
M is defined as given above;

a coumarine derivative of the formula



(6);

benzoxazole derivatives of the formula



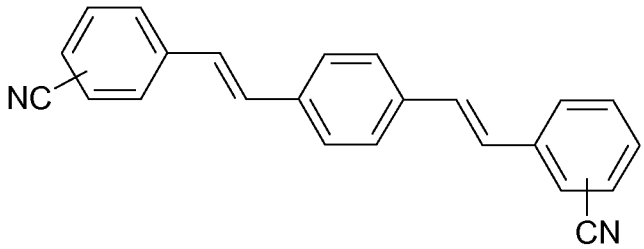
(7),

in which

B is a radical of formula -CH=CH-, 2,5-thiophenylene or 1,4-naphthylene, and

R_7 and R_8 independently of one another are hydrogen, C_1-C_4alkyl, C_1-C_4alkoxy or halogen; and

dicyanstyrylbenzene derivatives of the formula



(8).

C_2-C_6hydroxyalkyl which may be interrupted by an oxygen atom as suitable substituent for M in the meaning of mono-, di-, tri- or tetra-substituted ammonium is suitably typically a radical of formula $-CH_2CH_2OH$, $-CH_2-CH(OH)-CH_3$, $-(CH_2)_2-O-(CH_2)_2-OH$, $-(CH_2)_2-O-CH_2-CH_2-OH$ or $-(CH_2)_2-O-CH_2-CH(CH_3)-OH$.

C_1-C_6alkyl as suitable substituent for M in the meaning of mono-, di-, tri- or tetra-substituted ammonium is, for example, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, isobutyl, pentyl or hexyl.

R_1 and R_2 defined as C_1-C_6alkyl independently of one another are, for example, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, isobutyl, pentyl or hexyl and, preferably, C_1-C_4alkyl, such as ethyl.

R_1 and R_2 defined as C_1-C_6alkoxy independently of one another are, for example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, pentyloxy or hexyloxy, preferably C_1-C_4alkoxy, such as methoxy or ethoxy.

R_1, R_2, R_5, R_6, R_7 and R_8 defined as halogen independently of one another are, for example, fluorine, chlorine or bromine, preferably chlorine or bromine and in particular chlorine, the definitions and preferences also applying to the following halogen radicals.

R_3 and R_4 defined as C_1-C_4alkylthio independently of one another are, for example, methylthio, ethylthio, n-propylthio, isopropylthio or n-butylthio, preferably ethylthio or n-propylthio.

R_3, R_4, R_5, R_6, R_7 and R_8 defined as C_1-C_4alkoxy independently of one another are, for example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy or isobutoxy, preferably methoxy or ethoxy, the definitions and preferences also applying to the following C_1-C_4alkoxy radicals.

R_5, R_6, R_7 and R_8 defined as C_1-C_4alkyl independently of one another are, for example, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl or isobutyl, the definitions and preferences also applying to the following C_1-C_4alkyl radicals.

R_3 and R_4 as substituted or unsubstituted amino independently of one another are, for example, amino which is unsubstituted or substituted on the N-atom by, for example, the following radicals:
N-mono- or N,N-di-C_1-C_4alkylamino, which includes both the unsubstituted radicals, for example, ethylamino or diethylamino, and the radicals substituted in the alkyl moiety by, for example, N,N-di-C_1-C_4alkylamino, C_1-C_4alkoxy, hydroxy, carboxy, sulfo or sulfato;
C_5-C_7cycloalkylamino, which includes both the unsubstituted radicals and the radicals substituted in the cycloalkyl ring, for example by C_1-C_4alkyl, in particular methyl;
phenylamino or N-C_1-C_4alkyl-N-phenylamino, which includes both the unsubstituted radicals and the radicals substituted in the phenyl ring, for example by C_1-C_4alkyl which in turn may be substituted by phenyl, C_1-C_4alkoxy, halogen, carbamoyl, carbamoyl which is mono- or di-substituted on the nitrogen by C_1-C_4alkyl or C_1-C_4hydroxyalkyl, carboxy, C_1-C_4alkoxycarbonyl or sulfo; these radicals are preferably unsubstituted in the phenyl ring or substituted by sulfo.

R_3 and R_4 in the meaning of an N-heterocycle which may contain further heteroatoms independently of one another are, for example, morpholino or piperidin-1-yl.

