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Title: METHOD FOR ENZYMATICAL PREPARATION OF TEXTILES

Abstract: Disclosed is a process for bioscouring a fabric based on the use of enzymes. The process provide ready to dye fabric at least equal to similar fabric scoured using a conventional chemical based process. The disclosed process is fast, uses smaller amounts of chemicals, water and energy compared to the conventional process and is therefore a more sustainable process. Further a multifunctional wetting agent is disclosed.
METHOD FOR ENZYMATIONAL PREPARATION OF TEXTILES

The following specification particularly describes the invention

FIELD OF THE INVENTION
The present invention relates to an improved method for preparing a Grey or 'Ready-for-Dyeing' (RFD) textile, in particular the invention relates to an improved method for enzymatic scouring of textiles.

BACKGROUND OF THE INVENTION
The processing of fabric, such as cellulosic material, into material ready for garment manufacture involves several steps including preparation, dyeing and finishing operations. The preparation process, which may involve desizing (for woven goods), scouring, and bleaching, produces a fabric suitable for dyeing and/or finishing.

Processing of fabrics has traditionally been performed using processes that requires significant amounts of chemicals, water and energy. Water and energy are limited resources and in many countries there is a desire to reduce the consumption of water and energy in order to sustain a growing population. Further the use of significant amounts of chemicals and energy often leads to pollution of the environment. There is, therefore, a desire to reduce the amount of chemicals, water and energy use for the processing of textiles.

SUMMARY OF THE INVENTION
The invention provides an eco-friendly method for preparing a Grey or 'Ready-for-Dyeing' (RFD) fabric comprising or consisting of the steps in following sequence:

- a. Washing the fabric in hot water
- b. Desizing
- c. Mercerizing
- d. Hot wash with a multifunctional wetting agent
- e. Bioscouring
- f. Optional hot wash with a multifunctional wetting agent
- g. Peroxide rinse/peroxide bleaching
- h. Bleach clean up and wash.

Further the invention provides a multifunctional wetting agent and the use thereof for treating fabrics.
This invention eliminates singeing process, which is a common practice to remove the protruding fiber. This helps avoiding the starch getting scorched at singeing temperature ( > 100 °C) which causes problem in its removal.

5 SHORT DESCRIPTION OF THE FIGURES

Figure 1 shows a dosage profile of a MWA of the invention with IPA. See Example 3 for details. Figure 2 shows a dosage profile of a MWA of the invention without IPA. See Example 3 for details.

10 DETAILED DESCRIPTION OF THE INVENTION

Definitions

HLB-value


25 Description of the invention

The present invention is directed towards providing an enzymatic desizing and scouring process that is more sustainable compared to the traditional chemical based desizing and scouring processes. The process of the invention may be carried out using traditional desizing equipment e.g., pad systems such as pad-batch, pad-steam, J-box, jet, jigger, etc. No additional process equipment is needed. This is accomplished by simultaneously treating the fabric with a combination of alpha-amylase and scouring enzyme in the process of the invention. The inventors have found that, beside the advantages obtained by carrying out desizing and scouring simultaneously, other advantages are obtained as well.

The invention uses a number of steps that as such are known in the art, but the order of the steps has been changed compared to the conventional process and the chemicals have been
replaced with enzymes where possible which surprisingly has resulted in a completely new process that can provide the desired desizing and scouring efficiency in a short time, with reduced use of chemicals, water and energy compared to a conventional chemical based processes. Further, the process provides ready for dyeing (RFD) fabrics that are at least equal to or better to fabrics prepared by conventional methods in respect of acceptance for dyeing. The weaving process demands a "sizing" of the warp yarn to protect it from abrasion and for improved weavability. Starches both, unmodified and modified, polyvinyl alcohol (PVA), carboxymethyl cellulose (CMC), lubricants, waxes and acrylic binders, and mixtures thereof, are examples of typically used sizing agents. The sizing agent(s) must be removed after the weaving process as the first step in preparing the woven goods.

Further, the cellulosic fibers contain natural non-cellulosic impurities, which must be removed before the subsequent processing steps, such as dyeing, printing and finishing. Scouring removes much of the natural non-cellulosic impurities, including especially waxes, pectins and proteins. A proper wax removal is necessary for obtaining a high wettability, being a measure for obtaining a good dyeing. Removal of pectins - improves wax removal and ensures a more even dyeing. In addition, scouring can remove dirt, soils and residual manufacturing introduced materials such as spinning, coning or sizing agents.

Method of the invention

Hot wash

In the first step of the method of the invention the textile is washed in hot water. In this step, water soluble sizing agent are partly or completely removed and starch based sizing agent swell and loosen and thereby becomes more accessible for the action of enzymes in subsequent steps.

The wash should be performed at an elevated temperature that is sufficiently high to swell and loosen any starch or starch based agents present in the textile. Thus, in general, the wash is performed at a temperature between the gelatinization temperature of the starch or the starch based agent and the boiling point of water. Thus, the wash is in general performed at a temperature between 65°C and 100 °C, preferably in the range of 70°C and 100 °C; more preferred in the range of 80°C and 100 °C; even more preferred in the range of 90°C and 100 °C and most preferred one in the range of 95°C and 99°C. In a preferred embodiment the water is boiling water which is understood as water having a temperature of 95°C or more.

The wash should be performed in a sufficient long time to allow an efficient swelling and loosening of the starch or starch based agent and at the other side it should be sufficiently short to se-
cure a satisfactory high capacity of the plant. The wash is in general performed in a period from a few minutes and up to about 1.5, such as in the range of 5 minutes and 90 minutes, preferably in the range of 10 minutes and 50 minutes, preferably in the range of 15 minutes and 40 minutes, preferably in the range of 20 minutes and 30 minutes, preferably about 30 minutes. In a particular embodiment the wash is performed in a period of about 40 minutes or 2 turns in jigger or a few minutes for continuous process such as in soaper/washer.

In a preferred embodiment the hot wash is performed at a temperature in the range of 80°C to 95°C for 30 minutes or less to accommodate the step in conventional machine set-up. The wash liquid is in general process water typically the softened water supply or tap water.

**Desizing**

In the second step of the method of the invention starch or starch based agents and pectins etc., are removed.

The second step is performed using a combination of enzyme treatment in the presence of a suitable wetting agent, such as an non-ionic wetting agent, as known in the art. Preferably this step also removes pectins and waxes naturally present in cotton fibres, and therefore is an enzyme cocktail comprising a pectinase often used in this step. This step may be performed at any conditions where the enzymes have good activity and are reasonably stable during the whole process.

The temperature is preferably selected sufficiently high to secure a high catalytic rate of the enzymes and sufficiently low to avoid excess heat denaturation of the enzymes. In general the temperature of the step is selected in the range of room temperature and 80°C, preferably in the range of 30°C and 80°C, more preferred in the range of 50°C to 70°C and most preferred in the range of 55°C to 65°C.

The pH should be selected to provide for good activity of the enzymes and reasonable stability thereof during the process time. In general the pH is selected in the range of 5 to 9, preferably in the range of 6 to 8 and most preferred in the range of 6.5 to 7.

The process in this step should be continued in a sufficient time to allow efficient removal of all starches and starch based material from the fabrics. In general a time in the range 16 to 24 hours for pad batch processes at ambient temperature, around 1.5 to 4 hours for exhause (jigger) or 10-20 minutes for continuous (pad stream) process would be adequate.

The enzyme treatment is performed using desizing enzymes such as amylases optionally in combination with a smaller amount of pectinases. The enzyme treatment is preferably performed using a combination of starch degrading enzymes and pectin degrading enzymes. Starch degrading enzymes include amylases, preferably alpha-amylases. Pectin degrading enzymes
include pectinases preferably pectate lyases. Enzymes for use according to the invention should be selected according to the intended conditions in the particular step where the enzymes are used, for example, should enzymes having sufficient activity at the intended pH of the particular step be selected and further should enzymes having a reasonable stability at the intended temperature of the particular step be selected for such use. All this is known in the art and it is within the scope of the skilled person to select suitable enzymes based on the intended conditions.

