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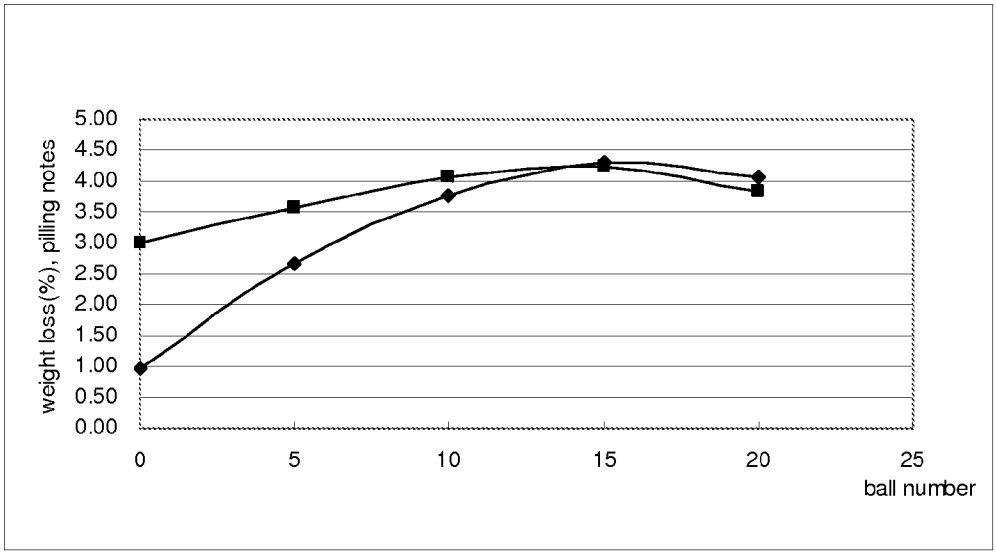


**Figure 3**

(57) **Abstract:** The present invention relates to amethod of bio-polishingfabrics containing man-made cellulosicpreferable rayon by the use of cellulase, comprising contacting the fabrics in an aqueous medium with enzyme system containing a cellulase of Family 45. The invention further comprises the composition for the method.

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# Biopolishing on Man-Made Cellulosic Fabric

## Field of the Invention

The present invention relates to a method of bio-polishing fabrics containing man-made cellulosics preferable rayon by the use of cellulase, comprising contacting the fabrics in an aqueous medium with enzyme system containing a cellulase of Family 45. The invention further comprises the composition using for the method.

## Background of the Invention

Rayon fabric as a textile is soft and comfortable, with good drapability and excellent absorbency properties. However, it loses a great deal of strength when wet, and has poor abrasion resistance due to inelasticity of the fibers. Regular rayon fabric looks fuzzy and pills are easily built upon abrasion on its surface. It has been claimed as one of the main hurdles for further development of rayon into the market. So far no effective enzymatic solution has been reported to address this issue.

## Summary of the Invention

The inventor of present invention surprisingly found out that enzyme solution of the present invention can be used efficiently for anti-fuzzy and anti-pilling on rayon fabric.

Therefore, the present invention provides a method of bio-polishing fabrics containing man-made cellulosics, comprising contacting the fabric in an aqueous medium with enzyme system containing a cellulase of Family 45.

Preferably, the cellulase is a CBD-containing cellulase (cellulase with cellulose binding domain), and more preferably the cellulase is derived from *Humicola insolens, Thielavia terrestris* or *Trichoderma sp.*. Even more preferably, said cellulase is the cellulase of SEQ ID NO:1 and/or SEQ ID NO:2.

A textile enzyme composition for bio-polishing man-made cellulosic fabrics, comprising Family 45 cellulase, wherein Family 45 cellulase is at least 30wt% on weight of total cellulase, preferably at least 50wt% Family 45 cellulase on weight of total cellulase in the composition.

This invention covers enzyme compositions and their process parameters to achieve anti-fuzzing and anti-pilling effect with acceptable weight loss and strength loss on the rayon, preferably viscose fabrics with cellulase preparation.

**Description of the Figures**

Figure 1: illustrates the anti-fuzzy effects on viscose by pre-treatment with sodium hydroxide. the Figure shows the dosage profile of Cellulase A on raw or pre-treated Rayon 1# in biopolishing at 55°C, pH 5.0 for 60 minutes; wherein dotted bar = Raw, wt loss; black bar = pre-treated, wt loss; triangle =raw, pilling notes; square= pre-treated, pilling notes; “wt” represents weight.

Figure 2: illustrates the anti-pilling effects by the use of cellulase B in miniwasher. Wherein A: Raw Rayon 2# fabrics treated with Nu-Martindale at 2000R; B: Cellulase B Bio-polished Rayon 2# fabrics treated with Nu-Martindale at 2000R.

Figure 3: illustrates the influence of mechanical action on the bio-polishing performance; the Figure shows the effects of steel balls number on the Rayon 3# biopolishing by Cellulase A at 55°C, pH 5.0 for 60 minutes; wherein diamond = weight loss(%); square = pilling notes.

Figure 4: illustrates the influence of treating time on the bio-polishing performance; the Figure shows time curve of biopolishing of Rayon 3# by Cellulase A at 55°C, pH 5.0 for 60 minutes; wherein diamond = weight loss(%); square = pilling notes.

Figure 5: illustrates the anti-pilling effects by the use of blend cellulase D/A on fabric Rayon 3#. The Figure shows the dosage profile of D/A blend on Rayon 3# in biopolishing at 55°C, pH 5.0 for 60 minutes; wherein striped bar = weight loss; black bar = pilling notes.

**Detailed Description of the Invention**

**Man-Made Cellulosic fabric**

As used herein, a "man-made cellulosic fabric" refers to the cellulosic substrate to be treated and comprises, without limitation, rayon, and their blends with other natural or synthetic fibers. The man-made cellulosic containing fabric may also comprise, without limitation, crude fiber, yarn, woven or knit textile or fabric, or a garment or finished product.