Carboxy generally means the group -COOM; Sulfo generally means the group -SO_3M, in which M is hydrogen, lithium, sodium, potassium, ammonium or ammonium that is mono-, di-, tri- or tetra-substituted by C_1-C_4alkyl or C_1-C_4hydroxyalkyl or a mixture thereof.

In one embodiment at least one of a distyryl biphenyl derivative and a triazolyl stilbene disulfonic acid derivative are used in accordance with the method described herein.

In another embodiment at least one of a distyryl biphenyl derivative of the formula (1a) and a triazolyl stilbene disulfonic acid derivative of the formula (2) are used in accordance with the method described herein.

In some embodiments the fluorescent whitening agent is provided at a concentration of about 0.05 to about 0.8 %, about 0.08 to about 0.5 %, about 0.1 to about 0.4 %, based on the weight of the textile material.

Organic Acid

An organic acid is appropriately used to adjust the pH for the uptake of the fluorescent whitening agent. The organic acid is suitably oxalic acid, citric acid and acetic acid. In one embodiment the organic acid is acetic acid.

In some embodiments the organic acid is provided at a concentration of about 100 to about 4000 ppm, about 600 to about 3000 ppm, about 800 ppm to about 1600 ppm, based on the total weight of the aqueous composition (bath) applied for treatment of the textile material.

Appropriately, the pH is adjusted to pH 4 to 5, for example, pH 4.5.

Enzymatic Textile Bleach-Whitening Method

Appropriately, the method of the invention utilizes a liquor ratio of about 2:1 to about 50:1, about 5:1 to about 20:1, for example, about 20:1 or 10:1. In some embodiments, the method according to the present invention is performed in a discontinuous process, for example, as a one-bath-two-steps exhaustion method. In these embodiments the enzymatic textile bleaching is carried out in the first step and in a subsequent second step whitening is carried out by adding the fluorescent whitening agent into the same bath after adjustment of the bath to pH 4 to 5, for example, pH 4.5, during 20 minutes at about 65°C to about 95°C. In some embodiments, the method is performed in a semi-continuous process like pad-batch or pad-roll.

Textiles are contacted with the enzymatic bleaching composition at a temperature of about 55°C to about 75°C, about 60°C to about 70°C, for a processing time of about 20 to about 60 minutes at a pH of about 6 to about 8. In one embodiment, the bleaching temperature is about 65°C and the processing time is about 50 minutes. In some embodiments, the temperature of the enzymatic bleaching composition is raised by about 2°C per minute from a starting

temperature of about 20°C to about 50°C, for example, about 20°C to about 40°C, until the processing temperature for bleaching is reached.

In some other embodiments the whitening step is carried out in a separate bath after enzymatic textile bleaching, washing-off and neutralization (two-baths-two-steps). One or more rinsing steps are performed after treatment of the textile material with the enzymatic bleaching composition, to remove the bleaching composition. Appropriately, the textile is rinsed with an aqueous composition (water or a composition containing water). In some embodiments, the rinsing temperature is about 40°C to about 60°C, for example, about 50°C. In some embodiments, the aqueous rinsing composition contains a catalase enzyme to catalyze the decomposition of hydrogen peroxide to water and oxygen. In one embodiment, the textile is rinsed twice with a catalase containing aqueous composition for about 10 minutes for each rinse. In one embodiment residual hydrogen peroxide is removed by rinsing twice with an aqueous composition containing catalase at about 50°C.

Appropriately, a rinsing step as described above is applied in the one-bath-two-steps method after the whitening step is finished.

Bioscouring Enzymes

In some embodiments, the methods for enzymatic textile bleach-whitening described herein include one or more bioscouring enzyme(s). One or more bioscouring enzyme(s) may be included in the enzymatic textile bleaching composition, or a textile may be treated with bioscouring enzyme(s) in a subsequent processing step after treatment in the enzymatic textile bleaching composition.

As the bioscouring enzymes there come into consideration for practicing the present invention pectinases, hemicellulases, cellulases, enzymes that hydrolyze polyester substrates, for example, cutinases or lipases and other bioscouring enzymes, for example, proteases.

Suitable pectinases, their application as well as assays to determine enzyme activity are described and referred to in more detail in WO 2007/136469 on pages 19 to 21, which are herein incorporated by reference.

