Title: COLOR MODIFICATION OF Sized FABRIC

Abstract: Described are methods relating to modifying the color of sized fabrics using a perhydrolase enzyme system, thereby eliminating the need for prior desizing.
COLOR MODIFICATION OF SIZED FABRIC

PRIORITY

[01] The present application claims priority to U.S. Provisional Application Serial No. 61/453,880, filed on March 17, 2011, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[02] The present methods relate to modifying the color of sized fabrics using a perhydrolase enzyme system, thereby elimination the need for prior desizing.

BACKGROUND

[03] The use of enzymes to process textiles is now well established. Amylases are used for desizing, cellulases are used for abrading and polishing, pectate lyases and pectinases are used for scouring, and catalases are used for bleach clean-up. More recently, enzymes such as perhydrolases and laccases have been used in textile processing to affect color modification.

[04] Although enzymatic textile treatments have greatly reduced the environmental impact of textile processing and produced significant cost saving to textiles producers, the complete manufacture of a textile products continues to require multiple discrete steps, frequently involving separate baths and multiple rinse cycles to remove the reaction components from one process prior to initiating a subsequent process. In particular, current textile processing procedures demand the desizing of sized fabrics prior to subsequent enzymatic process, such as abrading, dying, and color modification.

SUMMARY

[05] Compositions and methods relating to color modification of sized fabrics are described.

[06] In one aspect, a method for modifying the color of a dyed, sized textile is provided, comprising, contacting the dyed, sized textile with a perhydrolase enzyme system to modify the color of the textile, wherein the contacting is performed without first desizing the textile.

[07] In some embodiments, the contacting is performed in the absence of desizing the textile. In some embodiments, the contacting is performed prior to desizing the textile.

[08] In some embodiments, the perhydrolase enzyme system comprises a perhydrolase enzyme and an ester substrate, wherein the perhydrolase enzyme catalyzes perhydrolysis of the ester substrate with a perhydrolysis :hydrolysis ratio equal to or greater than 1.
In some embodiments, the perhydrolase enzyme system comprises *Mycobacterium* perhydrolase or a variant, thereof. In some embodiments, the perhydrolase is a *Mycobacterium smegmatis* perhydrolase. In some embodiments, the perhydrolase enzyme is a variant of *Mycobacterium smegmatis* perhydrolase comprising the amino acid substitution S54V.

In some embodiments, the ester substrate is PGDA or triacetin.

In some embodiments, the size comprises starch, a starch-like material, or polyvinyl alcohol (PVA). In some embodiments, the size comprises starch, or a starch-like material. In some embodiments, the size comprises starch.

In some embodiments, the textile is denim. In some embodiments, the textile comprises cotton. In some embodiments, the dye is a sulphur dye.

In another aspect, a textile produced by any of the described methods is provided.

These and other aspects and embodiments of present compositions and methods will be further apparent from the description.

**DETAILED DESCRIPTION**

**Overview**

Described are methods for the enzymatic color-modification of dyed, sized textile materials, such as fibers, yarns, fabrics, and garments. The compositions and methods are particularly relevant to the processing of textile fabrics that are dyed prior to sizing, for example, denim fabrics.

Conventionally, such fabrics are desized prior to color-modification, because size is well known to interfere with subsequent color-modification processes, such as bleaching. It has now been discovered that a perhydrolase system can be used to modify the color of sized fabric, eliminating the need for desizing, or allowing desizing to be performed subsequent to color-modification. This discovery obviates a step in fabric processing and/or provides manufacturers with an option in terms of when a sized fabric is desized with relation to a color-modification step. These and other features and advantages of the present methods are further described, herein.

**Definitions**

Prior to describing the present compositions and methods in detail, the following terms are defined for clarity. Terms not defined should be given their ordinary meanings as using in the relevant art.
As used herein, a "perhydrolase" is an enzyme capable of catalyzing a perhydrolysis reaction that results in the production of a sufficiently high amount of peracid for use in an oxidative dye decolorization method as described. Generally, the perhydrolase enzyme exhibits a high perhydrolysis to hydrolysis ratio. In some embodiments, the perhydrolase enzyme 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 acyltransferase and/or arylesterase activity.

As used herein, the terms "perhydrolyzation," "perhydrolyze," or "perhydrolysis" refer to a reaction wherein a peracid is generated from ester and hydrogen peroxide substrate. In some embodiments, 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 \( R_1 C(=0)OR_2 \), where \( R_1 \) and \( R_2 \) are the same or different organic moieties, in the presence of hydrogen peroxide \( (H_2O_2) \). In some embodiments, \( -OR_2 \) is \(-OH\). In some embodiments, \(-OR_2\) is replaced by \(-NH_2\). In some embodiments, a peracid is produced by perhydrolysis of a carboxylic acid or amide substrate.

As used herein, an "effective amount of perhydrolase enzyme" refers to the quantity of perhydrolase enzyme necessary to produce the decolorization effects described herein. Such effective amounts are determined by the skilled artisan in view of the present description, and are based on several 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 whiteness).

As used herein, the term "peracid" refers to a molecule derived from a carboxylic acid ester that has been reacted with hydrogen peroxide to form a highly reactive product having the general formula \( RC(=0)OOH \). Such peracid products are able to transfer one of their oxygen atoms to another molecule, such as a dye. It is this ability to transfer oxygen atoms that enables a peracid, for example, peracetic acid, to function as a bleaching agent.

As used herein, an "ester substrate," with reference to an oxidative dye decolorization system containing a perhydrolase enzyme, 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 an acetate ester. In some embodiments, the ester source is selected from one or more of propylene glycol diacetate, ethylene glycol diacetate, triacetin, ethyl acetate and tributyrin. 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.

[23] As used herein, the term "hydrogen peroxide source" refers to a molecule capable of generating hydrogen peroxide, e.g., in situ. Hydrogen peroxide sources include hydrogen peroxide, itself, as well as molecules that spontaneously or enzymatically produce hydrogen peroxide as a reaction product. Such molecules include, e.g., perborate and percarbonate.