As used here, "rayon" is the generic term for fiber (and the resulting yarn and fabric) manufactured or regenerated cellulose. There are several types of rayon fibers in commercial use today, named according to the process by which the cellulose is converted to the soluble form and then regenerated. The following four types of rayon are commonly used in textile industry.

1) Regular Rayon, also known as Viscose, is made by converting purified cellulose to xanthate, dissolving the xanthate in dilute caustic soda and then regenerating the

cellulose from the product as it emerges from the spinneret. Most rayon is made by the viscose process. It is typically found in apparel and home furnishings. The distinguishing property of viscose is its low wet strength. As a result, it becomes unstable and may stretch or shrink when wet. Dry cleaning is usually recommended to preserve the appearance of fabrics made from this fiber. If machine washed, untreated viscose can shrink as much as 10%.

2) High Wet Modulus Rayon, known as Polynosic rayon or Modal, is a modified viscose that has virtually the same properties as regular rayon, plus high wet strength. Modal can be machine washed and tumble dried and perform much like cotton in similar end uses. Modal can be mercerized, like cotton, for increased strength and luster.

3) High Tenacity Rayon, known as Tencel, is a modification of “regular rayon” to provide exceptional strength (two times that of Modal). Tencel is primarily found in tire cord and industrial end uses. It may be finished, chemically coated, or rubberized for protection from moisture and potential loss of dimensional stability and strength during use.

4) Cupramonium rayon is another type with properties similar to these of viscose. The manufacturing process differs somewhat from that of viscose and is less environmentally friendly. As a result, cupramonium rayon is no longer produced in the United States.

The fabric may be treated in the form of unsewn fabric or a sewn garment made of such fabric. It is of particular interest to apply the process of the invention to new, clean fabric or garment.

***Enzyme composition***

***Cellulase:***

The term "cellulase" refers to an enzyme that contributes to the hydrolysis of cellulose, such as cellobiohydrolase (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 cellubiose hydrolyzing capability. The cellulase used in practicing the present invention may be derived from microorganisms which are known to be capable of producing cellulolytic enzymes, such as, e.g., species of *Humicola, Thermomyces, Bacillus, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Irpex, Scytalidium, Schizophyllum,*

*Penicillium, Thielavia, Aspergillus*, or *Geotricum,* 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. WO91/17244, WO91/17243 and WO98/12307.

It is also known that cellulases may or may not have a cellulose binding domain (CBD). The CBD enhances the binding of the enzyme to a cellulose-containing fiber and increases the efficacy of the catalytic active part of the enzyme. Preferably, cellulase used in the present invention is CBD-containing cellulase.

Cellulases are classified into families on the basis of amino-acid sequence similarities according to the classification system described in Henrissat, B. et al.: *Biochem. J.*, (1991), 280, p. 309-16, and Henrissat, B. et al.: *Biochem. J.*, (1993), 293, p. 781-788. At present are known cellulases belonging to the families 5, 6, 7, 8, 9, 10, 12, 26, 44, 45, 48, 60, and 61 of glycosyl hydrolases.

The cellulases used in this invention can be monocomponent, wherein the cellulase of monocomponent, is a cellulase which is essentially free from other proteins, in particular other cellulases. Monocomponent family 45 cellulase 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.

The cellulases used in this invention can further be a cellulase composition comprising one or more Family 45 cellulases, or comprising Family 45 cellulase(s) and other cellulase (non family 45 cellulase) of BG, CBH and EG, wherein Family 45 cellulase is at least 30wt%, preferably 50wt% or 70wt% or 80wt% or 90wt% on weight of total cellulase in the composition, so as to ensure good anti-pilling effect and at the same time with low level of weight loss on fabric. In a further embodiment of the invention, the enzyme system used in the invention can be family 45 cellulases blended with any 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 International, USA), and Youteer 800 (Youteer Co. Ltd, China).

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).

In a preferred embodiment, the cellulase according to present invention is a Family 45 cellulase, and more preferably Family 45 endoglucanase. Preferably, the cellulase for the use in the invention may be derived from a strain of *Humicola*, preferably *H. insolens*. One embodyment is an endoglucanase denoted EG V derived from *H. insolens* strain DSM 1800

having a molecular weight of ~43 kD. The cellulase and its amino acid sequence are described in WO 91/17243 (Novo Nordisk). It has a specific activity of 430 ECU/mg.

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.*, a *Piromyces sp.*, a *Penicillium sp.*, an *Agaricus sp.*, or a *Phanerochaete sp.*

In one embodiment of the invention, the cellulase is an Family 45 endoglucanase comprising the amino acid sequence of the *Thielavia terrestis* endoglucanase shown in SEQ ID No. 1 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 1 (as described in WO 96/29397), 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 Family 45 endoglucanase comprising the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 2 (as described 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.

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 kluyveri* 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, variants 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 differences 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).

***Additional components***:

In some embodiments of the invention, the aqueous solution or wash liquor further comprises other components, including without limitation other enzymes, as well as surfactants, stabilizer, wetting agent, dispersing agents, antifoaming agents, lubricants, builder systems, and the like, or a mixture thereof, that enhance the bio-polishing processes and/or provide superior effects related to, e.g., strength, resistance to pilling, water absorbency, and dyeability.

The enzymes may be isolated from their cell of origin or may be recombinantly produced, and may be chemically or genetically modified. Typically, the enzymes are incorporated in the aqueous solution at a level of from about 0.0001% to about 99% of enzyme protein by weight of the composition. It will be understood that the amount of enzymatic activity units for each additional enzyme to be used in the methods of the present invention in conjunction with a particular bio-polishing enzyme can be easily determined using conventional assays.

Surfactants suitable for use in practicing the present invention include, without limitation, nonionic, anionic; cationic; and zwitterionic surfactants; which are typically present at a concentration of between about 0.2% to about 15% by weight, preferably from about 1% to about 10% by weight. Anionic surfactants include, without limitation, linear alkylbenzenesulfonate, α-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid, and soap. Non-ionic surfactants include, without limitation, alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, and N-acyl N-alkyl derivatives of glucosamine (“glucamides”).