Suitable cellulases, their application as well as assays to determine enzyme activity are described and referred to in more detail in WO 2007/136469 on pages 22 and 23, which are herein incorporated by reference.

As the other bioscouring enzymes which come into consideration for practicing the present invention, may be used, for example, proteases, protease variants, and lipases. These enzymes, their application as well as assays to determine enzyme activity are described and referred to in more detail in WO 2007/136469 on pages 23 to 25, which are herein incorporated by reference.

As the enzymes that hydrolyze polyester substrates suitable for use in the present invention may be used, for example, cutinases or lipases. These enzymes, their application as well as assays to determine enzyme activity are described and referred to in more detail in WO 2007/136469 on pages 21 and 22, which are herein incorporated by reference.

In one embodiment, the bioscouring enzyme is a pectinase.

Desizing Enzymes

In some embodiments, the methods for enzymatic textile bleach-whitening described herein include one or more desizing enzyme(s). One or more desizing enzyme(s) may be included in the enzymatic textile bleaching composition, or a textile may be treated with desizing enzyme(s) in a previous processing step before treatment in the enzymatic textile bleaching composition.

Any suitable desizing enzyme may be used in the present invention. In some embodiments, the desizing enzyme is an amylolytic enzyme. Mannanases and glucoamylases may also be used. In some embodiments, the desizing enzyme is an α- or β-amylase and combinations thereof.

Suitable amylases, their application as well as assays to determine enzyme activity are described and referred to in more detail in WO 2007/136469 on pages 18 and 19, which are herein incorporated by reference.

The following examples are intended to illustrate, but not limit, the invention. Temperatures are in degrees Celsius, parts are parts by weight and the percentage data are percentages by weight, unless noted otherwise. Parts by weight bear the same relation to parts by volume as the kilogram to the litre.

Experimental Part

A comparison between the method of the present invention and conventional reduction bleach with sodium dithionite is performed according to the procedures given below by treating the fabric in exhaust using a Mathis AG Lab Vistacolor apparatus (ZELTEX).

Examples 1A, 2 and 3

Enzymatic bleach-whitening carried out as a one-bath-two-steps exhaustion method:
Nylon 66 fabric, prescoured and heat-set at 175°C, is treated in a bath containing the bleaching processor, soda ash, the ester substrate, hydrogene peroxide and the perhydrolase enzyme in the amounts given in Table 1 using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 65°C at a rate of 2°C per minute. The bath is then held at 65°C for 30 minutes and afterwards adjusted to pH 4.5 by addition of acetic acid (80%). Subsequently, the fluorescent whitening agent in the amount given in Table 1 is added to the bath and treatment is continued at a temperature of 65°C for further 20 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and then dried at 70°C. 0.5 g/l of a 25% solution of Catalase T100 (available from Genencor) is included in each rinse.

Table 1: Examples 1A, 2 and 3 - enzymatic bleach-whitening

Example #	1A	2	3
Bleaching processor[1)] [g/l]	0.9	0.9	0.9
Sodium carbonate buffer [g/l]	2.0	2.0	2.0
Ester substrate[2)] [g/l]	3.0	3.0	3.0
Hydrogen peroxide 35% [ml/l]	6.0	6.0	6.0
Perhydrolase enzyme[3)] [mg/l]	1.7	1.7	1.7
FWA(1a)[4)] [%]	0.4	0.36	
FWA(2)[5)] [%]			0.26
Shading dye[6)]		6)	

1) CLARITE® LTC (commercial product of Huntsman)

2) Propylene glycol diacetate

3) corresponds to 1.0 g/l Primagreen® EcoWhite (1x) (product of Genencor)

4) Fluorescent whitening agent of the formula (1a); [%] based on the weight of fabric

5) Fluorescent whitening agent of the formula (2); [%] based on the weight of fabric

6) Mixture of 0.00025% of Benzo phenazinium, 7-phenyl-5,9-bis(phenylamino)-4,10-disulfo-, hydroxide, inner salt, monosodium salt and 0.00019% of Benzo phenazinium, 7-phenylsulfo-5-[(4-sulfophenyl)amino]-, hydroxide, inner salt, monosodium salt; [%] based on the weight of fabric

Comparative Examples 1 to 3

Conventional reduction bleach with sodium dithionite:

Nylon 66 fabric, prescoured and heat-set at 175°C, is treated in a bath containing the components in the amounts given in Table 2 using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 95°C at a rate of 2°C per minute. The bath is then held at 95°C for 30 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and subsequently dried at 70°C.