The desizing enzymes are used in sufficient amounts to remove the starches and starch based materials. Thus the amount of enzymes used may depend on the particular used sizing agent, the amount of sizing agent used for the particular fabric, the intended time for the desizing step and the conditions applied during the process and the amount can be determined based on the manufacturers recommendations or the skilled person can determine a suitable dosing of the enzymes based on the simple experiments completely within the skills of the average practitioner.

The recommend concentration of a commercial desizing alpha-amylase, such as AQUAZYM™ SD-L (Novozymes A/S, Denmark), lies in the range from about 180 to 240 KNU/L, corresponding to about 180-240 KNU per kg fabric. Such a concentration also applies for the present invention.

In a preferred embodiment the alkaline alpha-amylase is present in a concentration of 0.05-150 KNU/L treating solution, preferably, 1-100 KNU/L treating solution, especially 2-20 KNU/L treating solution or 0.05-150 KNU/Kg fabric, preferably, 1-100 KNU/kg fabric, especially 2-20 KNU/kg fabric.

Further, the recommended concentration of a commercial pectinase for scouring, such as SCOURZYME™ L (Novozymes A/S, Denmark), lies in the range of about 1500-1875 APSU/L, which corresponding to about 1500-1875 APSU per kg fabric.

In a preferred embodiment the pectinase enzyme is a pectate lyase present in a concentration in the range from 1-1,500 APSU/kg fabric, preferably 10-1,200 APSU/kg fabric, especially 100-1,000 APSU/kg fabric.

The wetting agent should be added in sufficient amounts to allow a satisfactory wetting of the fabrics. It is within the skills of the average practitioner to find a suitable dosage of the wetting agent based on simple routine experiments. In general the wetting agent is added in an amount of between 0.5 g/l and 10 g/l based on the total volume of the desizing fluid, preferably in the range of 1 g/l and 5g/l, more preferred in the range of 1 g/l and 4 g/l and most preferred in the range of 1 g/l and 3 g/l.
The multi wetting agent (MWA) of the invention performs not only as a wetting agent, it also serves as a scouring aid to remove non-cellulosic by emulsification. After the desizing procedure the fabrics are washed in order to remove the degraded/liquefied starches, preferably by hot washes in multi box washer.

In a preferred embodiment the second step in the method of the invention is performed using the conditions of a temperature of 60°C and pH of 6.5 for 1.5 hour or 4 turns in case of jigger process for several hours exceeding eight hours in case of pad-batch system, followed by hot washes at 85°C giving 2 turns in case of jigger washing.

10 **Mercerize and wash**

The third step of the method of the invention is the mercerization step which is performed in a mercerization machine as known in the art.

The mercerization step is performed by treating the fabrics with base in a mercerization machine. A suitable base for this step is NaOH even though also other strong bases may be used.

Suitable NaOH is used in amounts of approximately 300 g/l. Further a suitable mercerizing wetting agent is added in the mercerization step preferably in amounts in the range of 1 g/l to 10 g/l preferably about 5 g/l.

The mercerization is preferably performed at low temperature in order to impart lustre to the fabric or at elevated temperatures, such as in the range of 10°C to 50°C, more preferred in the range of 15°C to 30°C and most preferred around 25°C.

After the mercerization the fabrics are washed conventionally in hot water, including in recuperator and scouring until the washed fabric is neutral.

When neutrality has been reached the fabrics are washed once more in water comprising a multifunctional wetting agent (MWA) in an amount in the range of 0.1 g/l to 10 g/l, preferably in the range of 0.5 g/l to 8 g/l and most preferred in the range of 2 g/l to 7 g/l.

This last treatment with MWA is performed at temperature between 40°C and 90°C, preferably 60°C and 85°C and most preferably between 70°C and 80°C for 10 minutes in case of soaper or by giving 2 turns in case of jigger.

30 **Bioscouring**

The next step in the method of the invention is the bioscouring where any residual pectins, pectinaceous materials, waxy materials are removed from the fabrics.

This step is performed as an enzymatic treatment of the fabrics using pectinases and a neutral cellulase.
This step may be performed at any conditions where the enzymes have good activity and are reasonably stable during the whole process.

The temperature is preferably selected sufficiently high to secure a high catalytic rate of the enzymes and sufficiently low to avoid excess heat denaturation of the enzymes. In general the temperature of the step is selected in the range of room temperature and 80°C, preferably in the range of 30°C and 80°C, more preferred in the range of 50°C to 70°C and most preferred in the range of 55°C to 65°C.

The pH should be selected to provide for good activity of the enzymes and reasonable stability thereof during the process time. In general the pH is selected in the range of 5 to 9, preferably in the range of 6 to 8 and most preferred in the range of 6.5 to 8.

The process in this step should be continued for a sufficient time to allow efficient removal of all pectins, pectinaceous materials from the fabrics. In general a time in the range of 5 minutes and 3 hours is sufficient, preferably the step is conducted at a time between 30 minutes and 2 hours, most preferred around 1.5 hours or 4 turns for jigger process or 16-24 hours batching for pad-batch process at ambient temperature or a brief steaming in case of pad-batch process.

After the enzymatic treatment, the enzymatic reactions are stopped by methods known in the art for stopping enzymes, e.g. by altering the pH to a pH value where the enzymes are inactive or even denatured, by inactivating the enzymes by heat treatment or even by adding compound(s) known to inhibit the particular used enzymes. It may even be possible to omit the step of stopping the enzymes by proceeding immediately to the next step where the pH is highly alkaline which will inactivate enzymes.

A convenient way to stop the enzymatic reaction is by treating the fabric with an alkaline solution such as a solution of sodium carbonate in water. Suitable conditions for this is treating the textile in a solution of up to 10 g/l Na₂C₀₃, preferably about 5g/l Na₂C₀₃ and a temperature of about 80°C.

After the enzymatic reactions have been stopped the fabrics is washed in hot water e.g. 85°C; where the multifunctional wetting agent is added or, may not be added for clean up.

Optionally a cold wash may be included as a final step.

**Peroxide rinse (for subsequent dyeing in dark shades)**

Next step in the method of the invention is a peroxide rinse where remaining coloured substances are bleached. Peroxide rinse denotes a mild bleach.

In the peroxide rinse the fabrics are treated by a solution of hydrogen peroxide in water.

Hydrogen peroxide is used in an amount of up to 10 ml of a concentrated solution (50%, ) per liter solution, preferably in the range of 5 to 10 ml/l, more preferred in the range of 8 to 10 ml/l.
pH should be regulated to 10.5 or higher, preferably 11 or higher using alkali. Stabilizer for peroxide, wetting agent and conventional additives to peroxide bath may also be used.

The temperature should be in the range of 50°C to 100°C, preferably in the range of 60°C to 95°C, more preferred in the range of 70°C to 90°C and most preferred around 85°C for a jigger process or a brief steaming of 10-20 minutes in Continuous Bleaching Range (CBR).

**Peroxide Bleaching (for Whites and Pestal Shades)**

If the treatment is for preparing white or pestal shaded fabrics it is in general recommended to use a stronger peroxide bleaching process in order to obtain satisfactory white. Such a treatment is similar to above except that the hydrogen peroxide (50%) is used in an amount of up to 50 ml of a concentrated solution (50%) per liter solution in a streamer allowing residence time of 10-20 minutes. Similar treatment in a jigger it also possible.

Peroxide bleaching as well as finding suitable conditions for a particular batch of fabric intended for dyeing is within the scope of the skilled person.

**Bleach clean-up and wash**

After the peroxide treatment any remaining peroxide is removed and the fabric is washed and dried. Thereafter the fabrics are ready for dyeing and/or other suitable treatments.

Preferably, the peroxide is removed enzymatically using an enzyme that degrades hydrogen peroxide such as a catalase. Methods for removal of hydrogen peroxide is known in the art and such methods may be applied according to the present invention.

This step may be performed at any conditions where the enzyme have good activity and are reasonably stable during the whole process.

The temperature is preferably selected sufficiently high to secure a high catalytic rate of the enzymes and sufficiently low to avoid excessive heat denaturation of the enzymes.