Builder systems include, without limitation, aluminosilicates, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, and metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid, which are included at a concentration of between about 5% to 80% by weight, preferably between about 5% to about 30% by weight.

Antifoam agents include without limitation silicones (U.S. Patent No. 3,933,672; DC-544 (Dow Corning), which are typically included at a concentration of between about 0.01% and about 1% by weight.

The compositions may also contain soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, and/or bactericides. All of such further components suitable for textile use are well know in the art.

***Process conditions***

The manner in which the aqueous solution containing the enzyme system for BioPolishing is contacted with the rayon-containing fabric will depend upon whether the processing regime is continuous, discontinuous pad-batch or batch. For example, for continuous or discontinuous pad-batch processing, the aqueous enzyme solution is preferably contained in a saturator bath and is applied continuously to the rayon-containing fabric as it travels through the bath, during which process the rayon-containing fabric typically absorbs the processing liquor at an amount of 0.5-1.5 times its weight. In batch operations, the rayon-containing fabric is exposed to the enzyme solution for a period ranging from about 5 minutes to 24 hours at a liquor-to-fabric ratio of 4:1-50:1.

The aqueous solution or wash liquor typically has a pH of between about 4 and about 11. Preferably, the pH of the treating composition is between about 5 and about 10, preferably between about 5 to about 9, and most preferably about 5 to about 7.

In one embodiment, the subjected method for treating rayon-containing fabric is carried out at a pH below 9, more preferably, below 8, and even more preferably below 7.

The temperature to carry out biopolishing processes will depend on the process used. In the case of cold pad batch process, the temperature for the bio-polishing processes is preferably between about 15°C and about 65°C, and most preferably between about 25°C and about 60°C. For continuous and other batch processes, the temperature for carrying out and the bio-polishing processes is preferably between about 35°C and about 75°C, and most preferably between about 45°C and about 65°C.

The family 45 cellulases for biopolishing are generally added in an amount which is effective to generate enough biopolishing effect on man-made cellulosic fabric. The enzyme(s) may preferably be dosed in an amount of from about 0.08 to 5 mg protein/ g fabric, preferably 0.09 to 3 mg protein/ g fabric, and even more preferably 0.12 to 3 mg protein/ g fabric.

Preferably, before biopolishing step, pretreatment (i.e. pre-fibrillation) step will be conducted to adjust pH to over 10, especially pH 10-12. The pretreatment step will render the biopolishing with cellulase more effective. Preferably, in the pretreatment step, NaOH or $Na_2CO_3$ was used to adjust pH.

It will be understood that the optimum dosage and concentration of the enzymes, the volume of the aqueous solution or wash liquor, and the pH and temperature will

vary, depending on: (i) the nature of the fiber, i.e., crude fiber, yarn, or textile; (ii) the particular enzyme(s) used, and the specific activity of the enzyme; (iii) the conditions of temperature, pH, time, etc., at which the processing occurs; (iv) the presence of other components in the wash liquor; and (v) the type of processing regime used, i.e., continuous, discontinuous pad-batch, or batch. The optimization of the process conditions can be determined using routine experimentation, such as, by establishing a matrix of conditions and testing different points in the matrix. For example, the amount of enzyme, the temperature at which the contacting occurs, and the total time of processing can be varied, after which the resulting cellulosic materials or textile is evaluated for (a) pilling result; and (b) weight loss and strength loss.

The following are intended as non-limiting illustrations of the present invention.

**Examples**

**Materials & Methods**

Enzymes

- Cellulase A: cellulase with *SEQ ID NO:2* (as described in WO 91/17243)

- Cellulase B: cellulase with SEQ ID NO: 1 (as described in WO 96/29397)

- Cellulase D: Cellusoft L™ (multi-components cellulases from *Trichoderma reesei*, commercially available from Novozymes A/S Denmark)

Fabric

Rayon fabric 1#: 30S/1 viscose+30D Dupont Lycra Jersey, which contained 5% polyvinyl chloride fibre and 95% viscose, and with the fabric weight of 200g/m$^2$ (Dongguan Fuan Textiles Limited, Chang An Town, Dongguan City 523840, Guangdong Province, China.)

Rayon fabric 2#: 30S/1 viscose+20D Dupont Lycra Jersey, which contained 2% Polyurethane Fiber and 98% viscose, and with the fabric weight of 170g/m$^2$ (Dongguan Fuan Textiles Limited)

Rayon fabric 3# (Shaoxing Rainbow Co. Ltd, China): 100% viscose

Rayon fabrics 4#: modal fabrics (Lenzing Aktiengesellschaft, 4860 Lenzing, Austria)

Rayon fabric 5#: Giditex Co., Binh Thanh Dist., Ho Chi Minh, Vietnam: 100% viscose

Buffer

Acetate buffer:

1) 50mM Acetate buffer (pH5.0)

2.873 g of Sodium acetate and 0.901 g of Acetic acid were dissolved in 1 L of de-ionized water.

2) 50mM Acetate buffer (pH5.5)

3.631 g of Sodium acetate and 0.345 g of Acetic acid were dissolved in 1 L of de-ionized water.

Phosphate buffer:

1) 50mM phosphate buffer (pH6.0)

2.203 g of Sodium Hydrogen Phosphate Dodecahydrate and 6.842 g of Sodium Dihydrogen Phoshate Dihydrate were dissolved in 1 L of de-ionized water.

2) 50mM phosphate buffer (pH6.5)

5.642 g of Sodium Hydrogen Phosphate Dodecahydrate and 5.344 g of Sodium Dihydrogen Phoshate Dihydrate were dissolved in 1 L of de-ionized water.