Table 2: Comparative Examples 1 to 3 - reduction bleach with sodium dithionite

Comparative Example #	1	2	3
Wetting agent[1)] [g/l]	1.0	1.0	1.0
Sodium dithionite[2)] [g/l]	3.0	3.0	3.0
FWA(1a)[3)] [%]	0.4	0.36	
FWA(2)[4)] [%]			0.26
Shading dye[5)] [%]		5)	

1) INVADINE® DA or ULTRAVON® EL (commercial wetting agents available from Huntsman)

2) Stabilized

3) Fluorescent whitening agent of the formula (1a); [%] based on the weight of fabric

4) Fluorescent whitening agent of the formula (2); [%] based on the weight of fabric

5) Mixture of 0.00025% of Benzo phenazinium, 7-phenyl-5,9-bis(phenylamino)-4,10-disulfo-, hydroxide, inner salt, monosodium salt and 0.00019% of Benzo phenazinium, 7-phenylsulfo-5-[(4-sulfophenyl)amino]-, hydroxide, inner salt, monosodium salt; [%] based on the weight of fabric

Example 1B

Enzymatic bleach-whitening carried out as a two-baths-two-steps exhaustion method:
(i) Nylon 66 fabric, prescoured and heat-set at 175°C, is treated in a bath containing
0.9 g/l of a bleaching processor as given in Table 1,
2.0 g/l of sodium carbonate buffer,
3.0 g/l of an ester substrate (propylene glycol diacetate),
6.0 ml/l of hydrogene peroxide 35%, and
1.7 mg/l of the perhydrolase enzyme which corresponds to 1.0 g/l of Primagreen® EcoWhite (1x) (product of Genencor)
using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 65°C at a rate of 2°C per minute. The bath is then held at 65°C for 50 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and subsequently dried at 70°C. 0.5 g/l of a 25% solution of Catalase T100 (product of Genencor) is included in each rinse.
(ii) In a separate bath containing
1.0 g/l of a wetting agent as given in Table 2,
0.4 % of the fluorescent whitening agent of the formula (1a), and
sufficient acetic acid (80%) to adjust the bath to pH 4.5,

the dried fabric obtained according to step (i) is treated using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 95°C at a rate of 2°C per minute. The bath is then held at 95°C for 30 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and subsequently dried at 70°C.

Example 4

Enzymatic bleach-whitening carried out as a one-bath-two-steps exhaustion method:
Nylon 6 texturized knitgood, prescoured and heat-set at 175°C, is treated in a bath containing
0.9 g/l of a bleaching processor as given in Table 1,
2.0 g/l of sodium carbonate buffer,
3.0 g/l of an ester substrate (propylene glycol diacetate),
6.0 ml/l hydrogene peroxide 35%, and
1.7 mg/l of the perhydrolase enzyme which corresponds to 1.0 g/l of Primagreen® EcoWhite (1x) (product of Genencor),
using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 65°C at a rate of 2°C per minute. The bath is then held at 65°C for 30 minutes and afterwards adjusted to pH 4.5 by addition of acetic acid (80%). Subsequently, 0.4 % of the fluorescent whitening agent of the formula (1a) is added to the bath and treatment is continued at a temperature of 65°C for further 20 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and subsequently dried at 70°C. 0.5 g/l of a 25% solution of Catalase T100 (product of Genencor) is included in each rinse.

Comparative Example 4

Conventional reduction bleach with sodium dithionite:
Nylon 6 texturized knitgood, prescoured and heat-set at 175°C is treated in a bath containing the components as given in Table 2 for Comparative Example 1 using a liquor ratio of 20:1. The temperature is raised from ambient temperature to a target temperature of 95°C at a rate of 2°C per minute. The bath is then held at 95°C for 30 minutes. After draining the fabric is rinsed twice for 10 minutes each at 50°C and subsequently dried at 70°C.