[24] As used herein, the phrase "perhydrolysis to hydrolysis ratio" refers to the ratio of enzymatically produced peracid to enzymatically produced acid (e.g., in moles) that is produced 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.

[25] 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., methanoyl chloride, CH$_3$CO-Cl, is the acyl chloride formed from methanoic acid, CH$_3$CO-OH).

[26] 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.

[27] 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.

[28] As used herein, the term "hydrogen peroxide generating oxidase" refers to an enzyme that catalyzes an oxidation/reduction reaction involving molecular oxygen (O$_2$) as the electron acceptor. In such a reaction, oxygen is reduced to water (H$_2$O) or hydrogen peroxide (H$_2$O$_2$). An oxidase suitable for use herein is an oxidase that generates hydrogen peroxide (as opposed to water) on its substrate. An example of a hydrogen peroxide generating oxidase and its substrate suitable for use herein is glucose oxidase and glucose. Other oxidase enzymes that may be used for generation of hydrogen peroxide include alcohol oxidase, ethylene glycol oxidase, glycerol oxidase, amino acid oxidase, etc. In some embodiments, the hydrogen peroxide generating oxidase is a carbohydrate oxidase.

[29] As used herein, the term "textile" refers to fibers, yarns, fabrics, garments, and non-wovens. The term encompasses textiles made from natural, synthetic (e.g., manufactured), and various natural and synthetic blends. Textiles may be unprocessed or processed fibers, yarns,
woven or knit fabrics, non-wovens, and garments and may be made using a variety of materials, some of which are mentioned, herein.

[30] As used herein, a "cellulosic" fiber, yarn or fabric is made at least in part from cellulose. Examples include cotton and non-cotton cellulosic fibers, yarns or fabrics. Cellulosic fibers may optionally include non-cellulosic fibers.

[31] As used herein, a "non-cotton cellulosic" fiber, yarn or fabric is comprised primarily of a cellulose based composition other than cotton. Examples include linen, ramie, jute, flax, rayon, lyocell, cellulose acetate, bamboo and other similar compositions, which are derived from non-cotton cellulosics.

[32] As used herein, a "non-cellulosic" fiber, yarn or fabric is comprised primarily of a material other than cellulose. Examples include polyester, nylon, rayon, acetate, lyocell, and the like.

[33] As used herein, the term "fabric" refers to a manufactured assembly of fibers and/or yarns that has substantial surface area in relation to its thickness and sufficient cohesion to give the assembly useful mechanical strength.

[34] As used herein, the term "dyeing," refers to applying a color, especially by soaking in a coloring solution, to, for example, textiles.

[35] As used herein, the term "dye" refers to a colored substance (i.e., chromophore) that has an affinity to a substrate to which it is applied. Numerous classes of dyes are described herein.

[36] As used herein, the terms "color modification," "color adjustment," "modifying or adjusting the color," or similar, are used to refer to any change to the color of a dyed textile resulting from the destruction, modification, or removal of a dye associated with the textile. In some embodiments, the color modification is decolorization (see below). Examples of color modification include but are not limited to, bleaching, fading, imparting a grey cast, altering hue, saturation, or luminescence, and the like. The amount and type of color modification can be determined by comparing the color of a textile following enzymatic treatment with a perhydrolase enzyme (i.e., residual color) to the color of the textile prior to enzymatic treatment (i.e., original color) using known spectrophotometric or visual inspection methods.

[37] As used herein, the terms "decolorizing" and "decolorization" refer to color elimination or reduction via the destruction, modification, or removal of dye, e.g., from an aqueous medium. In some embodiments, decolorizing or decolorization is defined as a percentage of color removal from aqueous medium. The amount of color removal can be determined by comparing the color of a textile following enzymatic treatment with a perhydrolase enzyme (i.e., residual color) to
the color of the textile prior to enzymatic treatment (i.e., original color) using known spectrophotometric or visual inspection methods.

[38] As used herein, the term "original color" refers to the color of a dyed textile prior to enzymatic treatment. Original color may be measured using known spectrophotometric or visual inspection methods.

As used herein, the term "residual color" refers to the color of a dyed textile prior to enzymatic treatment. Residual color may be measured using known spectrophotometric or visual inspection methods.

[40] As used herein, the terms "size" or "sizing material" refer to compositions used in the textile industry to improve weaving performance by increasing the abrasion resistance and/or strength of threads or yarns. Size is usually made of, for example, starch or starch-like material (i.e., chemically-modified starch), polyvinyl alcohol (PVA), carboxymethyl cellulose (CMC), acrylates, and the like.

[41] As used herein, the terms "desize" or "desizing" refer to the process of removing or eliminating size from textiles, usually prior to applying special finishes, dyes or bleach. Desizing can be performed enzymatically, e.g., using enzymes such as amylases and/or mannanases, or using caustic conditions.

[42] As used herein a "desizing enzyme" is an enzyme used to remove size. Exemplary enzymes are amylases and mannanases.

[43] As used here, the expression "dyed prior to sizing," refers to a fabric that has been dyed, completely or in part, or includes a component, such as a fiber or yarn, that has been dyed completely or in part, before the application of a sizing material to the fabric.

[44] As used herein, a "cellulase" is an enzyme capable of hydrolyzing cellulose.

[45] As used herein, the term "abrading" refers generally to contacting a textile comprising cellulose fibers with one or more cellulases to produce an effect. Such effects include but are not limited to softening, smoothing, defuzzing, depilling, biopolishing, and/or intentionally distressing the textile, locally or in its entirety. In some cases, more than one abrading step may be desirable.

[46] As used herein, an "aqueous medium" is a solution and/or suspension primarily comprising water as a solvent. The aqueous medium typically includes at least one dye to be decolorized, as well as any number of dissolved or suspended components, including but not limited to surfactants, salts, buffers, stabilizers, complexing agents, chelating agents, builders, metal ions, additional enzymes and substrates, and the like. Exemplary aqueous media are
textile dying solutions. Materials such as textile articles, textile fibers, and other solid materials
amy also be present in or in contact with the aqueous medium.