**Methods**

Weight loss determination

The swatches were placed in the conditioned room(65%+/-5% humidity, 20+/-1°C) for 24 hours before they were numbered, weighed by the analytical balance (for samples below 100g) or a precision balance(for samples over 100g) and recorded. After treatment, all samples were tumbled dried for 1hr and conditioned for 24 hr in conditioned room same as above. For each sample, the weight loss was defined as below:

$$Weight\ loss\ \% : \frac{(weight\ before - weight\ after) * 100}{weight\ before\ treatment}$$

Pilling Notes test

Fabrics including treated and untreated which had been pre-conditioned in norm-climate(65% humidity, 20°C) for at least 24 hours were tested for the pilling notes with Nu-Martindale Tester (James H. Heal Co. Ltd, England), with untreated fabrics of the same type as the abraded fabrics. A standard pilling test (Swiss Norm (SN) 198525) was carried out after 2000 Revolutions by marking from 1-5, with the meaning defined as below:

| | |
|---|---|
| Note 5: | No pilling |
| Note 4: | Slight Pilling |
| Note 3: | Moderate Pilling |
| Note 2: | Distinct Pilling |

Note 1: Heavy Pilling

1/2, 1/4 notes are allowed

To make the test result more reliable, 3 separate readings were carried out by different persons for each sample, and the average of the 3 readings was adopted as the final result of pilling notes.

Anti-fuzzy Effect

Fabrics treated with different cellulases or processes were evaluated by visual observation and comparison for their hairness on the surface. A better anti-fuzzy effect of a certain cellulase or process refers to a cleaner fabric surface treated thereby.

Protein Content

BCA kit (available from PIERCE) was used for detecting the total protein content in the enzyme product.

**Example 1**

Bio-polishing of rayon 1# with Cellulase A and B in Launder-Ometer

Fabric swatches were cut to about 16.5cm*16.5cm (about 5g each). The swatches were conditioned and weighed. The bio-polishing was conducted with a Launder-Ometer. Two conditioned swatches and 20 big steel balls (220g) were placed in each beaker. The beaker was filled with enzymes and buffer (pH 5.0, 50mM acetate) to a total volume of 100ml, which would create a liquid ratio about 10:1.

1) Enzymatic wash: The beaker with pre-conditioned fabrics was filled with about 100ml buffer. Cellulase A and B were added to each beaker as specified. The Launder-Ometer was loaded with such beakers and kept running for 60 minutes when the system temperature was raised to 55°C.
2) Inactivation: After the required time reached, removed all beakers from Launder-Ometer and transferred the swatches to the inactivation solution (2g/L soda ash) at 85 for 10 minutes.
3) Post rinse: rinsed with hot water for 2 times and cold water for another 2 times.
4) Spinned off the water on the fabrics and tumble dried.
5) Conditioned for 24hr in a standard condition room(65% humidity, 20°C).
6) Measured the weight after treatment and test the pilling notes of the treated fabrics after martindale-treatment for 2000R.

The result was shown as Table 1:

Table 1

| Cellulase | Dosage(mg/g fabrics) | Weight loss(%) | Pilling notes |
|---|---|---|---|
| Blank | 0 | -0.17±0.06 | 2.08±0.14 |
| A | 1.2 | 2.56±0.25 | 4.75±0.25 |
| B | 1.2 | 1.87±0.50 | 4.08±0.38 |

The dosage of "mg/g fabrics" in above table and also in other examples referred to "mg protein/ g fabrics".

Blank group did not use any enzyme but only buffer, while conducting the same process as other two experimental groups.

Both cellulases showed good performance in bio-polishing on rayon 1# at acid pH, with good anti-pilling effect and low level of weight loss.

**Example 2**

pH and dosage file of Bio-polishing of rayon 1# with Cellulase A at different buffers in Launder-Ometer

The same fabric swatch was prepared as in Example 1. Buffers were prepared: 50mM acetate buffers with pH 5.0 and pH 5.5; 50mM phosphate buffers with pH 6.0 and pH 6.5; 5mM acetate buffer with pH 5. The procedure was same with Example 1, while only Cellulase A was used, and buffers with different pHs and concentrations were included. The results were summarized as Table 2.

Table 2

| Cellulase | Buffers | Dosage(mg/g) | Pilling notes |
|---|---|---|---|
| Blank | pH 5, 50mM | 0 | 1.67±0.29 |
| A | pH 5, 50mM | 1.2 | 4.58±0.13 |
| | pH 5, 5mM | | 4.83±0.14 |
| | pH 5.5, 50mM | | 4.33±0.20 |
| | pH 6.0, 50mM | | 4.38±0.34 |
| | pH 6.5, 50mM | | 4.02±0.14 |
| | pH 5, 5Mm | 0.6 | 4.50±0.00 |

Cellulase A showed good performance in pH range from 5.0-6.5, and thus could also be applied in buffers with different concentrations.

**Example 3**

Bio-polishing of rayon 1# with Cellulase A in mini washer (XGP-2)

The Mini washer (XGP-2, JunYe) was a belly washer like washing device. Cellulase A was tested in the bio-polishing of rayon 1#, with the procedure as below:

1) Enzymatic wash: Temperature 55°C; Time 1hr; Fabrics loaded about 300g; Liquid/fabric ratio 10; pH 5.0 adjusted by sodium acetate and/or acetic acid; Cellulase dosage 1.2 mg protein/g fabric;
2) Inactivation: when time for enzymatic wash was up, drained out the treating solutions, followed by treatment in inactivation solution(2g/L soda ash) at 80 for 10 minutes;
3) Post Rinse: rinsed fabrics with hot water for 2 times and cold water for another 2;
4) Spinned off the water on the fabrics and tumble dried;
5) Conditioned for 24hr in a standard condition room (65% humidity, 20°C).
6) Measured the weight after treatment and test the pilling notes of the treated fabrics after matindale-treatment for 2000R.

The result suggested that Cellulase A could lead a good anti-pilling effect in mini washer, representing by the pilling notes above 4.5 at 2000R.

**Example 4**

Bio-polishing of pre-treated rayon 1# with Cellulase A in Launder-Ometer

Rayon 1# fabrics were pre-treated with procedure as below:

1) incubated in 1g/L of sodium hydroxide at 80°C for 2hr;
2) rinsed with hot water for 2 times;
3) rinsed with cold water for 2 times;
4) tumble dry for 1 hr and conditioned at for 24 hr.