Results

The degree of whiteness of the fabrics obtained according to the Examples and the Comparative Examples is determined according to the method described by Ganz (Journal of Color and Appearance 1, No. 5 (1972)). Differences in Whiteness above 5 units Ganz are visually significant. The results are given in Tables 3 to 6.

Table 3: Degree of whiteness

Treatment	Degree of whiteness [Ganz]	
	Base white	Full white
Nylon 6.6 untreated	52	-
Conventional (Comparative Example 1)	58	231
Enzymatic/2 baths (Example 1B)	66	248
Enzymatic/1 bath (Example 1A)	70	257

The enzymatic bleach at 65°C clearly improves the base white of Nylon 66 fibers, the bleaching effect being more efficient than the bleaching effect achieved by conventional reduction bleach. Superior full white effects (with fluorescence) are achieved when the fabric is treated according to the enzymatic bleach-whitening method when compared to a fabric treated in accordance with the conventional bleach-whitening method. One-bath-two-steps bleach-whitening allows to achieve highest full white effects.

Table 4: Degree of whiteness

Bleach-Whitening Treatment	Degree of whiteness [Ganz]
Conventional (Comparative Example 2)	260
Enzymatic/1 bath (Example 2)	288

Superior full white effects are achieved when the fabric is treated in accordance with the enzymatic bleach-whitening method when compared to the fabric treated in accordance with the conventional method.

Table 5: Degree of whiteness

Bleach-Whitening Treatment	Degree of whiteness [Ganz]
Conventional (Comparative Example 3)	233
Enzymatic/1 bath (Example 3)	241

Superior full white effects are achieved when the fabric is treated in accordance with the enzymatic bleach-whitening method when compared to the fabric treated in accordance with the conventional method.

Table 6: Degree of whiteness

Bleach-Whitening Treatment	Degree of whiteness [Ganz]
Conventional (Comparative Example 4)	226
Enzymatic/1 bath (Example 4)	258

Superior full white effects are achieved when the fabric is treated in accordance with the enzymatic bleach-whitening method when compared to the fabric treated in accordance with the conventional method.

What is claimed is:

1. A method for bleach-whitening of synthetic textile material comprising contacting said textile material with
(a) an enzymatic textile bleaching composition comprising
(i) a perhydrolase enzyme,
(ii) an ester substrate for said perhydrolase enzyme,
(iii) a hydrogen peroxide source,
(iv) a surfactant and/or an emulsifier,
(v) a peroxide stabilizer,
(vi) a sequestering agent,
(vii) a buffer which maintains a pH of about 6 to about 8, and
(b) at least one fluorescent whitening agent, and, optionally,
(c) at least one acid or disperse shading dye for shaded variations of white,
for a length of time and under conditions suitable to permit measurable whitening of the textile material, thereby producing a bleached-whitened textile material.

2. A method according to claim 1, wherein said perhydrolase enzyme comprises the amino acid sequence set forth in SEQ ID NO:1 or a variant or homolog thereof.

3. A method according to either claim 1 or 2, wherein said perhydrolase enzyme is the S54V variant of SEQ ID NO:1.

4. A method according to any one of claims 1 to 3, wherein said perhydrolase enzyme comprises a perhydrolysis to hydrolysis ratio greater than 1.

5. A method according to any one of claims 1 to 4, wherein said ester substrate is selected from propylene glycol diacetate, ethylene glycol diacetate, glycerol triacetate, ethyl acetate, and glycerol tributyrate, for example, propylene glycol diacetate.

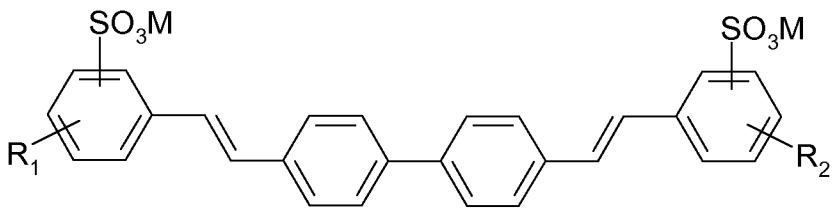
6. A method according to any one of claims 1 to 5, wherein said hydrogen peroxide source is hydrogen peroxide.

7. A method according to any one of claims 1 to 6, wherein said surfactant and/or emulsifier comprises a non-ionic surfactant, for example, a fatty alcohol ethoxylate or isotridecanol ethoxylate.

