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Aged-look vat dyed cotton with anti-bacterial/anti-fungal properties by treatment with nano clay and enzymes

Ali Sadeghian Maryan[a], Majid Montazer[b,*], Tina Harifi[b], Mahnaz Mahmoudi Rad[c]

[a] Chemical Engineering Department, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[b] Department of Textile Engineering, Amirkabir University of Technology, Tehran, Iran
[c] Skin Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

In this research, nanotechnology as a route to functional finishing of textiles was used along with bio-finishing to enhance the cotton fabrics performance. For this purpose, quaternary modified montmorillonite and common enzymes such as cellulase, laccase and their mixture were applied on vat dyed cotton fabric. Characteristic analysis of the treated samples and the dispersed nano clays in the effluent of the treatment was performed by various analyzing methods. The nano/bio-finishing is believed to impart antibacterial and antifungal activities with simultaneously higher lightness, advanced softness and handle properties into cotton fabrics. Moreover, cotton fabrics were proved to have no adverse effects (low toxicity) on human dermal fibroblasts. Findings suggest the potential of the proposed method in reducing the risk of microorganism for textile applications and imparting better handle and appearance properties.

1. Introduction

Textile industry has been searching for new technologies to accomplish the consumers' demands. Especially in recent years, new developments including bio and nano technology allowed the production of functional and smart textiles.

Biotechnology is the application of living organisms and their components to industrial products and processes and has found many applications in textile industry (Chena, Wang, Hua, & Du, 2007). Use of enzymes in textile processing and finishing is one of the best examples of the application of biotechnology to textiles (Csisza'r et al., 2001; Sarkar & Etters, 2004). Enzymes are biological catalysts, which can be used to develop eco-friendly alternative processes on textile materials to improve their properties (Montazer & Seifollahzadeh, 2011a,b). There is a range of enzymes such as amylase, cellulase, catalase, pectinase, protease and laccase for various textile wet-processing applications including desizing, bio-polishing, denim finishing, bleaching and bio-scouring. Bio-stoning of denims and surface modification of cellulosic fabrics to improve their appearance and handle are attracting most current attention in the area of enzyme processing (Belghith, Ellouz-Chaabouni, & Gargouri, 2001; Gusakov et al., 2001). Bio-polishing is a finishing process applied to cellulosic textiles, producing permanent effects (Cavaco-Paulo, 1998; Ibrahim, EL-Badry, Eid, & Hassan, 2011; Koo, Ueda, & Wakida, 1994; Rousselle & Howley, 1998; Saravanan, Vasanthi, & Ramachandran, 2009). It removes protruding fiber from fabrics, significantly reduces pilling, softens fabric hand and provides a smooth fabric appearance (Ibrahim, Fahmy, Hassan, & Mohamed, 2005). Bio-polishing uses a group of enzymes called cellulase which can synergistically hydrolyze cellulose and perform a specific catalytic activity on the 1,4-β-glucosidic bonds of the cellulose molecule (Cavaco-Paulo, 1998; Koo et al., 1994). There have been many studies on the use of cellulase on cotton fabric, regarding its effects on fabric softness, luster and resistance to pilling (Cavaco-Paulo, 1998; Ibrahim et al., 2011; Koo et al., 1994; Rousselle & Howley, 1998; Saravanan et al., 2009). Improving the dimensional stability of cellulosic fabrics using cellulase under pad-batch conditions has been reported (Cortez, Ellis, & Bishop, 2002). Enzymatic treatment has been proved to have a positive impact on dyeing regardless of the used dyestuffs which could probably be explained by creation of new dye-absorbing surface, alteration of pore structure along with simultaneous removal of fibrillar matters, enhancing the extent of dye diffusion or penetration into the treated fabric structure (Mori, Haga, & Takagishi, 1992; Mori, Haga, & Takagishi, 1996). On the other hand, dyeing with reactive dyes prior to enzymatic treatment may have inhibitory effect on enzyme activity, depending on size, molecular weight and concentration of dyes (Buschle-Diller & Traore, 1998; Czilik

* Corresponding author at: Textile Department, Amirkabir University of Technology, Center of Excellence in Textile, Hafez Avenue, Tehran, Iran.
Tel.: +98 21 64542657; fax: +98 21 66400245.
E-mail address: tex5mm@aut.ac.ir (M. Montazer).