The pH should be selected to provide for good activity of the enzymes and reasonable stability thereof during the process time. In general the pH is selected in the range of 5 to 9, preferably in the range of 6 to 8 and most preferred in the range of 6.5 to 7.

The process in this step should be continued for a sufficient time to allow efficient removal of all hydrogen peroxide.

In one preferred embodiment, the bleach clean up process is performed after the peroxide rinse/bleach step using Terminox catalase enzyme (available from Novozymes A/S Bagsvaerd, Denmark).

The method of the invention provide ready to dye fabrics in a process that is fast, uses less water, chemicals and energy, and further provides less pollutants. Further the fabrics prepared by
the method have a better preserved mechanical properties & tactile and it is in good shape for accepting dye.

The method of the invention provides several benefits in comparison with the traditional chemical based process, particularly in Jigger processing and in oxidative pad-batch desizing.

The process is fast and a significant time reduction can be achieved, such as a time reduction of at least 5%, preferably at least 10%, more preferred at least approximately 15%, compared with the traditional process, meaning that the capacity of a plant is increased.

The process uses less water than the conventional process and up to at least 5%, preferably at least 10%, preferably at least 15%, preferably at least 20% an most preferred 24% or more water can be saved. This also means that the volume of waste water can be reduced.

The process uses less chemicals meaning that the pollutant load on the environment is reduced.

The fiber-loss and damage is minimized meaning that the mechanical properties & tactile is better preserved. It also helps in maintaining the weight of the fabric.

Less energy in form of heating water and electricity to run the equipment is used.

The resultant fabrics is better to accept dye.

Amylases

Any alpha-amylase may be used according to the invention, however, it is preferred to use an alkaline or a neutral alpha-amylase. An amylase is "alkaline" in context of the present invention when the pH optimum under the conditions present during simultaneously desizing and scouring is above 7, preferably above 8, especially above 9. An amylase is "neutral" in context of the present invention when the pH optimum under the conditions present during simultaneously desizing and scouring is around 7 such as in the range of 5.5 to 8.5, preferably in the range of 6 to 8.

Suitable alpha-amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included. A preferred alkaline alpha-amylase is derived from a strain of Bacillus, such as Bacillus licheniformis, Bacillus amylo liquefaciens, Bacillus stearothermophilus, Bacillus subtilis, or other Bacillus sp., such as Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513, DSM 9375, DSMZ no. 12649, KSM AP1378 (WO 97/00324), KSM K36 or KSM K38 (EP 1,022,334). Preferred are the Bacillus sp. alpha-amylases disclosed in WO 95/26397 as SEQ ID NOS. 1 and 2 (i.e., SEQ ID NO: 4 herein), respectively, the alpha-amylase disclosed as SEQ ID NO: 2 in WO 00/60060 (i.e., SEQ ID NO: 6 herein), and the #707 alpha-amylase disclosed by Tsukamoto et al., Biochemical and Biophysical Research Communications, Vol. 151, pp. 25-31 (1988).
Commercially available alkaline alpha-amylase products or products comprising alpha-amylases include product sold under the following tradenames: NATALASE™, STAINZYME™ (Novozymes A/S), BIOAMYLASE - D(G), BIOAMYLASE™ L (Biocon India Ltd.), KEMZYM™ AT 9000 (Biozym Ges. m.b.H, Austria), PURASTAR™ ST, PURASTAR™ HPAmL, PURAFECT™ OxAm, RAPIDASE™ TEX (Genencor Int. Inc, USA), KAM (KAO, Japan).

For purposes of the present invention, the degree of identity between two amino acid sequences is determined by the Clustal method (Higgins, 1989, CABIOS 5: 151-153) using the LASERGENE™ MEGALIGN™ software (DNASTAR, Inc., Madison, WI) with an identity table and the following multiple alignment parameters: Gap penalty of 10, and gap length penalty of 10. Pairwise alignment parameters were Ktupel=1, gap penalty=3, windows=5, and diagonals=5].

In an embodiment of the process of the invention the alkaline alpha-amylase may preferably be present in a concentration of 0.05-150 KNU/L treating solution, preferably, 1-100 KNU/L treating solution, especially 2-20 KNU/L treating solution or 0.05-150 KNU/Kg fabric, preferably, 1-100 KNU/kg fabric, especially 2-20 KNU/kg fabric.

**Scouring enzymes**

Any scouring enzyme may be used according to the invention, however, it is intended to use an alkaline or a neutral scouring enzyme. The alkaline scouring enzyme may be an alkaline enzyme selected from the group consisting of pectinase, cellulase, lipase, protease, xylolucanase, cutinase and a mixture thereof. A scouring enzyme is "alkaline" in context of the present invention when the pH optimum under the conditions present during scouring is above 7, preferably above 8, and up to 9. A scouring enzyme is "neutral" in context of the present invention when the pH optimum under the conditions present during simultaneously desizing and scouring is around 7 such as in the range of 5.5 to 8.5, preferably in the range of 6 to 8.

In a preferred embodiment the alkaline pectinase is a pectate lyase, a pectin, lyase, a polygalacturonase, or a polygalacturonate lyase.

**Pectinase**

The term "pectinase" is intended to include any pectinase enzyme. Pectinases are a group of enzymes that hydrolyse glycosidic linkages of pectic substances mainly poly-1,4-alpha-D-galacturonide and its derivatives (see reference Sakai et al., Pectin, pectinase and propectinase: production, properties and applications, in: Advances in Applied Microbiology, Vol. 39, pp. 213-294 (1993)) which enzyme is understood to include a mature protein or a precursor form thereof, or a functional fragment thereof, which essentially has the activity of the
full-length enzyme. Furthermore, the term pectinase enzyme is intended to include homologues or analogues of such enzymes.

Preferably the alkaline pectinase is an 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. Also preferred is a pectinase enzyme which catalyzes the random hydrolysis of alpha-1,4-glycosidic linkages in pectic acid such as the enzyme class polygalacturonase (EC 3.2.1.15) (PG) also known as endo-PG. Also preferred is a pectinase enzyme such as polymethylgalacturonate lyase (EC 4.2.2.10) (PMGL), also known as Endo-PMGL, also known as poly(methoxygalacturonate) lyase also known as pectin lyase which catalyzes the random cleavage of alpha-1,4-glycosidic linkages of pectin. Other preferred pectinases are galactanases (EC 3.2.1.89), arabinanases (EC 3.2.1.99), pectin esterases (EC 3.1.1.1), and mannanases (EC 3.2.1.78).

The enzyme is preferably derived from a micro-organism, preferably from a bacterium, an archa or a fungus, especially from a bacterium such as a bacterium belonging to the genus Bacillus, preferably to an alkalophilic Bacillus strain which may be selected from the group consisting of the species Bacillus licheniformis and highly related Bacillus species in which all species are at least 90% homologous (identical) to Bacillus licheniformis based on aligned 16S rDNA sequences. Specific examples of such species are the species Bacillus licheniformis, Bacillus alcalophilus, Bacillus pseudoalcalophilus, and Bacillus clarkii. A specific and highly preferred example is the strain Bacillus licheniformis, ATCC 14580 (U.S. Patent No. 6,284,524). Other useful pectate lyases are derivable from the species Bacillus agaradhaerens, especially from the strain deposited as NCIMB 40482; and from the species Bacillus subtilis, Bacillus stearothermophilus, Bacillus pumilus, Bacillus cohnii, Bacillus pseudoalcalophilus, Erwinia sp. 9482, especially the strain FERM BP-5994, and Paenibacillus polymyxa.

The pectinase may be a component occurring in an enzyme system produced by a given micro-organism, such an enzyme system mostly comprising several different pectinase components including those identified above.