The pre-treated and raw rayon fabrics were cut into the same size and bio-polishing trial in Laundry-O-Meter was carried out towards these two types of fabrics with different loads of Cellulase A, the treating conditions of which was same as Example 1.

The result as shown in Fig 1 suggested that the pre-treatment with sodium hydroxide at a high temperature could boost the anti-pilling effects. It was also observed that the pre-treatment could increase the anti-fuzzy effects in bio-polishing.

**Example 5**

Bio-polishing of rayon 1# with Cellulase A in two steps in mini washer

Rayon 1# fabrics were treated with Cellulase A for 2 times (i.e. 2-step process):

1) 1st Enzymatic wash: 0.84mg protein/g fabrics of Cellulase A was loaded, the other conditions same as Example 3;
2) Inactivation: when time for enzymatic wash was up, drained out the treating solutions, followed by treatment in inactivation solution(2g/L soda ash) at 80 for 10 minutes;
3) Post Rinse: rinsed fabrics with hot water for 2 times and cold water for another 2 times;
4) Spinned off the water on the fabrics and tumble dried;
5) One part of fabrics were conditioned and evaluated as defined in Example 1, another part of fabrics were subjected for further enzymatic wash;
6) 2nd Enzymatic wash: 0.36mg Enzyme protein/g of Cellulase A was loaded, with other conditions same as step 1);
7) Repeated step 2)-4);
8) Conditioned and evaluated as defined in Example 1;

Control: Rayon 1# fabrics were treated with Cellulase A for 1 time (i.e. 1-step process):

Steps 1) to 5) were conducted as above, while dose of Cellulase A added in step 1) were 1.2 mg protein/g fabrics or 0.84 mg protein/g fabrics.

Table 3

| Bio-polishing steps | Dosage(mg/g fabrics) | Pilling notes | Anti-fuzzy effects |
|---|---|---|---|
| Control | 1.2 | 4.75±0.00 | Average |
| | 0.84 | 4.42±0.38 | Average |
| 2-step process | 0.84 in 1st wash; 0.36 in 2nd wash | 4.92±0.14 | Very Good |

The results as shown in Table 3 suggested that Cellulase A could lead good anti-pilling effects in both 1-step and 2-step process when the enzyme protein dosage was

above 0.84 mg of enzyme proteins per gram of rayon fabrics. And additional Bio-polishing step with 0.36 mg of enzyme proteins per gram of rayon fabrics could further boost the anti-pilling effects and dramatically increase the anti-fuzzy effects.

**Example 6**

Bio-polishing of rayon 1# with Cellulase A in wascator

Bio-polishing of rayon 1# was carried out in wascator (Electrolux, Switzerland) with the procedure as below:

1) Pre-treatment: rayon 1# fabrics were grouped in 3 parts: 1st part was pre-treated with 1g/L of sodium hydroxide at 80°C for 2hr and tumble dried(Pre-treated part); the 2nd part was bio-polished with 1.2mg proteins per gram of fabric in wascator (Temperature 55°C, Time 1hr, Fabrics loaded about 500g viscose and about 500g cotton interlock fabric as the filler, Liquid ratio 10;pH 5.0 adjusted by Sodium acetate and/or Acetic acid; Cellulase A dosage 1.2 mg Enzyme protein/g fabric) and tumble dried(Pre-washed); and the 3rd part was raw fabrics(Raw);
2) Enzymatic wash: Temperature 55°C; Time 1hr; Fabrics loaded about 300g for each kind from step 1) with the total weight of about 1kg; Liquid ratio 10;pH 5.0 adjusted by Sodium acetate Acetic acid; Cellulase A dosage 1.2 mg Enzyme protein/g fabric;
3) Inactivation: when time for enzymatic wash was up, drained out the treating solutions, followed by treatment in inactivation solution(1g/L soda ash) at 80 for 10 minutes;
4) Post rinse: rinsed with hot water(40°C) and cold water(25°C) for 10 min separately;
5) Spinned off the water on the fabrics and tumble dried;
6) Conditioned for 24hr in a standard condition room (65% humidity, 20°C).
7) Measured the weight after treatment and test the pilling notes of the treated fabrics after martindale-treatment for 2000R.

Table 4

| Pre-treatment | Dosage(mg/g fabrics) | Weight loss | Pilling notes | Anti-fuzzy effects |
|---|---|---|---|---|
| Pre-treated | 1.2 | 3.11±0.00 | 4.46±0.29 | Very Good |
| Pre-washed | | 3.85±0.05 | 4.92±0.13 | Very Good |
| Raw | | 2.72±0.01 | 3.83±0.44 | Average |
| Control | 0 | - | 2.00±0.50 | Fuzzy |

Control: raw fabrics with only martindale treatment

The results suggested that Cellulase A could lead significant anti-pilling effects in wascator. And proper pre-treatments such as pre-fibrillation with sodium hydroxide at a

high temperature and double Bio-polishing could significantly boost the anti-pilling and anti-fuzzy effects.

**Example 7**

Bio-polishing of rayon 2# with Cellulase B in mini washer(XGP-2)

Cellulase B was tested for their effects in anti-pilling effects on rayon 2# in the same mini washer and with the same procedure and treating conditions as Example 3.

The result suggested that Cellulase B (1.2mg protein/g fabric) could lead a good anti-pilling effect in mini washer, representing by the pilling notes about 4.0 at 2000R, as compared to about 2.0 for untreated rayon 2# fabrics at 2000R. The improvement could also be observed in SEM photos as shown in Fig 2.

**Example 8**

Bio-polishing of rayon 3# with Cellulases A,B,D in Launder-Ometer

Another kind of rayon was cut to about 16.5cm*16.5cm(about 4.5g each). The procedures were same as Example 1, while Cellulase A,B,D were tested, and different amounts of steel balls and treating time were applied according to specialization.