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et al., 2002). The modes of action of cellulase on cotton become more complicated due to the existence of dyes (Yamada, Amano, Horikava, Nozaki, & Kanda, 2005). Also vat dyes are considered to be firmly trapped inside the fibers and seem not to interfere with the cellulase hydrolysis process (Cavaco-Paulo, 1998).

Laccase as an oxidoreductase enzyme with an intrinsic electron-donating tendency has been found to have applications in oxidations of dyes, polymerization of lignin and lignosulfonates, preparation of musts and wastewater treatment to improve the whiteness in a conventional bleaching of cotton, and recently, bio-stoning (Montazer & Sadeghian, 2008, 2010; Pereira, Bastos, Tzanov, Cavaco-Paulo, & Guebitz, 2005; Riva, 2006; Tian, White, Wang, Nie, & Zhu, 2012; Tzanov, Basto, Gübitz, & Cavaco-Paulo, 2003). In recent years, biological decolorization with laccase has been considered as an alternative and eco-friendly method to dye degradation and color removal from effluents (Montazer & Sadeghian, 2008, 2010; Murugesan, Dhamija, Nam, Kim, & Chang, 2007). Due to the discoloration effect of laccase on dyes, it can be used along with cellulase in the bio-stoning of denim garment. It is able to degrade indigo both in solution and on denim, leading to various bleaching effects on the fabric. We have already reported the use of laccase with cellulase for repeatable bio-washing to save water, enzyme, and energy (Montazer & Sadeghian, 2008). Also, minimizing the staining on white pocket and back of the denim by adding laccase in the washing bath was investigated (Montazer & Sadeghian, 2010).

There have been many researches focusing on using nano-size substances, generating nanostructures during manufacturing and finishing processes (Dastjerdi & Montazer, 2010). The field of nanofinishing in textile technology is very promising due to various end uses. Growing awareness of health has increased the demand for UV-protecting and antimicrobial textiles. Coating the surface of textiles with nanoparticles is a new way to the production of highly active surfaces, having UV blocking, antimicrobial, flame retardant, water repellant and self-cleaning properties. Metal oxide nanoparticles such as TiO_2 and ZnO, carbon nano tubes, silver nanoparticles, and clay nanoparticles are among the particles have been investigated (Montazer & Morshedi, 2012; Nazari, Montazer, Rashidi, Yazdanshenas, & Anary-Abbasinejad, 2009).

Moreover, textile industry generates colored effluents containing different dyes and pigments which are toxic and have to be adequately treated before discharging into the environment. A variety of materials have been used for removing of dyes from effluents and recently use of nanophoto catalysts has attracted great attention due to their ability to degrade organic pollutants under UV irradiation (Murugesan et al., 2007).