Alternatively, the pectinase may be a single component, i.e., a component essentially free of other pectinase enzymes which may occur in an enzyme system produced by a given micro-organism, the single component typically being a recombinant component, i.e., produced by cloning of a DNA sequence encoding the single component and subsequent cell transformed with the DNA sequence and expressed in a host. Such useful recombinant enzymes, especially pectate lyases, pectin lyases and polygalacturonases are described in detail in, e.g., WO 99/27083 and WO 99/27084 which are hereby incorporated by reference in their entirety.
including the sequence listings. The host is preferably a heterologous host, but the host may
under certain conditions also be the homologous host.
In a preferred embodiment the pectate lyase used according to the invention is derived from
the genus *Bacillus*, preferably the species *Bacillus licheniformis*, *Bacillus alcalophilus*, *Bacillus
pseudoalcalophilus*, and *Bacillus clarkia*, especially the species *Bacillus licheniformis*, ATCC
14580.
In an even more preferred embodiment the pectate lyase is a mature pectase lyase derived
from a strain of *Bacillus licheniformis*. The pectate lyase is also disclosed in U.S. Patent No.
6,284,524, which is hereby incorporated by reference.
The pectinase, such as especially pectate lyase, may preferably be present in a concentration
in the range from 1-1,500 APSU/kg fabric, preferably 10-1,200 APSU/kg fabric, especially 100-
1,000 APSU/kg fabric.
Commercially available alkaline pectate lyases include BIOPREP™ and SCOURZYME™ L from
Novozymes A/S, Denmark.

Cellulase
The term "cellulase" denotes an enzyme that contributes to the hydrolysis of cellulose, such a
cellbiohydrolase (abbreviated as "CBH"; Enzyme Nomenclature E.C. 3.2.1.91), an
endoglucanase (hereinafter abbreviated as "EG", E.C. 3.2.1.4), or a beta-glucosidase
(abbreviated as "BG", E.C. 3.2.1.21). Cellulases are classified in a series of enzyme families
encompassing endo- and exo- activities as well as cellobiose hydrolyzing capability. The
cellulase used in practicing the present invention may be derived from microorganisms which
are known to be capable of producing cellulosolytic enzymes, such as, e.g., species of *Humicola*,
Thermomyces, *Bacillus*, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Irpex,
Scytalidium, Schizophyllum, Penicillium, Thielavia, Aspergillus, or Geotrichum, particularly
*Humicola insolens*, *Fusarium oxysporum*, or *Trichoderma reesei*. Non-limiting examples of
suitable cellulases are disclosed in U.S. Patent No. 4,435,307; European patent application No.
0 495 257; PCT Patent Application No. W091/17244, W091/17243, W098/12307; and
The term "neutral" cellulase is according to the invention intended to mean a cellulase that have
significant activity at a neutral pH, such as at a pH between 6 and 8, preferably about 7.
The cellulases used in this invention can be monocomponent or multi-components.
Monocomponent, i.e. a cellulase which is essentially free from other proteins, in particular other
cellulases. Monocomponent enzymes can be prepared economically by recombinant DNA
technology, i.e. they can be produced by cloning of a DNA sequence encoding the
monocomponent, subsequently transforming a suitable host cell with the DNA sequence and expressing the component in the host. Cellulases of multi-components contain more than one cellulase. Cellulases of multi-components can be blends of two or more cellulases, or can be derived from wild type strain.

The DNA sequence coding for a useful cellulase may for instance be isolated by screening a cDNA library of the microorganism in question and selecting for clones expressing the appropriate enzyme activity (i.e. cellulase activity).

A DNA sequence coding for a homologous enzyme, i.e. an analogous DNA sequence, may be obtainable from other microorganisms. For instance, the DNA sequence may be derived by similarly screening a cDNA library of another fungus, such as a strain of an *Aspergillus* sp., in particular a strain of *A. aculeatus* or *A. niger*, a strain of *Trichoderma* sp., in particular a strain of *T. reesei*, *T. viride*, *T. longibrachiatum*, *T. harzianum* or *T. koningii* or a strain of a *Neocallimastix* sp., *Humicola* sp., *Pirromyces* sp., *Penicillium* sp., *Agaricus* sp., or a *Phanerochaete* sp.

Preferably, the cellulase is derived from or producible by a strain of *Scytalidium* (*f.* *Humicola*), *Fusarium*, *Myceliophthora*, more preferably derived from or producible by *Scytalidium thermophilum* (*f.* *Humicola insolens*), *Fusarium oxysporum* or *Myceliophthora thermophila*, most preferably from *Humicola insolens*, DSM 1800, *Fusarium oxysporum*, DSM 2672, or *Myceliophthora thermophila*, CBS117.65.

In one embodiment of the invention, the cellulase is an endoglucanase, preferably cellulase from family 45 EG, such as the amino acid sequence of the *Thielavia* terrestis endoglucanase shown in SEQ ID No. 1 in WO 96/29397 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 1, reacts with an antibody raised against said endoglucanase, and/or is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase. In another embodiment of the invention, the cellulase is an endoglucanase comprising the amino acid sequence of the *Humicola* insolens endoglucanase shown in SEQ ID No. 2 in WO 91/17243 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 2, reacts with an antibody raised against said endoglucanase, and/or is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase. In a further embodiment of the invention, the cellulases used in the invention are commercially available multi-components cellulase enzyme product such as Cellusoft L, Cellish L (Novozymes A/S, Denmark), Primafast 100, Primafast 200 (Genencor International Inc.), Rocksoft ACE (Dyadic), and Youteer 800 (Youteer Co. Ltd, China).
The host cell which is transformed with the DNA sequence is preferably a eukaryotic cell, in particular a fungal cell such as a yeast or filamentous fungal cell. In particular, the cell may belong to a species of *Aspergillus* or *Trichoderma*, most preferably *Aspergillus oryzae* or *Aspergillus niger*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplast followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host microorganism is described in EP 238 023 (Novo Nordisk A/S), the contents of which are hereby incorporated by reference. The host cell may also be a yeast cell, e.g. a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*, *Saccharomyces kluveri* or *Saccharomyces uvarum*, a strain of *Schizosaccharomyces* sp., such as *Schizosaccharomyces pombe*, a strain of *Hansenula* sp., *Pichia* sp., *Yarrowia* sp. such as *Yarrowia lipolytica*, or *Kluyveromyces* sp. such as *Kluyveromyces lactis*.

In the context, an analogue of the proteins comprises "variant proteins". In some preferred 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. In the present context, the term "homologous" or "homologous sequence" is intended to indicate an amino acid sequence differing from another protein, by one or more amino acid residues, preferably 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50 or more amino acid residues. For example, in some embodiments, variant proteins have one to ten difference from the parent protein. The homologous sequence may be one resulting from modification of an amino acid sequence shown in these listings, e.g. involving substitution of one or more amino acid residues at one or more different sites in the amino acid sequence, deletion of one or more amino acid residues at either or both ends of the enzyme or at one or more sites in the amino acid sequence, or insertion of one or more amino acid residues at one or more sites in the amino acid sequence.

However, as will be apparent to the skilled person, amino acid changes are preferably of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding or activity of the protein, small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain. See in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. Examples of conservative substitutions are within the group of basic amino acids (such as arginine, lysine, histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine, isoleucine, valine), aromatic amino acids (such as phenylalanine, tryptophan, tyrosine) and small amino acids (such as glycine, alanine, serine, threonine, methionine).
The modification of the amino acid sequence may suitably be performed by modifying the DNA sequence encoding the enzyme, e.g. by site-directed or by random mutagenesis or a combination of these techniques in accordance with well-known procedures. Alternatively, the homologous sequence may be one of an enzyme derived from another origin than the cellulases corresponding to the amino acid sequences shown in each of the sequence listings shown hereinafter, respectively. Thus, "homologue" may e.g. indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for the cellulase with the amino acid sequence in question under certain specified conditions (such as presoaking in 5 x SSC and prehybridising for 1 h at -40°C in a solution of 20% formamide, 5 x Denhardt's solution, 50 mM sodium phosphate, pH 6.8, and 50 mg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 mM ATP for 18 h at -40°C). The homologous sequence will normally exhibit a degree of homology (in terms of identity) of at least 50%, such as at least 60%, 65%, 70%, 75%, 80%, 85%, 90% or even 95% with the amino acid sequences shown in each of the sequence listings shown hereinafter, respectively.

The homology referred to above is determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Needleman, S.B. and Wunsch, C.D., Journal of Molecular Biology, 48: 443-453, 1970).