As shown in Table 5, dosage response on weight loss and anti-pilling effects on rayon 3# when different amounts of Cellulase A and B were loaded, this suggested that both cellulases were effective in bio-polishing of rayon.

The influence from mechanical action and treating time was as shown in Fig 3 and Fig 4, respectively. Appropriate mechanical action and treating time was necessary for the bio-polishing of rayon by Cellulase A.

Table 5

| Cellulase | Dosage(mg/g fabrics) | Weight loss | Pilling notes |
|---|---|---|---|
| Blank | 0 | 0.39±0.18 | 1.00±0.00 |
| A | 0.24 | 1.47±0.04 | 1.08±0.14 |
| | 0.48 | 2.55±0.06 | 2.50±0.00 |
| | 0.96 | 3.75±0.02 | 3.33±0.14 |
| | 1.2 | 3.58±0.26 | 4.08±0.14 |
| B | 0.24 | 2.35±0.33 | 1.42±0.14 |
| | 0.48 | 2.67±0.24 | 2.58±0.14 |
| | 0.96 | 4.03±0.18 | 3.50±0.00 |
| | 1.2 | 3.77±0.46 | 3.67±0.14 |
| D | 28.6 | 7.71±0.04 | 1.17±0.29 |

When only Cellulase D, without Family 45 cellulase, was used in biopolishing, the result suggested poor anti-pilling effect even at dosage of 28.6 mg Cellulase D /g fabrics and high level of weight loss. It was concluded that Cellulase D alone cannot be applied in biopolishing of rayon.

**Example 9**

Bio-polishing of rayon 3# with Cellulases blends in Laundry-Ometer

The blends of Cellulase A/Cellulase D and Cellulase B/ Cellulase D were also tried on rayon 3# with the same procedure and condition as Example 8.

As shown in Table 6, the blends of Cellulase D/A or D/B with 10-30% (by weight of protein) of Cellulase D could significantly improve the anti-pilling effects on rayon 3#, when the blends were dosed at 1.2 mg protein/g fabrics. The results suggested that the blends of D /A or D/B with different ratios also had the anti-pilling effects on rayon.

As shown in Fig 5, a significant dosage response was observed towards rayon 3# with different dosages of Cellulase D/A blends(with 20% Cellulase D and 80% Cellulase A proteins in the total proteins of the blends), both in terms of weight loss and pilling notes, which suggested that the blends could be applied in the bio-polishing of rayon.

Table 6

| Cellulase | Dosage(mg/g fabrics) | Ratio (D/ A or D/B ) | Weight loss | Pilling notes |
|---|---|---|---|---|
| Blank | 0 | 0 | 0.39±0.18 | 1.00±0.00 |
| D / A blends | 1.2 | 3/7 | 3.72±0.01 | 3.25±0.00 |
| | | 2/8 | 3.93±0.16 | 3.42±0.14 |
| | | 1/9 | 3.88±0.09 | 3.50±0.00 |
| D / B blends | 1.2 | 3/7 | 3.84±0.34 | 3.25±0.00 |
| | | 2/8 | 4.22±0.04 | 3.25±0.00 |
| | | 1/9 | 4.14±0.29 | 3.33±0.14 |

**Example 10**

Bio-polishing of rayon 4# with Cellulase B and D/A blend in Launder-Ometer

Rayon 4# fabric, which was a modal from Lenzing AG was also tried with Cellulases B and D with the procedure and condition same as Example 1 and the dosage specialized in Table 7.

As shown in Table 7, an obvious dosage response was observed when different amounts of Cellulase B and/or D/A blend(6:4 on weight basis) were loaded to rayon 4#, both in terms of weight loss and pilling notes. The results suggested that both Cellulases B and D/A blend were effective in Biopolishing of this type of rayon fabrics.

Table 7

| Cellulase | Dosage(mg/g fabrics) | Weight loss | Pilling notes |
|---|---|---|---|
| Blank | 0 | 0.32±0.07 | 3.08±0.14 |
| B | 0.72 | 1.14±0.19 | 4.08±0.14 |
| | 1.2 | 1.75±0.06 | 4.25±0.00 |
| | 2 | 2.00±0.06 | 4.50±0.00 |
| D/A blend | 0.72 | 1.23±0.05 | 4.25±0.00 |
| | 1.2 | 1.51±0.02 | 4.50±0.00 |
| | 2 | 1.98±0.00 | 4.50±0.00 |

**Example 11**

Bio-polishing of Rayon 5# with Cellulase A in Laundry-Ometer

Rayon 5# was pre-treated and prepared as Example 4, and then tested with Cellulase A in Laundry-Ometer with the same procedure and condition as Example 1. Buffer was prepared: 1g/L sodium acetate and adjusted to pH 5.0 with acetate acid. The results were summarized as Table 2.

Table 8

| Cellulase | Buffers | Dosage(mg/g) | Pilling notes |
|---|---|---|---|
| Blank | pH 5.0 | 0 | 1.5 |
| A | | 0.12 | 3.3 |
| | | 0.36 | 3.7 |
| | | 0.6 | 4.3 |
| | | 1.2 | 4.5 |

All patents, patent applications, and literature references referred to herein are hereby incorporated by reference in their entirety. Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. Such obvious variations are within the full-intended scope of the appended claims.

## Claims

1. A method of bio-polishing man-made cellulosic fabrics, comprising contacting the fabrics in an aqueous medium with enzyme system containing cellulase of Family 45.

2. The method of claim 1, wherein the cellulase is endoglucanase.

3. The method of claim 1 or 2, wherein the cellulase is a CBD-containing cellulase.

4. The method of claim 1 or 2 or 3, wherein the cellulase is derived from Humicola or Thielavia, preferably Humicola insolens, Thielavia terrestris or Trichoderma sp.