8. A method according to any one of claims 1 to 7, wherein said peroxide stabilizer is phosphonic acid.

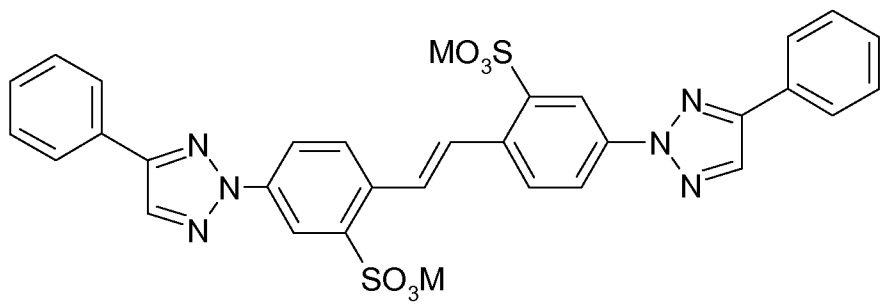
9. A method according to any one of claims 1 to 8, wherein said sequestering agent is polyacrylic acid.

10. A method according to any one of claims 1 to 9, wherein the fluorescent whitening agent is selected from the group comprising:
a distyryl biphenyl derivative of the formula



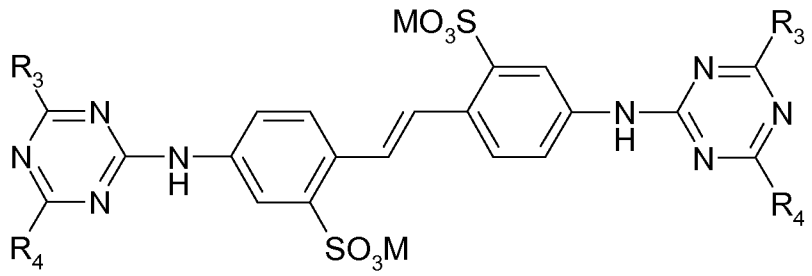
(1),

in which
M is hydrogen, lithium, sodium, potassium, ammonium or ammonium that is mono-, di-, tri- or tetra-substituted by C_1-C_6alkyl or C_2-C_6hydroxyalkyl which may be interrupted by an oxygen atom or a mixture thereof, and
R_1 and R_2 independently of one another are hydrogen, C_1-C_6alkyl, C_1-C_6alkoxy or halogen;
a triazolyl stilbene disulfonic acid derivative of the formula



(2),

in which
M is defined as given above;
a diamino stilbene derivative of the formula



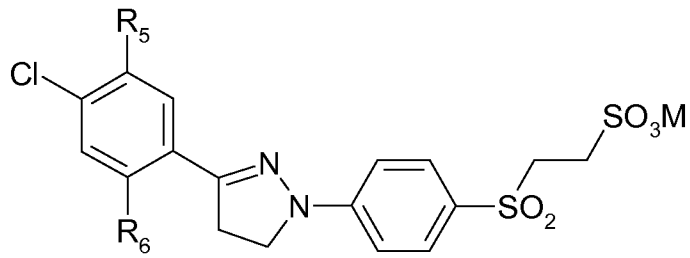
(3),

in which

M is is defined as given above, and

R_3 and R_4 independently of one another are C_1-C_4alkoxy, C_1-C_4alkylthio, substituted or unsubstituted amino or an N-heterocycle which may contain further heteroatoms;

a diphenyl pyrazoline derivative of the formula



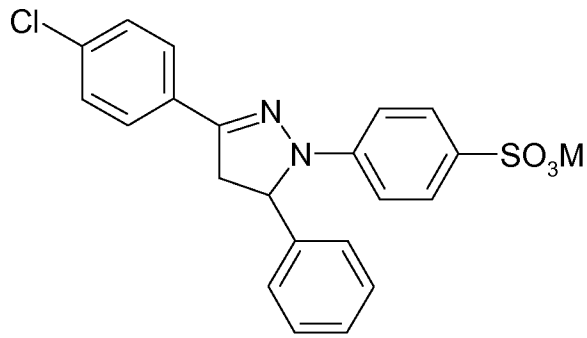
(4),

in which

M is defined as given above, and

R_5 and R_6 independently of one another are hydrogen, C_1-C_4alkyl, C_1-C_4alkoxy or halogen;