**Bleach clean-up Enzymes:**

Bleach clean-up enzymes refer to enzymes which can catalyze for the conversion of hydrogen peroxide into water and oxygen, such as catalase (EC 1.11.1.6). Preferred catalases that are suitable for use in a process according to the invention are catalases that is derived from bacteria such as *Bacillus*, *Pseudomonas* or *Streptomyces strain*; yeast such as *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Yarrowia* or *Schizosaccharomyces*; fungal such as *Acremonium*, *Scytalidium*, *Aspergillus*, *Coprinus*, *Aureobasidium*, *Bjerkandera*, *Humicola*, *Ceriporiopsis*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Magnaporthe*, *Mucor*, *Myceliphthora*, *Neocallichemis*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tokyoiodro* or *Trichoderma strain*; or animal such as pig liver, beef liver. Non-limiting examples of suitable catalases are disclosed in W092/17571, CN1563373, US20031001 12-A1, EP1336659-A, US2003074697-A1, US6201 167-B1, US6022721-A, EP931831-A, JP1 1046760-A, W09317721-A, WO9309219-A, JP1086879-A and/or JP63003788-A. Non-limiting examples...
are T 100; Oxy-Gone 400 (GCI); Fermcolase 1000 (Mitsubishi Gas Chemical) or Thermocatalase CTL 200 or JH CT 1800 (Mitsubishi Gas Chemical).

In one embodiment of the present invention, the catalase used is derived from Scytalidium thermophilum with SEQ ID No:3 (as described in US 5,646,025), or analogue or particularly variant proteins of it.

Depending on the activity of the catalase and the pH of the liquor used to apply the catalase, preferably the amount of catalase used is from 0.001 to 1 g/l, especially about 5 g/l of liquor used to apply the catalase.

Wetting agent

The invention provides a new Multifunctional Wetting Agent (MWA) based on HLB concept that have been specially developed for the purpose of deep cleansing action. Enzymes are large molecules that provides action of the fiber surface for the removal of the particular molculed the enzymes have specificity for such as non-cellulosic impurities, particular the waxy materials.

The MWA of the invention provides for the removal of waxy materials and hydrophobic impurities from within the fibre also. Thus, the MWA of the invention functions also as scouring aid providing assistance to enzymatic scouring. The MWA is not only a wetting agent but functions also as an emulsifying agent, dispersing agent, penetrant and sequestrant in addition to having matching HLB in respect of waxy impurities.

A wetting agent is according to the invention intended to mean a compound or composition hav- ing surfactant character and which is capable of wetting / emulsifying/ dispersing components , such as waxes, waxy materials normally found in connection with cotton. The HLB value of the wetting system should be selected to match that of cotton impurities, in particular match the wax and/or waxy materials usually found connected to native cotton.

In one preferred embodiment the wetting agent of the invention is a MWA which is understood as a composition that is capable of functioning as one or more of wetting agent, penetrant, surfactant, dispersant, emulsifier, detergent and sequestrant. The MWA comprises or consists of at least three component selected from at least three of the following groups: non-ionic surfactants, anionic surfactant, penetrants and sequestrant. Preferably the MWA comprises of consists of at least four component selected from at least four of the following groups: non-ionic surfactants, anionic surfactant, penetrants & sequestrant.

In a particular preferred embodiment the MWA comprises or consist of two or more non-ionic surfactants, one anionic surfactant a penetrant and optionally a sequestrant. In a preferred embodiment the MWA comprises one non-ionic surfactant having a low HLB, such as 1-
5, preferably 1.5 to 4.5; and one non-ionic surfactant having a high HLB, such as 10-15, preferably 12-14.

The HLB for the MWA should be selected in the range of 5 to 12. Preferably in the range of 8 to 12 and more preferred in the range of 10 to 12.

Those skilled in the art can make a suitable combination for obtaining a desired HLB value of the MWA.

A proper combination of the components of MWA results in a definite HLB value. Persons skilled in the art can make a suitable combination for obtaining a desired HLB value of the MWA.

For example, a combination of Sorbitan trioleate 150 g, Sodium oleate 150 g, Iso propyl alcohol 400 g, Ethylene oxide condensate comprising in average 9-12 moles ethylene oxide units per molecule 300 g results in HLB value 6.8; similarly a combination of Ethylene glycol monostearate 300 g, Sodium oleate 350 g, Isopropyl alcohol 50 g, and Ethylene oxide condensate comprising in average 9-12 moles ethylene oxide units per molecule 250 g, sodium pyrophosphates 50 g results in HLB value 10.37.

In another example the MWA comprises or consists of 10-20% by weight of a non-ionic surfactant having a HLB in the range of 1.5 to 4.5; 20-30% by weight of a non-ionic surfactant having a HLB in the range of 12-14; 15-23% by weight of an anionic surfactant and 35-45% by weight of alkyl alcohol.

The non-ionic surfactants have the properties of good dispersing and emulsifying power, excellent grease removal, efficient soil removal, resistant to water hardness de-activation and have low foaming properties. The non-ionic surfactants are preferably selected among ethylene oxide condensates comprising in average 5-20 ethylene oxide units per molecule with sorbitan oleate, stearate, palmitate, stearic acid steryl alcohol, lauryl alcohol, cetyl alcohol, glycerol oleate, ethylene glycol monostearate or any mixture thereof.

Fatty alcohol ethylene oxides condensates should in average contain between 5 and 20 ethylene oxide units per molecule. The fatty alcohol is preferably selected among cetosteryl alcohol, lauryl alcohol and cetyl alcohol.

The anionic surfactants have the properties of excellent cleaning, good wetting and re-wetting properties coupled with disperse properties and good at keeping the dirt (e.g. grease, waxy matters, stains, particulate dirt) away from the fabric, preventing re-deposition of removed dirt onto the fabric. The anionic surfactant is preferably selected among alkyl sulphonate/ sulphate, sodium oleate, dioctyle sulfosuccinate or any mixture thereof.
The combination of both non-ionic and anionic surfactants, complement each other’s cleansing properties. The non-ionic surfactants contribute to making the surfactant system less water hardness sensitive.

Additionally, an incorporation of a penetrant can endow the formulation with wetting, properties and de-aering properties, which are quite important for an effective fabric-preparation leading to an uniform dyeing.

The penetrant is preferably selected among alkyl alcohols such as methanol, ethanol, propanol, butanol including isomers thereof such as iso-propyl alcohol, iso-butanol and any mixtures thereof. It is preferred to use alkyl-alcohols that have a boiling point, or a boiling point for an azeotroph of the alcohol and another component of the mixture, whatever is the lower, of at least 5°C above the temperature used in this step in order to avoid evaporation of the alkyl-alcohol from the solution, preferably at least 10°C above the temperature used, more preferred at least 15°C above the temperature.

The sequestrant is preferably selected among phosphonate, phosphate or carboxylic acid based products. Examples are sodium hexa meta phosphate (SHMP), sodium tripolyphosphate, sodium trimeta phosphate; phosphonated aminopolyoxylates such as deiethylene triamine pentaacetic acid (DTPA), or N-(hydroxyl ethyl) ethylene diamine triacetic acid (HEDTA).

The invention is further defined in the following paragraphs:

1. A composition being a multifunctional wetting agent, comprising at least three components selected from at least two of the following groups: non-ionic surfactants, anionic surfactant, penetrants and sequestrant.

2. The composition of paragraph 1, having a HLB value in the range of 5 to 12, preferably 8-12 and most preferred in the range of 10-12.

3. The composition of paragraph 1 or 2, comprising or consisting of two or more non-ionic surfactant, one anionic surfactant and one penetrant.

4. The composition of paragraph 3, further comprising a sequestrant.

5. The composition according to any of paragraphs 1-4, comprising or consisting of one non-ionic surfactant having a low HLB, one non-ionic surfactant having a high HLB, one anionic surfactant, one sequestrants and optionally one sequestrant.

6. The composition of paragraph 5, wherein the non-ionic surfactant having a low HLB has a HLB in the range of 1-4, preferably in the range of 1.5 to 1.4.