5. The method of claim 3, wherein the cellulase is the cellulase with SEQ ID NO:1, or a variant thereof.

6. The method of claim 3, wherein the cellulase is the cellulase with SEQ ID NO:2, or a variant thereof.

7. The method of any of the preceding claims, wherein the man-made cellulosic fabrics is viscose and/or modal.

8. The method of any of the preceding claims, wherein family 45 cellulase is dosed in an amount of from about 0.08 to 5 mg protein/ g fabric, preferably 0.09 to 3 mg protein/ g fabric, and even more preferably 0.12 to 3 mg protein/ g fabric.

9. The method of any of the preceding claims, wherein the pH of the solution is about 4 to about 11, preferably about 5 to about 10, and even preferably about 5 to about 7.

10. The method of any of the preceding claims, wherein the process is carried out at a temperature between about 15°C to about 75°C, preferably 25°C and about 60°C.

11. The method of any of the preceding claims, wherein the step of pretreatment of adjusting pH to the range of 10-12 is conduct before contacting the fabrics with enzyme.

12. The method of claim 11, wherein NaOH or $Na_2CO_3$ is used for the pretreatment.

13. The method of any of the preceding claims, wherein the enzyme system further comprise one or more cellulase of EG, CBH and BG.

14. The method of any of preceding claims, wherein the enzyme system further comprises one or more additional components selected from surfactants, stabilizer, wetting agent, dispersing agents, antifoaming agents, lubricants, and builder system, or a mixture thereof.

15. A textile enzyme composition for bio-polishing man-made cellulosic fabrics, comprising Family 45 cellulase, wherein Family 45 cellulase is at least 30% on weight of total cellulase.

16. A textile enzyme composition of claim 15, wherein Family 45 cellulase is at least 50% on weight of total cellulase.

17. A textile enzyme composition of claim 16, wherein Family 45 cellulase is at least 70% on weight of total cellulase.

18. A textile enzyme composition of any of claims 15-17, wherein the cellulase is derived from Humicola, preferably Humicola insolens.

19. A textile enzyme composition of claim 18, wherein the cellulase is the cellulase with SEQ ID NO:2, or a variant thereof.

20. A textile enzyme composition of any of claims 15-17, wherein the cellulase is derived from Thielavia, preferably Thielavia terrestris or Trichoderma sp.

21. A textile enzyme composition of claim 20, wherein the cellulase is the cellulase with SEQ ID NO:1, or a variant thereof.

22. A textile enzyme composition of any of the preceding claims, further comprising one or more additional components selected from surfactants, stabilizer, wetting agent, dispersing agents, antifoaming agents, lubricants, and builder system, or a mixture thereof.