47 As used herein, the term "contacting," means bringing into physical contact, such as by
incubating a subject item (e.g., a textile) in the presence of an aqueous solution containing a
reaction component (e.g., an enzyme).

48 As used herein, "packaging" refers to a container capable of providing a perhydrolase
enzyme, substrate for the perhydrolase enzyme, and/or hydrogen peroxide source in an easy to
handle and transport form. Exemplary packaging includes boxes, tubs, cans, barrels, drums,
bags, or even tanker trucks.

49 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.

50 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.

51 As used herein, "polypeptide" refers to any composition comprising amino acids linked
by peptide bonds 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.

52 As used herein, functionally and/or structurally similar proteins are considered to be
"related proteins." In some embodiments, these proteins are derived from a different genus
and/or species, including differences between classes of organisms (e.g., a bacterial protein and
a fungal protein). In additional embodiments, related proteins are provided from the same
species. Indeed, it is not intended that the processes, methods and/or compositions described herein be limited to related proteins from any particular source(s). In addition, the term "related proteins" encompasses tertiary structural homologs and primary sequence homologs. In further embodiments, the term encompasses proteins that are immunologically cross-reactive.

[53] As used herein, the term "derivative" refers to a protein which is derived from a protein by addition of one or more amino acids to either or both the C- and N-terminal end(s), substitution of one or more amino acids at one or a number of different sites in the amino acid sequence, and/or deletion of one or more amino acids at either or both ends of the protein or at one or more sites in the amino acid sequence, and/or insertion of one or more amino acids at one or more sites in the amino acid sequence. The preparation of a protein derivative is preferably achieved by modifying a DNA sequence which encodes for the native protein, transformation of that DNA sequence into a suitable host, and expression of the modified DNA sequence to form the derivative protein.

[54] Related (and derivative) proteins comprise "variant proteins." In some embodiments, variant proteins differ from a parent protein, e.g., a wild-type protein, and one another by a small number of amino acid residues. The number of differing amino acid residues may be one or more, for example, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more amino acid residues. In some aspects, related proteins and particularly variant proteins comprise at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or even 99% or more amino acid sequence identity. Additionally, a related protein or a variant protein refers to a protein that differs from another related protein or a parent protein in the number of prominent regions. For example, in some embodiments, variant proteins have 1, 2, 3, 4, 5, or 10 corresponding prominent regions that differ from the parent protein. Prominent regions include structural features, conserved regions, epitopes, domains, motifs, and the like.

[55] 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. Note that where a particular mutation in a variant polypeptide is specified, further variants of that variant polypeptide retain the specified mutation and vary at other positions not specified.

[56] As used herein, the term "analogous sequence" refers to a sequence within a protein that provides similar function, tertiary structure, and/or conserved residues as the protein of interest (i.e., typically the original protein of interest). For example, in epitope regions that contain an alpha-helix or a beta-sheet structure, the replacement amino acids in the analogous sequence
preferably maintain the same specific structure. The term also refers to nucleotide sequences, as well as amino acid sequences. In some embodiments, analogous sequences are developed such that the replacement amino acids result in a variant enzyme showing a similar or improved function. In some embodiments, the tertiary structure and/or conserved residues of the amino acids in the protein of interest are located at or near the segment or fragment of interest. Thus, where the segment or fragment of interest contains, for example, an alpha-helix or a beta-sheet structure, the replacement amino acids preferably maintain that specific structure.

[57] As used herein, the term "homologous protein" refers to a protein that has similar activity and/or structure to a reference protein. It is not intended that homologs necessarily be evolutionarily related. Thus, it is intended that the term encompass the same, similar, or corresponding enzyme(s) (i.e., in terms of structure and function) obtained from different organisms. In some embodiments, it is desirable to identify a homolog that has a quaternary, tertiary and/or primary structure similar to the reference protein. In some embodiments, homologous proteins induce similar immunological response(s) as a reference protein. In some embodiments, homologous proteins are engineered to produce enzymes with desired activity(ies).


[59] 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, pair-wise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle, (Feng and Doolittle (1987) J. Mol. Evol. 35:351-360). The method is similar to that described by Higgins and Sharp (Higgins and Sharp (1989) CABIOS 5:151-153). Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps. Another example of a useful algorithm is the BLAST algorithm, described by Altschul et al. (Altschul et al. (1990) J. Mol. Biol. 215:403-410; and Karlin et al. (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One particularly useful BLAST program is the WU-BLAST-2 program (See, Altschul et al. (1996) Meth. Enzymol. 266:460-480). Parameters "W," "T," and "X" determine the sensitivity and speed of the alignment. The
BLAST program uses as defaults a word-length (W) of 11, the BLOSUM62 scoring matrix (See, Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M'>5, N'=4, and a comparison of both strands.

[60] As used herein, the phrases "substantially similar" and "substantially identical," in the context of at least two nucleic acids or polypeptides, typically means that a polynucleotide or polypeptide comprises a sequence that has at least about 40% identity, more preferably at least about 50% identity, yet more preferably at least about 60% identity, preferably at least about 75% identity, more preferably at least about 80% identity, yet more preferably at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% sequence identity, compared to the reference (i.e., wild-type) sequence. Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (See e.g., Altschul, et al. (1990) J. Mol. Biol. 215:403-410; Henikoff et al. (1989) Proc. Natl. Acad. Sci. USA 89:10915; Karin et al. (1993) Proc. Natl. Acad. Sci USA 90:5873; and Higgins et al. (1988) Gene 73:237-244). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Also, databases may be searched using FASTA (Pearson et al. (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448). One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions (e.g., within a range of medium to high stringency).