7. The composition of paragraph 5 or 6, wherein the non-ionic surfactant having a high HLB has a HLB in the range of 10 to 15, preferably in the range of 12 to 14.
8. The composition of any of the paragraph 1 to 7, wherein the non-ionic surfactant is selected among sorbitan trioleate, glycerol dioleate, ethylene glycol monostearate or any mixture thereof.
9. The composition of any of the paragraph 1 to 8, wherein the anionic surfactant is selected among alkyl sulphonate, alkyl sulphate, sodium oleate, dioctyle sulfosuccinate or any mixture thereof.
10. The composition of any of the paragraph 1 to 9, wherein the penetrant is selected among alkyl alcohols such as ethanol, propanol, butanol, including isomers thereof such as iso-propyl alcohol, iso-butanol and any mixtures thereof.
11. The composition of any of the paragraphs 1 to 10, wherein the sequestrant is selected among phosphonate or carboxylic based products such as sodium hexa meth phosphate (SHMP), sodium tripolyphosphate, sodium trimeta phosphate; phosphonated aminocarboxylates, such as EDTMP, DETMP, ATMP, HEDP and DTPMP.
12. The composition of any of the paragraphs 1 to 11 comprising or consisting of 10-20 % by weight of a non-ionic surfactant having a HLB in the range of 1.5 to 4.5; 20-30 % by weight of a non-ionic surfactant having a HLB in the range of 12-14; 15-23 % by weight of an anionic surfactant and 35-45% by weight of alkyl alcohol.
13. Use of a MWA according to any of the paragraphs 1-12 for treating a fabric.
14. A method of treating a fabric comprising or consisting of the steps in following sequence:
   i. Washing the fabric in hot water
   ii. Desizing
   iii. Mercerizing
   iv. Hot wash with a multifunctional wetting agent according to any of the paragraphs 1-12
   v. Bioscouring
   vi. Optional hot wash with a multifunctional wetting agent according to any of the paragraphs 1-12
   vii. Peroxide rinse/ peroxide bleaching
   viii. Bleach clean up and wash.
15. The method of paragraph 14, wherein the MWA in step iv has a HLB of 7 and the MWA in step vi has a HLB of 7.9.
16. The method of paragraph 14 or 15, wherein the desizing in step (ii) is performed enzymatically using an alpha-amylase and a wetting agent, preferably a nonionic wetting agent.
17. The method of paragraph 16, wherein the desizing in step (ii) further uses a pectinase.
18. The method of paragraph 14 or 15, wherein step (v) is performed enzymatically using a pectinase.
19. The method of paragraph 18, wherein step (v) further uses at least one further enzyme selected among alpha-amylases and neutral cellulases, preferably further uses both an alpha-amylase and a neutral cellulase.
20. The method of paragraph 14 or 15, wherein step (vii) is performed by the action of a bleach clean-up enzyme preferably a catalase at the end to destroy residual peroxide from the fabric.
21. The method of paragraph 14 or 15, comprising or consisting of the steps:
   a. Washing the fabric in water at a temperature in the range of 85°C to 100°C;
   b. Treating the fabric with a desizing amylase, a pectinase and a wetting agent;
   c. Mercerizing the fabric at ambient temperature preferably in the range of 15°C to 30°C;
   d. Treating mercerised fabric with hot water containing multifunctional wetting agent according to any of the paragraphs 1-12
   e. Treating the fabric with a pectinase, a desizing amylase and a neutral cellulase at a pH in the range of 6-8;
   f. Treating the fabric with hydrogen peroxide at alkaline conditions preferably in the range of 10 to 11; and
   g. Treating the fabric with catalase until all remaining hydrogen peroxide has been degraded.

The invention is now further described by the following examples that should not in any way be considered limiting for the invention.

25 EXAMPLES

Materials and Methods
Machine used: Lab-Jigger, fabricated by R.B. Electronics & Engg Pvt. Ltd, Mumbai, India. Capacity 100 kg

Specifications of the Grey fabric used:
   Warp (Cotton) X Weft (cotton)
   Yarn count: 40ª Ne X 40ª Ne
   Construction: 132 X 72,
   Weight: 145 Gram per Sq. meter
Chemicals used:
NaOH: Maghmani Fine Chem Ltd, Technical grade, Assay 96-98%
Hydrogen Peroxide: GACL, Vadodara, 50% w/w pure
Commercial Non-ionic surfactant: Instawet SFLF from Microgenix, India.
Commercial anionic surfactant: Primasol NF from BaSF, India
Commercial Sequestering agent: Decol SNS from BaSF, India
Mercerising wetting agent: Mercerol QWNI from Clariant, India

10 Reference example 1, Batch Process
A grey fabric was desized and scoured by a conventional process which provided as reference to the invention of the method.

Enzymes used:
15 Desizing enzyme: Pretex L

The following Mill procedure, recipe and sequence were employed simulating jigger conditions:

The grey fabric is passed through singeing machine to remove protruding hairs from fabric surface

1. Desizing and Hot wash: the fabric was treated with 5g/l desizing enzyme in lab-jigger giving 4 turns (= 1 hour) at 1 m/min machine speed. The fabric washed in a solution of 5g/l Commercial non-ionic surfactant at pH 5.0 and a temperature of 60°C. followed by two hot washes in water.
2. The fabric was boiled in a solution of 5 g/l NaOH in lab-jigger, giving 4 turns (= 1 hour) at 1 m/min machine speed at a temperature of 95°C. After the alkaline boil fabric was washed twice in hot water, at 95°C followed by one cold wash at room temperature.
3. The fabric was then treated with a solution of 38 g/l sodium hydroxide, 13 g/l hydrogen peroxide, 3.5 g/l Commercial anionic surfactant (Primasol NF), 2 g/l peroxide stabilizer and 0.25 g/l sequestering agent (Decol SNS) for 4 turns (= 1 hour) at a temperature of 95°C. After this treatment the fabric was washed using 2 turns in hot water at 95°C, followed by one neutralisation wash and one cold wash at room temperature.
4. Next, the fabric was mercerized in a mercerization machine (Kuster's Mercerisation Machine) using a solution of 300 g/l sodium hydroxide & 5 gpl mercerising wetting agent (Mercerol QWNI), and finally washed, neutralized and dried.

5 The fabric treated as above was used as a reference for comparison with fabrics treated according to the invention.

**Example 1. Batch process**

Grey fabric was treated according to the invention in the jigger machine.

Enzymes used:
- Aquazyme SD-L: Alpha-amylase
- Scourzyme L: Pectinase
- Cellusoft CR: Neutral cellulase
- Terminox: Catalase

All enzymes are available from Novozymes A/S, Bagsvaerd Denmark.

Steps involved in the pretreatment of the fabric:
1. A boil wash was given to the grey fabric at 95°C, in a jigger for 30 min (2 turn).
2. Desizing was carried out at temp 60°C, for 1 hour (4 turn), pH 6.5+/-0.2 receipe: Aquazyme SD-L: 2.5 g/l, Scourzyme L: 1 g/l, Commercial non-ionic surfactant: 2 g/l

boil wash at 85°C for 30 min (2 turn)
boil wash at 85°C for 30 min with (2 turn) with 1 g/l MWA of HLB value 8.
cold wash for 30 min (2 turn) at room temperature

Evaluation of fabric after desizing:
Desizing efficiency: 80 % based on tegawa scale

3. Mercerisation was carried out on a mercerisation machine:

Receipte: Sodium hydroxide - 300 g/l, mercerising wetting agent - 5 g/l.

Postwash treatment was given till neutralization of the fabric.
4. A hot wash was given to the mercerised fabric at 70°C for 30 min (2 turn) in a jigger, containing 2 g/l MWA of HLB value 8.

5. Enzymatic scouring was carried out at temp 55°C for 1 hour (4 turn) in a jigger, pH 7.5 +/- 0.2 Receipe: Scourzyme L: 5 g/l, Cellusoft CR: 0.3 g/l, MWA 5 g/l.

6. Boil wash at 85°C (2 turn)

7. Cold wash (2 turn)

8. Peroxide rinse was carried out at temp: 85°C (2 turn)

Receipe: Hydrogen peroxide: 2 cc per litre, pH 10.5+/− 0.2 adjusted by sodium carbonate

9. Bleach clean up:

10. Terminox: 5 g/l (2 turn) at room temperature

11. Cold wash (2 turn) at room temperature & dried.

The fabric was now ready for dyeing (RFD), medium and dark shades.