a diphenyl pyrazoline derivative of the formula

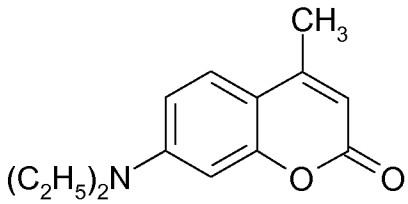


(5),

in which

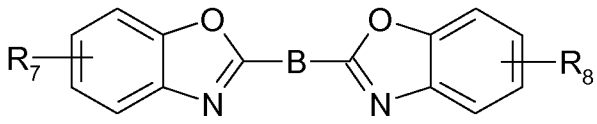
M is defined as given above;

a coumarine derivative of the formula



(6);

a benzoxazole derivative of the formula



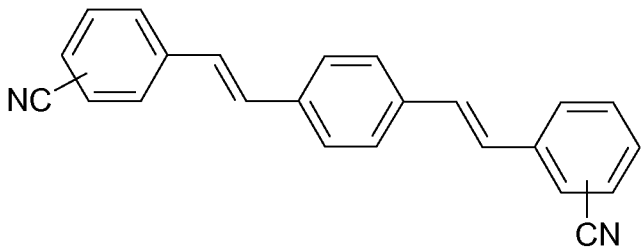
(7),

in which

B is a radical of formula -CH=CH-, 2,5-thiophenylene or 1,4-naphthylene, and

R_7 and R_8 independently of one another are hydrogen, C_1-C_4alkyl, C_1-C_4alkoxy or halogen; and

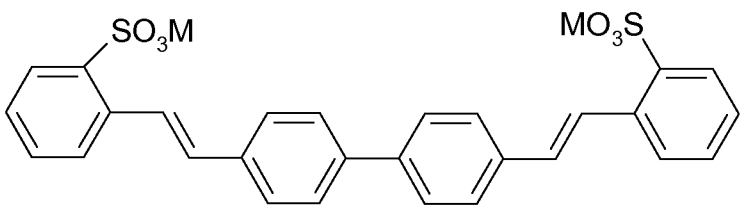
a dicyanstyrylbenzene derivative of the formula



(8).

11. A method according to any one of claims 1 to 10, wherein the fluorescent whitening agent is selected from the group of

a distyryl biphenyl derivative of the formula

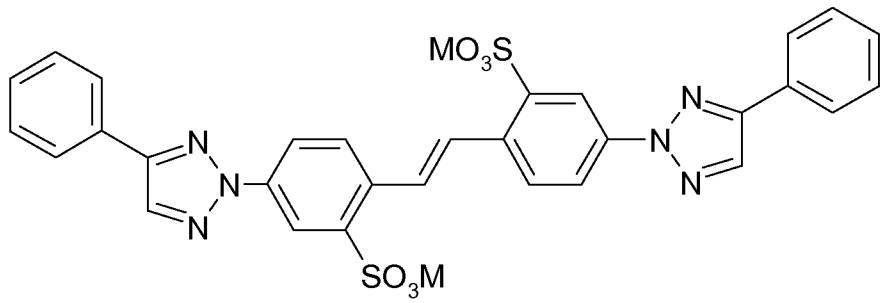


(1a),

in which

M is hydrogen, lithium, sodium, potassium, ammonium or ammonium that is mono-, di-, tri- or tetra-substituted by C_1-C_6alkyl or C_2-C_6hydroxyalkyl which may be interrupted by an oxygen atom or a mixture thereof; and

a triazolyl stilbene disulfonic acid derivative of the formula



(2),

in which

M is defined as given above.

12. A method according to any one of claims 1 to 11, further comprising hydrolyzing said hydrogen peroxide with a catalase enzyme after said bleached textile is produced.

13. A method according to any one of claims 1 to 12, wherein the liquor ratio is about 20:1 or 10:1.

14. A method according to any one of claims 1 to 13, wherein said method is performed in a process selected from a batch process, an exhaust process, and a discontinuous process.

15. A method according to any one of claims 1 to 14, wherein the textile material is contacted with the enzymatic textile bleaching composition at a bleaching temperature of about 60°C to about 70°C, for example 65°C, for a processing time of about 40 to about 60 minutes, for example 50 minutes.