[61] As used herein, "wild-type" and "native" proteins are those found in nature. The terms "wild-type sequence," and "wild-type gene" are used interchangeably herein, to refer to a sequence that is native or naturally occurring in a host cell. In some embodiments, the wild-type sequence refers to a sequence of interest that is the starting point of a protein engineering project. The genes encoding the naturally-occurring protein may be obtained in accord with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protein of interest, preparing genomic libraries from organisms expressing the protein, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.
As used herein, the singular articles "a," "an," and "the" encompass the plural referents unless the context clearly dictates otherwise. All references sited herein are hereby incorporated by reference in their entirety.

The following abbreviations/acronyms have the following meanings unless otherwise specified:

- cDNA: complementary DNA
- DNA: deoxyribonucleic acid
- EC: enzyme commission
- kDa: kiloDalton
- MW: molecular weight
- SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
- w/v: weight/volume
- w/w: weight/weight
- v/v: volume/volume
- wt%: weight percent
- °C: degrees Centigrade
- H₂O: water
- H₂O₂: hydrogen peroxide
- dH₂O or DI: deionized water
- dffl₂O: deionized water, Milli-Q filtration
- g or gm: gram
- μg: microgram
- mg: milligram
- kg: kilogram
- μL and μl: microliter
- mL and ml: milliliter
- mm: millimeter
- μm: micrometer
- M: molar
- mM: millimolar
- μM: micromolar
- U: unit
- ppm: parts per million
- sec and "s": second
- min and "m": minute
- hr: hour
- ETOH: ethanol
- eq.: equivalent
- N: normal
- CI: Colour (Color) Index
- CAS: Chemical Abstracts Society
- PGDA: propylene glycol diacetate

Color modification of sized fabric

The present methods relate to the enzymatic color-modification of textile materials using an enzymatic perhydrolase system, wherein the textile is sized prior to color-modification. Such methods eliminate the need to desize the textile prior to color modification. In some cases, the
textile is color-modified in the absence of desizing, either before or after color-modification. In some cases, the textile is color-modified prior to desizing. In some cases, the process of color-modification using the perhydrolyase system may remove at least a portion of the size. The ability to color modify a sized textile saves a step in processing, and/or provides textile manufacturers with options in terms of the sequence of color modification and desizing steps.

[65] Although not limited to a theory, it is believed that the present methods can be applied to a fabric sized with any sizing material that can be removed using a conventional oxidative desizing step, e.g., using a desizing agent such as potassium persulfate, sodium persulfate, sodium bromite, permanganate, and the like. In such cases, the present methods eliminate the need to desize the textile by contacting it with an oxidizing agent prior to color modification. In some cases, the textile is color-modified without contacting it with an oxidizing agent, either before or after color-modification. In some cases, the textile is color-modified prior to contacting it with an oxidizing agent. In some cases, the process of color-modification using the perhydrolyase system may remove at least a portion of the sizing material.

[66] Where the size is starch or starch-like material (e.g., chemically modified starch), the present methods eliminate the need to desize the textile by contacting it with an amylase enzyme prior to color modification. In some cases, the textile is color-modified without contacting it with an amylase enzyme, either before or after color-modification. In some cases, the textile is color-modified prior to contacting it with an amylase enzyme. In some cases, the process of color-modification using the perhydrolyase system may remove at least a portion of the starch, or starch-like sizing material.

[67] The present methods are particularly useful when applied to fabrics that are sized following dyeing, or include at least some fibers or yarns that are dyed prior to sizing. An exemplary fabric is denim, which is formed by weaving dyed weft fibers with non-dyed warp fibers. These fibers are sized prior to weaving, and the resulting denim fabric is conventionally desized prior to enzymatic or chemical color modification. The present compositions and methods allow the direct color-modification of denim, in the absence of, or prior to, desizing.

[68] The methods are useful for color modifying fabrics dyed with a number of different dyes, including but not limited to sulphur-based dyes (such as sulphur black) and indigo dyes. Sulphur and indigo dyes are the most common dyes applied to denim fabrics.

[69] The extent of color modification resulting from the present methods may be similar to that achieved using a conventional color-modification process, in which the fabric is desized prior to color modification. In other cases, the extent of color modification resulting from the present methods may be less than that achieved using a conventional color-modification process.
but may nonetheless be sufficient to produce a desired effect, while offering the advantage of not having to desize the fabric prior to treatment. For example, the extent of color modification resulting from the present methods may be about 95%, about 90%, about 85%, about 80%, about 75%, or even about 70% or less of the amount of color modification achieved using a conventional color-modification process that involved a prior desizing step.

**Perhydrolase Enzyme System**

[70] A feature of the present methods is the use of a perhydrolase enzyme system, comprising a perhydrolase enzyme capable of generating peracids in the present of a suitable ester substrate and hydrogen peroxide source.

[71] In some embodiments, the perhydrolase enzyme is naturally-occurring enzyme. In some embodiments, the perhydrolase enzyme comprises, consists of, or consists essentially of an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even 99.5% identical to the amino acid sequence of a naturally-occurring perhydrolase enzyme. In some embodiments, the perhydrolase enzyme is from a microbial source, such as a bacterium or fungus.

[72] In some embodiments, the perhydrolase enzyme is a naturally occurring *Mycobacterium smegmatis* perhydrolase enzyme or a variant thereof. This enzyme, its enzymatic properties, its structure, and numerous variants and homologs, thereof, are described in detail in International Patent Application Publications WO 05/056782A and WO 08/063400A, and U.S. Patent Publications US2008 145353 and US2007 167344, which are incorporated by reference.

[73] In some embodiments, the perhydrolase enzyme has a perhydrolysis:hydrolysis ratio of at least 1. In some embodiments, the perhydrolase enzyme has a perhydrolysis:hydrolysis ratio greater than 1. In some embodiments, the perhydrolysis:hydrolysis ratio is greater than 1.5, greater than 2.0, greater than 2.5, or even greater than 3.0. These high perhydrolysis:hydrolysis ratios are features unique to of *M. smegmatis* perhydrolyase and variants, thereof.