Evaluation of fabric made Ready-for-dyeing:

15. Tegava rating of treated fabric: 8

Absorbency: Instant

Assessment:

The spectrophotometric evaluation was carried out by Datacolor Spectroflash 650 on reflection mode with D65 illuminant, 10 Degree Observer.

Whiteness index: 55.02.

Dyeing procedure:

The RFD fabric thus prepared was dyed using following recipe & standard conditions followed in commercial dyeing:

Dye: Reactive HE dye- Blue

Machine: Jigger % shade: 2%

Soda ash: 10 g/l

Glauber salt: 20 g/l

Temperature: 85°C for 60 min (4 turn)

Chemically treated fabric as per Example 1 was used as the reference fabric for dyeing. The same process was carried for RFD fabric prepared in this example.
**Post dyeing treatment:**

Hot wash at 60°C for 30 min (2 turn) Followed by soaping with 5 g/l nonionic surfactant at 70°C for 30 min (2 turn)

Two hot wash at 70°C for 30 min (2 turn) followed by cold wash at room temperature, 30 min (2 turn).

**Evaluation of dyed fabric:**

The spectrophotometric evaluation was carried out by Datacolor Spectroflash 650 on reflection mode with D65 illuminant, 10 Degree Observer % R at 420 nm, L*, a* & b* values are measured.

<table>
<thead>
<tr>
<th>Sample Particulars</th>
<th>%R</th>
<th>k/s</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatically treated</td>
<td>23.52</td>
<td>1.1492</td>
<td>51.73</td>
<td>-16.29</td>
<td>-7.28</td>
</tr>
<tr>
<td>Chemically treated [Ref. Fabric]</td>
<td>22.71</td>
<td>1.1883</td>
<td>50.44</td>
<td>-16.78</td>
<td>-7.88</td>
</tr>
</tbody>
</table>

The evaluation results of the dyed fabrics showed that the enzymatically treated fabric is equal to the chemically treated fabrics.

**Example 2 - Pad-Batch process**

Grey fabric was treated according to the invention in the padding mangle & jigger machine.

Enzymes used:

- Aquizyme SD-L Alpha-amylase
- Scourzyme L Pectinase
- Cellusoft CR Neutral cellulase
- Terminox Catalase

All enzymes are available from Novozymes A/S, Bagsvaerd Denmark.

The fabric was selected as already stated.

Steps involved in the pretreatment of the fabric

1. Desizing was carried out at temp 60°C, pH 6.5+/−0.2
Recipe: Aquazime SD-L: 7 g/l, Scourzyme L: 1 g/l, commercial nonionic wetting agent: 2 g/l
The fabric was padded at 75% expression & batch was prepared. The batch was wrapped with polythene sheet & kept rotating at 3-5 rpm for 8 hrs.

After completion of batching time, after wash treatment was given in jigger as follows:

5. Boil wash at 85°C for 2 turn
   Cold wash with two turn at room temperature

2. Mercerisation was carried out on a mercerisation machine:
   Recipe: Sodium hydroxide - 300 g/l, mercerising wetting agent - 5 g/l.
   Postwash treatment was given till neutralization of the fabric.

3. The desized and mercerized cotton fabric was treated with MWA of HLB value 8.0, 2 g/l at temperature 65°C +/-2°C, for two turn

4. In the bio-scouring step, the fabric was padded (90-95% pick up) using the recipe:
   Scourzyme L: 10 g/l,
   Aquazyme SD-L: 1 g/l,
   Cellusoft CR: 0.4 g/l
   pH: 6.5, 55-58°C,
   The batch was wrapped with polythene sheet & kept rotating 3-5 rpm for 14-16 hrs.

5. After completion of batching time, the fabric was again loaded in Jigger for after wash treatment. A hot wash treatment was given to the fabric at temperature 80°C +/-2°C for 30 min (two turn).

6. Hot wash (70°C +/- 2°C) containing 5 g/l of MWA of HLB value of 8 was given to the fabric (2 turn).

7. Bleaching was carried out with following recipe: Hydrogen peroxide: 20 cc/litre, pH 11, temperature 85°C +/-2°C.

25. After-wash treatment was given to the fabric like hot wash -neutralisation- hot wash-cold wash.

8. The fabric thus prepared is dyed.

Evaluation of fabric made Ready-for-dyeing:

30. Tegava of treated fabric: 9

Absorbency: Instant

Whiteness Index:
The spectrophotometric evaluation was carried out by Datacolor Spectroflash 650 on reflection mode with D65 illuminant, 10 Degree Observer Whiteness index was measured.

**Whiteness index (CIE 76): 70.49**

**Dyeing procedure:**
The RFD fabric thus prepared was dyed by following recipe & conditions:
Dye: Reactive HE dye- Orange, 0.5% pastel shade
Soda ash: 10 g/l
Glauber salt: 20 g/l
Temperature: 85°C for 60 min

**Post dyeing treatment:**
Two hot wash at 60°C for 30 min (2 turn) followed by
soaping with 5 g/l nonionic surfactant at 70°C for 4 turns
two hot wash at 70°C for 30 minutes (2 turn) followed by
cold wash at room temperature, 30 minutes (2 turn)

**Evaluation of dyed fabric:**
The spectrophotometric evaluation was carried out by Datacolor Spectroflash 650 on reflection mode with D65 illuminant, 10 Degree Observer % R at 420 nm, L*, a* & b* values were measured. Both reference and experimental fabrics displayed similar L, a, b values.

**Example 3 - Batch Process**
The fabric prepared in example 1 was treated for full bleaching as follows:
Bleaching was carried out at temp : 90°C, 4 turn (45 min) using Hydrogen peroxide : 20 cc per litre, pH 11+- 0.2
Post bleach treatment :
terminox : 5 g/l, 1 turn at room tempertaure
cold wash 2 turn at room temperature & dried
The fabric was now ready for dyeing

The whiteness index (CIE 76) of the fabric thus prepared was 79.22

**EXAMPLE: 4**
Desize (Pad batch) - continuous bleaching (Pad Steam) method of fabric Preparation:
The enzyme used for combined desizing - scouring e
were a amylase (Aquazyme SDL -5 g/l), pectinase (Scourzyme L 5 g/l) and Neutral cellulase (Cellusoft CR 0.3 g/l). The multifunctional wetting agent (5 g/l) was also used. Fabric after saturating with desizing recipe (pH 7.5 to 8, bath temperature 60 - 70 °C) was squeezed (90 plus % pick up) and batched. The batch was wrapped with plastic sheet to avoid drying and kept rotating (about 5 rpm) for at least 16 hours. The fabric was then taken for continuous bleaching following the standard sequence namely, wash (3 boxes), heavy squeezing, saturation with peroxide bleaching recipe, light squeezing and steaming in combi steamer for 10 to 20 minutes followed by washing (4 boxes) and drying.

The peroxide concentration in the bleaching recipe depended on degree of whiteness intended. In any case, much less peroxide, caustic soda and peroxide stabilizer were used in light of enzymatic cleansing already done in desizing-scouring step.

The process provided satisfactory results measured by whiteness and Tegawa.

Example 5. Comparison of the MWAs of the invention with commercial wetting agents

MWA of the invention were compared with following commercial wetting agents:

- Leophen FR-M (BASF, Germany)
- SNS (BASF, Germany)
- Huntsman Invatex DA (Huntsman, Leeds, UK)
- Huntsman InvatexDE (Huntsman, Leeds, UK)

A Lab-O-Mat, was used for this experiment.

The wetting agents were added to the beaker together with 0.4 g/l Scourzyme L and raw knitted fabric in a total volume of 100 ml. The fabric was treated under the conditions of 55°C, 15 min, LR=1:10 pH 8, 30 rpm, 90s turnaround.

After the scouring the wax was removed by adding further wetting agent under following conditions: 80°C, 10 min, LR=1:10, 30 rpm, 90s turnaround. After the treatment the fabrics was rinsed with 10 l distilled water two times at 50°C, 5 min and two times at 30°C, and dried at 105 °C for 30 min.