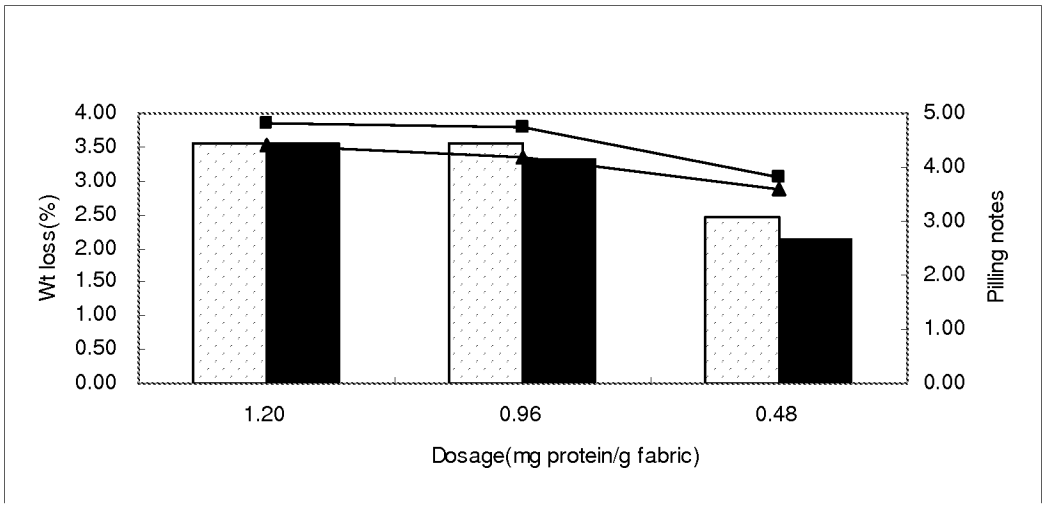


Figure 1

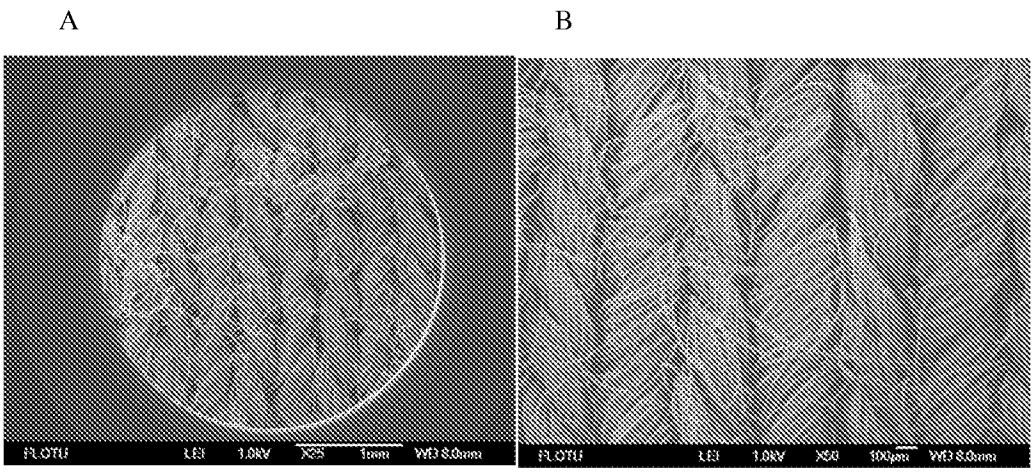


Figure 2

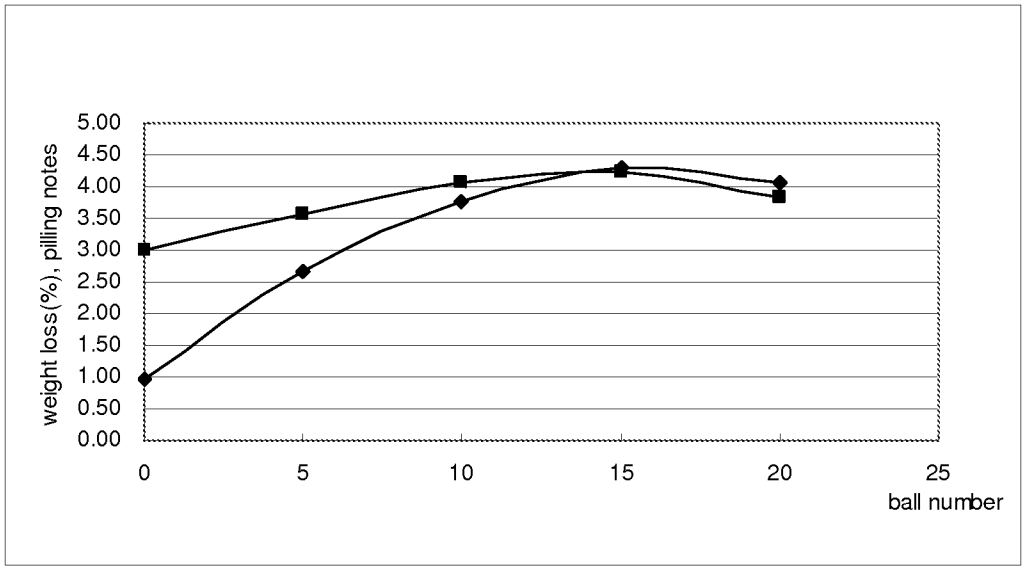


Figure 3

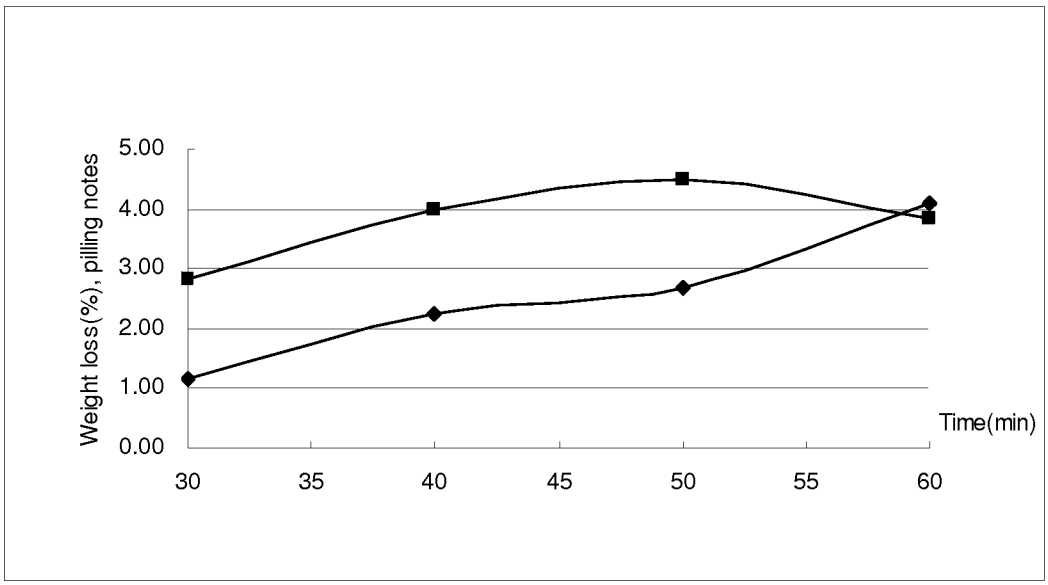


Figure 4

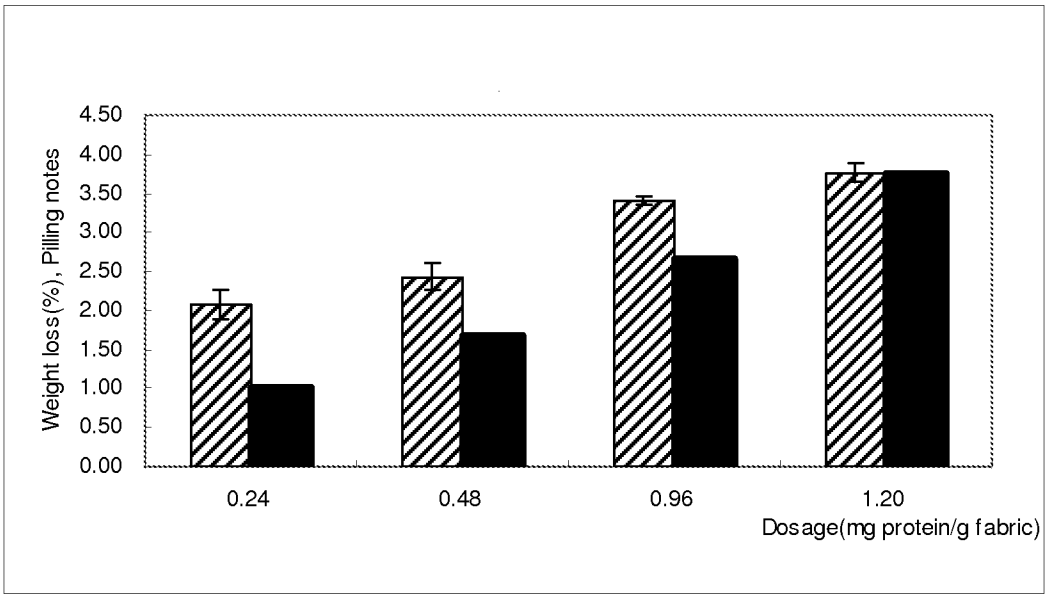
Weight loss(%), Pilling notes
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.24
0.48
0.96
1.20
Dosage(mg protein/g fabric)

**Figure 5**