[74] The amino acid sequence of *M. smegmatis* perhydrolyase is shown below (SEQ ID NO: 1):

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MAKRILCFGDSLTWGWVPOVEDGAPTERFAPDRVORTGWLACQQLLGAEVIEGLSARTT
NIDDPTDPRNLNGASLYPSCLATHLPLDLVIMLGLTNDTAYFRRTPLDIALGMSVLTQV
LTSAGGVGTTYPAPKVLVSPPLAMPMPHPFWQLIFEGGEQKTTELARVS ALASFMKV
PFDAGSVSTDGGVDFHTEANNRDLGVLAEQVRSLL
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[75] In some embodiments, a 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 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even 99.5% identical to the amino acid sequence set forth in SEQ ID NO: 1.


[77] 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.

[78] 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.

[79] In particular embodiments, the perhydrolase enzyme is the S54V variant of the M. smegmatis perhydrolase, which is shown, below (SEQ ID NO: 2); S54V substitution underlined:

MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLAQQLGADFEVIEEGLVART
TNIDDPTDPRLNGASLYPSCLATHPLDLVIIMLGTNDTKAYFRRTPLDIALGMSVLVTQ
VLTSAGGVGTTPAPKVLVSSPLAPMPHPWFQLIFEGGEQKTTELARVYSALASFMK

[80] In some embodiments, the perhydrolase enzyme includes the S54V substitution but is otherwise at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even 99.5% identical to the amino acid sequence set forth in SEQ ID NOs: 1 or 2.
In some embodiments, the perhydrolase enzyme is provided at a concentration of about 1 to about 100 ppm, or more. In some embodiments, the perhydrolase enzyme is provided at a molar ratio with respect to the amount of dye on the textile. In some embodiments, the molar ratio is from about 1/10,000 to about 1/10, or even from about 1/5,000 to about 1/100. In some embodiments, the concentration of carboxylic acid ester moieties in the aqueous medium is about 20-500 mM, for example, ethyl acetate, propyl acetate, etc., attached to glycerol or another multivalent alcohol.

The perhydrolase enzyme system may include at least one ester molecule that 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 or alcohol. The ester substrate may be a mono-, di-, or multivalent ester, or a mixture thereof. For example, the ester substrate may be a carboxylic acid and a single alcohol (monovalent, e.g., ethyl acetate, propyl acetate), two carboxylic acids and a diol (e.g., propylene glycol diacetate (PGDA), ethylene glycol diacetate (EGDA), or a mixture, for example, 2-acetyloxy 1-propionate, where propylene glycol has an acetate ester on alcohol group 2 and a propyl ester on alcohol group 1), or three carboxylic acids and a triol (e.g., glycerol triacetate or a mixture of acetate/propionate, etc., attached to glycerol or another multivalent alcohol).

In some embodiments, the ester substrate is an ester of a nitroalcohol (e.g., 2-nitro-1-propanol). In some embodiments, the ester substrate is a polymeric ester, for example, a partially acylated (acetylated, propionylated, etc.) poly carboxy alcohol, acetylated starch, etc. 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, triacetin, tributyric-acid, and other esters serve as acyl donors for peracid formation. 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.

As noted above, suitable substrates may be monovalent (i.e., comprising a single carboxylic acid ester moiety) or pluri-valent (i.e., comprising more than one carboxylic acid ester moiety). The amount of substrate used for color modification may be adjusted depending on the number carboxylic acid ester moieties in the substrate molecule. In some embodiments, the concentration of carboxylic acid ester moieties in the aqueous medium is about 20-500 mM, for...
example, about 40 mM to about 400 mM, about 40 mM to about 200 mM, or even about 60 mM to about 200 mM. Exemplary concentrations of carboxylic acid ester moieties include about 60 mM, about 80 mM, about 100 mM, about 120 mM, about 140 mM, about 160 mM, about 180 mM, and about 200 mM.

[85] In some embodiments, where the ester substrate is divalent (as in the case of PGDA) it is provided in an amount of about 10-200 mM, for example, about 20 mM to about 200 mM, about 20 mM to about 100 mM, or even about 30 mM to about 100 mM. Exemplary amounts of ester substrate include about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, and about 100 mM. The skilled person can readily calculate the corresponding amounts of trivalent, or other plurivalent ester substrates based on the number of carboxylic acid esters moieties per molecule.

[86] In some embodiments, the ester substrate is provided in a molar excess with respect to the molar amount of dye on the textile to be subjected to color modification. In some embodiments, the carboxylic acid ester moieties of the ester substrate are provided at about 20 to about 20,000 times the molar amount of dye. Exemplary molar ratios of carboxylic acid ester moieties to dye molecules are from about 100/1 to about 10,000/1, from about 1,000/1 to about 10,000/1, or even 2,000/1 to about 6,000/1. In some cases, the molar ratio of ester substrate to dye molecules is at least 2,000/1, or at least 6,000/1.

[87] In some embodiments, where the ester substrate is divalent (as in the case of PGDA) the ester substrate is provided at about 10 to about 10,000 times the molar amount of dye. Exemplary molar ratios of ester substrate to dye molecules are from about 50/1 to about 5,000/1, from about 500/1 to about 5,000/1, or even 1,000/1 to about 3,000/1. In some cases, the molar ratio of ester substrate to dye molecules is at least 1,000/1, or at least 3,000/1. As before, the skilled person can readily calculate the corresponding amounts of trivalent, or other plurivalent ester substrates based on the number of carboxylic acid esters moieties per molecule.