The fabrics were tested for residual pectin and CIE whiteness, using the methods described. Following experiments were performed:

<table>
<thead>
<tr>
<th>Experiments No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</tbody>
</table>
Wax removing 80°C*10min in Lab-O-Mat, 30rpm, 90s turnaround

IPA* = Isopropylalcohol
Following results were obtained:

<table>
<thead>
<tr>
<th>Scourzyme L, g/L</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
<th>0.4</th>
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<tbody>
<tr>
<td>MWA</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Hunstman Invatex DA 1, g/L</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Leophen FR-M, g/L</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Leophen+ IPA*, g/L</td>
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<td>1</td>
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<tr>
<td>IPA*, g/L</td>
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<td></td>
<td>0.4</td>
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</tbody>
</table>

Scouring 55°C*15min in Lab-O-Mat, 30rpm, 90s turnaround

| MWA- g/L          |  1  |  1  |  1  |     |     |     |     |     |     |     |     |     |
| Hunstman invatex DE, g/L |     |     |     |  1  |  1  |  1  |     |     |     |     |     |     |
| SNS, g/L          |     |     |     |  1  |  1  |  1  |     |     |     |     |     |     |
| SNS + IPA*, g/L   |     |     |     |     |     |     |  1  |  1  |  1  |     |     |     |
| IPA*, g/L         |     |     |     |     |     |     |     |     |     |     |  0.4 |  0.4 |  0.4 |

Wax removing 80°C*10min in Lab-O-Mat, 30rpm, 90s turnaround

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Wetting time, s</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
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<td>180</td>
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<tr>
<td>Average wetting time</td>
<td>4</td>
<td></td>
<td>&gt;180</td>
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<td>&gt;180</td>
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<td>&gt;180</td>
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<td>&gt;180</td>
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<td>&gt;180</td>
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<tr>
<td>Pectin left, %</td>
<td>8.7</td>
<td>13.1</td>
<td>12.5</td>
<td>18.8</td>
<td>16.7</td>
<td>16.2</td>
<td>17.5</td>
<td>16.2</td>
<td>17.1</td>
<td>10.2</td>
<td>18.2</td>
<td>18.2</td>
<td>22.2</td>
<td>32.0</td>
<td>23.1</td>
<td>29.5</td>
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<tr>
<td>Average Pectin</td>
<td>11.4</td>
<td>17.2</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
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<td></td>
<td>28.2</td>
<td>35.1</td>
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<td>CIE Whiteness, %</td>
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<tr>
<td>left, %</td>
<td>20.0</td>
<td>19.0</td>
<td>19.4</td>
<td>16.7</td>
<td>18.1</td>
<td>17.3</td>
<td>16.8</td>
<td>16.2</td>
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<tr>
<td>Average whiteness</td>
<td>19.44</td>
<td>17.37</td>
<td>16.75</td>
<td>17.45</td>
<td>17.62</td>
<td>16.2</td>
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</table>

The results demonstrate that the MWA of the invention has a significant shorter wetting time than the commercial wetting agents used for comparison.

The MWA of the invention also provides for a significant lower amount of pectin left and a higher whiteness compared with the commercial wetting agents.

**Example 6. Dosage profile of MWA of the invention**

The dosage profile of the MWA of the invention for bioscouring was determined on samples of Raw knitted Fabrics.

For the test a Lab-O-Mat was used with a test volume of 100 ml, with following conditions: 0.4 g/l Scourzyme L, 55°C, 15 min, LR=1:10, pH=8, followed by wax removal at 80°C, 10 min, LR=1:10. Various amounts of MWA were added to the beaker in both the scouring phase and in the wax removal phase.

The test was performed with the MWA alone and with MWA plus isopropyl alcohol. The results of the experiments are shown in figure 1 and figure 2.
WE CLAIM:

1. A composition being a multifunctional wetting agent, comprising at least three components selected from at least two of the following groups: non-ionic surfactants, anionic surfactant, penetrants and sequestrant.

2. The composition of claim 1, having a HLB value in the range of 5 to 12, preferably 8-12 and most preferred in the range of 10-12.

3. The composition of claim 1 or 2, comprising or consisting of two or more non-ionic surfactant, one anionic surfactant and one penetrant.

4. The composition of claim 3, further comprising a sequestrant.

5. The composition according to any of claims 1-4, comprising or consisting of one non-ionic surfactant having a low HLB, one non-ionic surfactant having a high HLB, one anionic surfactant, one sequestrants and optionally one sequestrant.

6. The composition of claim 5, wherein the non-ionic surfactant having a low HLB has a HLB in the range of 1-4, preferably in the range of 1.5 to 1.4.

7. The composition of claim 5 or 6, wherein the non-ionic surfactant having a high HLB has a HLB in the range of 10 to 15, preferably in the range of 12 to 14.

8. The composition of any of the claims 1 to 7, wherein the non-ionic surfactant is selected among sorbitan trioleate, glycerol dioleate, ethylene glycol monostearate or any mixture thereof.

9. The composition of any of the claims 1 to 8, wherein the anionic surfactant is selected among alkyl sulphonate, alkyl sulphate, sodium oleate, dioctyle sulfosuccinate or any mixture thereof.

10. The composition of any of the claims 1 to 9, wherein the penetrant is selected among alkyl alcohols such as ethanol, propanol, butanol, including isomers thereof such as iso-propyl alcohol, iso-butanol and any mixtures thereof.

11. The composition of any of the claims 1 to 10, wherein the sequestrant is selected among phosphonate or carboxylic based products such as sodium hexa meth phosphate (SHMP), sodium tripolyphosphate, sodium trimeta phosphate; phosphonated aminocarboxylates, such as EDTMP, DETMP, ATMP, HEDP and DTPMP.

12. The composition of any of the claims 1 to 11 comprising or consisting of 10-20 % by weight of a non-ionic surfactant having a HLB in the range of 1.5 to 4.5; 20-30 % by weight of a non-ionic surfactant having a HLB in the range of 12-14; 15-23 % by weight of an anionic surfactant and 35-45% by weight of alkyl alcohol.

13. Use of a MWA according to any of the claims 1-12 for treating a fabric.

14. A method of treating a fabric comprising or consisting of the steps in following sequence:
   i. Washing the fabric in hot water
ii. Desizing
iii. Mercerizing
iv. Hot wash with a multifunctional wetting agent according to any of the claims 1-12
v. Bioscouring
vi. Optional hot wash with a multifunctional wetting agent according to any of the claims 1-12
vii. Peroxide rinse/peroxide bleaching
viii. Bleach clean up and wash.

15. The method of claim 14, wherein the MWA in step iv has a HLB of 7 and the MWA in step vi has a HLB of 7.9.

16. The method of claim 14 or 15, wherein the desizing in step (ii) is performed enzymatically using an alpha-amylase and a wetting agent, preferably a nonionic wetting agent.

17. The method of claim 16, wherein the desizing in step (ii) further uses a pectinase.

18. The method of claim 14 or 15, wherein step (v) is performed enzymatically using a pectinase.

19. The method of claim 18, wherein step (v) further uses at least one further enzyme selected among alpha-amylases and neutral cellulases, preferably further uses both an alpha-amylase and a neutral cellulase.

20. The method of claim 14 or 15, wherein step (vii) is performed by the action of a bleach clean-up enzyme preferably a catalase at the end to destroy residual peroxide from the fabric.

21. The method of claim 14 or 15, comprising or consisting of the steps:

   a. Washing the fabric in water at a temperature in the range of 85°C to 100°C;
   b. Treating the fabric with a desizing amylase, a pectinase and a wetting agent;
   c. Mercerizing the fabric at ambient temperature preferably in the range of 15°C to 30 °C;
   d. Treating mercerised fabric with hot water containing multifunctional wetting agent according to any of the claims 1-12
   e. Treating the fabric with a pectinase, a desizing amylase and a neutral cellulase at a pH in the range of 6-8;
   f. Treating the fabric with hydrogen peroxide at alkaline conditions preferably in the range of 10 to 11; and
   g. Treating the fabric with catalase until all remaining hydrogen peroxide has been degraded.