[88] In some embodiments, the ester substrate is provided at a concentration of about 100 ppm to about 100,000 ppm, ppm, or about 2500 to about 3500 ppm. In some embodiments, the ester substrate is provided in a molar excess with respect to the perhydrolase enzyme. In some embodiments, the molar ratio of carboxylic acid ester moieties to perhydrolase enzyme is at least about 2 x 10^5/1, at least about 4 x 10^5/1, at least about 1 x 10^6/1, at least about 2 x 10^6/1, at least about 4 x 10^6/1, or even at least about 1 x 10^7/1, or more. In some embodiments, the ester substrate is provided in a molar excess of from about 4 x 10^5/1, to about 4 x 10^6/1, with respect to the perhydrolase enzyme.
In some embodiments, where the ester substrate is divalent (as in the case of PGDA), the molar ratio of ester substrate to perhydrolase enzyme is at least about $1 \times 10^5/1$, at least about $2 \times 10^5/1$, at least about $5 \times 10^5/1$, at least about $1 \times 10^9/1$, at least about $2 \times 10^9/1$, or even at least about $5 \times 10^9/1$, or more. In some embodiments, the ester substrate is provided in a molar excess of from about $2 \times 10^5/1$ to about $2 \times 10^9/1$, with respect to the perhydrolase enzyme. The skilled person can readily calculate the corresponding amounts of trivalent, or other plurivalent ester substrates based on the number of carboxylic acid esters moieties per molecule.

The perhydrolase enzyme system further includes at least one hydrogen peroxide source. Generally, hydrogen peroxide can be provided directly (i.e., in batch), or generated continuously (i.e., in situ) by chemical, electro-chemical, and/or enzymatic means.

In some embodiments, the hydrogen peroxide source is hydrogen peroxide, itself. In some embodiments, the hydrogen peroxide source is a compound that generates hydrogen peroxide upon addition to water. The compound may be a solid compound. 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.

In some embodiments, the hydrogen peroxide source is an inorganic perhydrate salt. Examples of inorganic perhydrate salts are perborate, percarbonate, perphosphate, persulfate and persilicate salts. Inorganic perhydrate salts are normally alkali metal salts. Additional hydrogen peroxide sources include adducts of hydrogen peroxide with zeolites, or urea hydrogen peroxide.

The hydrogen peroxide source may be in a crystalline form and/or substantially pure solid form without additional protection. For certain perhydrate salts, preferred forms are granular compositions involving a coating, 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.

Glucose oxidase

\[
\text{Glucose} + \text{H}_2\text{O} \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2
\]

+ 

Perhydrolase

\text{H}_2\text{O}_2 + \text{ester substrate} \rightarrow \text{alcohol} + \text{peracid}

It is not intended that the generation of \( \text{H}_2\text{O}_2 \) be limited to any specific enzyme, as any enzyme that generates \( \text{H}_2\text{O}_2 \) with a suitable substrate may be used. For example, lactate oxidases from \textit{Lactobacillus} species known to create \( \text{H}_2\text{O}_2 \) from lactic acid and oxygen may be used. One advantage of such a reaction is the enzymatic generation of acid (e.g., gluconic acid in the above example), which reduces the pH of a basic aqueous solution to within the pH range in which peracid is most effective in bleaching (i.e., at or below the pKa). Such a reduction in pH is also brought about directly by the production of peracid. Other enzymes (e.g., alcohol oxidase, ethylene glycol oxidase, glycerol oxidase, amino acid oxidase, etc.) that are capable of generating hydrogen peroxide may also be used with ester substrates in combination with a perhydrolase enzyme to generate peracids.

Where hydrogen peroxide is generated electrochemically, it may be produced, for example, using a fuel cell supplied with oxygen and hydrogen gas.

In some embodiments, hydrogen peroxide is provided at a concentration of about 100 ppm to about 10,000 ppm, about 1,000 ppm to about 3,000 ppm, or about 1,500 to about 2,500 ppm. In some embodiments, hydrogen peroxide is provided at about 10 to about 1,000 times the molar amount of dye.

In some embodiments, hydrogen peroxide is provided in an amount of about 10-200 mM, for example, about 20 mM to about 200 mM, about 20 mM to about 100 mM, or even about 30 mM to about 100 mM. Exemplary amounts of hydrogen peroxide include about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, and about 100 mM.

In some embodiments, hydrogen peroxide is provided in a molar excess with respect to the molar amount of dye to be subjected to color modification. In some embodiments, the hydrogen peroxide is provided at about 10 to about 10,000 times the molar amount of dye. Exemplary molar ratios of hydrogen peroxide to dye molecules are from about 500/1 to about
5,000/1, or even 1,000/1 to about 3,000/1. In some cases, the molar ratio of hydrogen peroxide to
dye molecules is at least 1,000/1, or at least 3,000/1.

[101] In some embodiments, the hydrogen peroxide is provided in a molar excess with respect to
the perhydrolase enzyme. In some embodiments, the molar ratio of hydrogen peroxide to
perhydrolase enzyme is at least about 1 x 10^5/1, at least about 2 x 10^5/1, at least about 5 x 10^5/1,
at least about 1 x 10^6/1, at least about 2 x 10^6/1, or even at least about 5 x 10^6/1, or more. In
some embodiments, the hydrogen peroxide is provided in a molar excess of about 2 x 10^5/1 to 2
x 10^6/1, with respect to the perhydrolase enzyme.

[102] It may in some circumstances be desirable to add catalase to the textile bath to destroy
residual hydrogen peroxide. In such cases, catalase can be added directly to the bath, without
prior rinsing of the textiles.

[103] The following numbered paragraphs further describe various aspects and embodiments
of the present compositions and methods:

1. In one aspect, a method for modifying the color of a dyed, sized textile is provided,
comprising, contacting the dyed, sized textile with a perhydrolase enzyme system to modify the
color of the textile, wherein the contacting is performed without first desizing the textile.

2. In some embodiments, the method of the preceding paragraph is performed in the
absence of desizing the textile.

3. In some embodiments, the method of paragraph 1 is performed prior to desizing the
textile.

4. In some embodiments, the perhydrolase enzyme system used to perform the method
of any of the preceding paragraphs comprises a perhydrolase enzyme and an ester substrate,
wherein the perhydrolase enzyme catalyzes perhydrolysis of the ester substrate with a
perhydrolysis:hydrolysis ratio equal to or greater than 1.

5. In some embodiments of the method of any of the preceding paragraphs, the
perhydrolase enzyme system comprises a Mycobacterium perhydrolase or a variant, thereof, for
example, a Mycobacterium smegmatis perhydrolase.

6. In some embodiments of the method of any of the preceding paragraphs, the
perhydrolase enzyme is a variant of Mycobacterium smegmatis perhydrolase comprising the
amino acid substitution S54V.

7. In some embodiments of the method the preceding paragraphs, the ester substrate is
PGDA or triacetin.

8. In some embodiments of the method of any of the preceding paragraphs, the size
comprises starch, a starch-like material, or PVA.
9. In some embodiments of the method of any of the preceding paragraphs, the textile is denim.

10. In some embodiments of the method of any of the preceding paragraphs, the textile comprises cotton.

11. In some embodiments of the method of any of the preceding paragraphs, the dye is a sulphur dye.

12. In another aspect, a textile produced by the method of any of the preceding paragraphs is provided.

[104] These and other aspects and embodiments of the present compositions and method will be apparent to the skilled person in view of the present description. The following examples are intended to further illustrate, but not limit, the compositions and methods.

**EXAMPLES**

[105] The following enzyme nomenclature is used in the Examples:

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMAGREEN® EcoLight 1</td>
<td>Mycobacterium smegmatis perhydrolase, S54V variant (i.e., SEQ ID NO: 2)</td>
</tr>
<tr>
<td>OPTISIZE® 160 amylase</td>
<td>Amylase from Bacillus amyloliquefaciens</td>
</tr>
</tbody>
</table>

**Example 1:** Color adjustment of sulphur-dyed, sized denim, using a perhydrolase system with a PGDA substrate

[106] The ability to modify the color of sulphur-dyed, sized fabric, using a perhydrolase system with a PGDA substrate was investigated. Standard samples of sulphur-dyed fabric or sulphur-indigo-dyed fabric were treated according to one of the following four different protocols:

**A. No desizing, single-step treatment with perhydrolase ("perhydrolase treatment")**

[107] Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg, was treated in a front loading commercial washing machine (Unimac UF 50) with perhydrolase according to the following protocol: 60 minutes at a 30:1 liquor ratio with 1 g/l perhydrolase (PRIMAGREEN® EcoLight 1,326 U/g, 1.5 mg enzyme protein/g), 6 g/l H₂O₂ solution (30%wt), 3 g/l PGDA (>99.7% pure), 0.5 g/L of non-ionic surfactant (ULTRAVON®)
RW; Huntsman), and 5 g/1 of disodium phosphate dihydrate, pH 8.1, at 60°C. This step was followed by 2 cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

B. Desizing only, no perhydrolase treatment (amylase desizing)

[S108] Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the following protocol: 60 minutes at 30:1 liquor ratio with 0.5 g/l (OPTISIZE® 160) and 0.5 g/L of a non-ionic surfactant (ULTRAVON® RW; Huntsman) at 60°C. This step was followed by 2 cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

C. Water wash only, no perhydrolase treatment ("water wash")

[S109] Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the following protocol: 60 minutes at a 30:1 liquor ratio at temperature of 60°C. This step was followed by 2 cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

D. Separate desizing and perhydrolase treatment ("2-bath process")

[S110] Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the following protocol: 15 minutes at a 30:1 liquor ratio with 0.5 g/l (OPTISIZE® 160) and 0.5 g/L of non-ionic surfactant (ULTRAVON® RW; Huntsman) at 60°C. This step was followed by 2 cold rinses for 5 minutes each. 60 minutes at a 30:1 liquor ratio with 1 g/l perhydrolase (PRIMAGREEN® EcoLight 1.326 U/g, 1.5 mg enzyme protein/g), 6 g/l H₂O₂ solution (30%wt), 3 g/l PGDA (>99.7% pure), 0.5 g/L of non-ionic surfactant (ULTRAVON® RW; Huntsman), and 5 g/l of disodium phosphate dihydrate, pH 8.1, at 60°C. This step was followed by 2 cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

Fabric evaluation

[S111] Color adjustment of each of the fabrics was evaluated using a Minolta Chromameter CR 310 in the CIE Lab color space with a D 65 light source. For each fabric, 10 measurements were taken and the results were averaged. The results are shown in Tables 1 and 2.
Table 1. Color adjustment of sulphur-dyed fabric

<table>
<thead>
<tr>
<th>Sulphur black denim</th>
<th>L/a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wash</td>
<td>24.00/0.12/-1.79</td>
</tr>
<tr>
<td>Amylase desizing</td>
<td>23.82/0.01/-1.96</td>
</tr>
<tr>
<td>Perhydrolase treatment</td>
<td>31.94/-0.90/-3.69</td>
</tr>
<tr>
<td>Desizing+perhydrolase 2-bath process</td>
<td>34.26/-1.11/-3.72</td>
</tr>
</tbody>
</table>

Table 2. Color adjustment of sulphur-indigo-dyed fabric

<table>
<thead>
<tr>
<th>Indy navy sulphur denim</th>
<th>L/a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wash</td>
<td>26.74/1.84/-11.67</td>
</tr>
<tr>
<td>Amylase desizing</td>
<td>26.26/1.96/-11.93</td>
</tr>
<tr>
<td>Perhydrolase treatment</td>
<td>43.30/-2.67/-18.92</td>
</tr>
<tr>
<td>Desizing+perhydrolase 2 bath process</td>
<td>47.54/-2.97/-19.34</td>
</tr>
</tbody>
</table>

The results demonstrate that perhydrolase treatment of sized fabric results in a level of color modification similar to that obtained in a two-step process involving separate desizing and perhydrolase treatment steps. Minimal color modification is observed following desizing, alone, or a water wash.

Example 2: Color adjustment of sulphur-dyed, sized denim, using a perhydrolase system with a triacetin substrate

The ability to modify the color of sulphur-dyed, sized fabric, using a perhydrolase system with a triacetin substrate was investigated. Standard samples of sulphur-dyed fabric or sulphur-indigo-dyed fabric were treated according to one of three different protocols:

A. No desizing, single-step treatment with perhydrolase ("perhydrolase treatment")

Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the following protocol: 60 minutes at a 30:1 liquor ratio, with 1 g/l perhydrolase (PRIMAGREEN® EcoLight 1,326 U/g, 1.5 mg enzyme protein/g), 6 g/l H₂O₂ solution (30% wt/wt) 3 g/l triacetin (>99.7% pure), 0.5 g/L non-ionic surfactant (ULTRAVON® RW; Huntsman), and 5 g/l disodium phosphate dehydrate, pH 8.1 at 60°C. This step was followed by 2 cold rinses for 5 minutes each. The denim was dried in an industrial dryer.

B. Desizing only, no perhydrolase treatment (amylase desizing)

Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1 kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the
following protocol: 60 minutes at 30:1 liquor ratio with 0.5 g/l (OPTISIZE® 160) and 0.5g/L of
a non-ionic surfactant (ULTRAVON® RW; Huntsman) at 60°C. This step was followed by 2
cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

C. Water wash only, no perhydrolase treatment ("water wash")

[116] Sulphur-dyed, sized fabric (2 pieces of 100 g each + ballast) weighing approximately 1.1
kg was treated in a front loading commercial washing machine (Unimac UF 50) according to the
following protocol: 60 minutes at a 30:1 liquor ratio at temperature of 60°C. This step was
followed by 2 cold rinses for 5 minutes each. The fabric was then dried in an industrial dryer.

Fabric evaluation

[117] Color adjustment of each of the fabrics was evaluated using a Minolta Chromameter CR
310 in the CIE Lab color space with a D 65 light source. For each fabric, 10 measurements were
taken and the results were averaged. The results are shown in Tables 3 and 2.

<table>
<thead>
<tr>
<th>Sulphur black denim</th>
<th>L/a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wash</td>
<td>24.00/0.12/-1.79</td>
</tr>
<tr>
<td>Amylase desizing</td>
<td>23.82/0.01/-1.96</td>
</tr>
<tr>
<td>Perhydrolase treatment</td>
<td>32.82/-1.00/-3.97</td>
</tr>
</tbody>
</table>

Table 3. Color adjustment of sulphur-dyed fabric

<table>
<thead>
<tr>
<th>Indy navy sulphur denim</th>
<th>L/a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wash</td>
<td>26.74/1.84/-11.67</td>
</tr>
<tr>
<td>Amylase desizing</td>
<td>26.26/1.96/-11.93</td>
</tr>
<tr>
<td>Perhydrolase treatment</td>
<td>42.99/-2.45/-18.98</td>
</tr>
</tbody>
</table>

Table 4. Color adjustment of sulphur-indigo-dyed fabric

[118] The results demonstrate that perhydrolase treatment of sized fabric using a triacetin
substrate results in a level of color modification that is similar to that obtained using a PGDA
substrate (Example 1). Minimal color modification is observed following desizing, alone, or a
water wash.

[119] Although the foregoing invention has been described in some detail by way of
illustration and examples for purposes of clarity of understanding, it will be apparent to those
skilled in the art that certain changes and modifications may be practiced without departing from
the spirit and scope of the invention. Therefore, the description should not be construed as
limiting the scope of the invention.
[120] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entireties for all purposes and to the same extent as if each individual publication, patent, or patent application were specifically and individually indicated to be so incorporated by reference.
What is claimed is:

1. A method for modifying the color of a dyed, sized textile, comprising, contacting the dyed, sized textile with a perhydrolase enzyme system to modify the color of the textile, wherein the contacting is performed without first desizing the textile.

2. The method of claim 1, wherein the contacting is performed in the absence of desizing the textile.

3. The method of claim 1, wherein the contacting is performed prior to desizing the textile.

4. The method of any of the preceding claims, wherein the perhydrolase enzyme system comprises a perhydrolase enzyme and an ester substrate, wherein the perhydrolase enzyme catalyzes perhydrolysis of the ester substrate with a perhydrolysis:hydrolysis ratio equal to or greater than 1.

5. The method of any of the preceding claims, wherein the perhydrolase enzyme system comprises a *Mycobacterium* perhydrolase or a variant, thereof.

6. The method of claim 5, wherein the perhydrolase enzyme is a variant of *Mycobacterium smegmatis* perhydrolase comprising the amino acid substitution S54V.

7. The method of any of the claims 4-6, wherein the ester substrate is PGDA or triacetin.

8. The method of any of the preceding claims, wherein the size comprises starch, a starch-like material, or PVA.

9. The method of any of the preceding claims, wherein the textile is denim.

10. The method of any of the preceding claims, wherein the textile comprises cotton.
11. The method of any of the preceding claims, wherein the dye is a sulphur dye.

12. A textile produced by the method of any of the preceding claims.
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/US2012/029009

### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** D96P5/ 13 D06P5/ 15

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)
D06P D06L

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

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X</td>
<td>wo 2010/ 13960 1 AI (HUNTSMAN ADV MAT SWITZERLAND [CH] ; VERMEERSCH LODE [DE] ; REDLING ERWIN N) 9 December 2010 (2010- 12-09 ) page 2, last paragraph page 8, paragraph 4 - page 11, paragraph 3 page 16, paragraph 1 page 17, paragraph 2; claims; examples 1, 2, 11</td>
<td>1 - 12</td>
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<td>A</td>
<td>wo 2010/030769 1 AI (DANISCO US INC [US]; AUTERI NEN ANNA-LIISA [US]; PROZZO BLANCAMARI [US]) 18 March 2010 (2010-03-18) page 32, last paragraph; claims</td>
<td>1 - 12</td>
</tr>
</tbody>
</table>

**□** Further documents are listed in the continuation of Box C.  
See patent family annex.

* Special categories of cited documents:
  * "A" document defining the general state of the art which is not considered to be of particular relevance,
  * "E" earlier application or patent but published on or after the international filing date,
  * "L" document which may throw doubts on priority claims(s) or which is cited to establish the publication date of another citation or other special reason (as specified),
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Blas, Valerie
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