Natural phyllosilicates, known as clays, such as montmorillonite (MMT) have high surface area and high cation exchange capacity (Uddin, 2008). MMT, the most used clay in textile, with the chemical structure of $(Na, Ca)_{0.33}(Al, Mg)_2(Si_4O_{10})(OH)_{2n}H_2O$ is a 2:1 type layered silicates which consists of packets of two tetrahedral silicate layers and an octahedral with adjacent margins. These sheets retain a negative charge which is neutralized by exchangeable cations such as Na^+ or Ca^{2+} located in the interlayer spacing (gallery) and on the surface. The replacement of the natural inorganic cations in the clays with other organic cations has been widely studied to alter the surface properties of clays in order to improve their adsorption capacity. Ion exchange with surfactant cations such as quaternary ammonium salts (QAS) has been extensively investigated (Avalo et al., 2009; Huang, Gao, & Wang, 2012; Jincheng, Xiaoyu, Wenli, Nan, & Xingchen, 2012; Kozak & Domk, 2004; Paiva, Morales, & Valenzuela Díaz, 2008; Sarier, Onder, & Ersoy, 2010). The modification process may induce an enormous change in the surface and pore structure of clays and mediate the practical applications of clays as adsorbents or catalysts. Considering the ability of clay minerals to be intercalated with selected organic or inorganic substances with antimicrobial properties, they have attracted much more attention to be used as antimicrobial agents due to their non toxic and environmentally friendly characteristics (Sajomsang, Gonil, & Tantayanon, 2009; Wu, Xie, Tan, & Cai, 2011). Many studies have been carried out using clay minerals in different nano- and bio-technological applications and they have been frequently used in the removal of organic pigments and dyes from effluents (Liu & Zhang, 2007; Wang & Wang, 2007, 2008; Wang et al., 2004). Nanoclay particles are one of modern technologies which bring revolutionary changes in textile industry. Incorporating of these particles into textiles by melt spinning or by producing polymer/nanoclay composite as a coating to finished textiles, which can play an important role in flame retardant textiles, has been widely studied (Ghosh, 2011; Sen, 2001). However, their usage as nano particle finishing agents has not been extensively investigated.

In this study, vat dyed cotton fabric as a cotton material, more susceptible to microorganisms was chosen and its anti-bacterial and anti-fungal characteristics along with bio-finishing ability were evaluated. For this purpose, montmorillonite (MMT) intercalated by quaternary ammonium salt (QAS) named as QAS-MMT along with conventional enzymes including neutral cellulase (NC), acid cellulase (AC), laccase (L) and their mixture (NC-L) was applied on vat dyed cotton fabric in one step. The possibility of imparting anti-bacterial and anti-fungal properties into dyed cotton fabric along with simultaneous enhancement in lightness, handle, softness and surface properties was investigated. The present research was successful in introducing a facile novel procedure, producing cotton fabric with desirable multifunctional properties.

2. Experimental

2.1. Materials

A vat dyed cotton fabric weighing 322 g/m^2 with warp density of 26 cm^{-1} and weft density of 20 cm^{-1} was used. Amylase (Hi CONS), neutral cellulase (Denimax XT, EC 3.2.1.4), acid cellulase (Denimax 992 L, EC 3.2.1.4) and laccase (Denilite IT, EC 1.10.3.2) were provided by Novozymes Co. Denmark with activities of 120 LAMU/g, 1600 DA U/g, 750 AC U/g and 120 LAMU/g, respectively. Modified montmorillonite with quaternary ammonium salt, QAS-MMT, (Cloisite® 10A) was supplied from SCP Co, U.S.A.

2.2. Methods

2.2.1. Clay/enzyme treatment

Vat dyed desized cotton fabrics were treated in aqueous solutions of QAS-MMT/enzyme in one step according to Fig. 1a. A rotary drum washer with 100 g capacity (steel basket, 25 r.p.m) was used for nano/bio finishing.

Consequently, five different nano/bio treated cotton fabrics labeled QAS-MMT/NC, QAS-MMT/AC, QAS-MMT/L, QAS-MMT/NC-L, and QAS-MMT/AC-L were obtained. Samples named as QAS-MMT (in two conditions: neutral and acidic), NC, AC and L were also prepared in a manner identical to the procedure shown in Fig. 1a in the absence of enzymes and clay, respectively and considered as control samples in order to investigate the effect of nano and bio finishing on each other.

Schematic diagram of the QAS-MMT/enzyme cotton treated fabric is shown in Fig. 1b.

2.2.2. Characterization techniques

Scanning electron microscopic (SEM) analysis was performed on gold coated samples using Philips Co, XL30, equipped with energy dispersive X-ray probe (Thermo Noran, EDX).

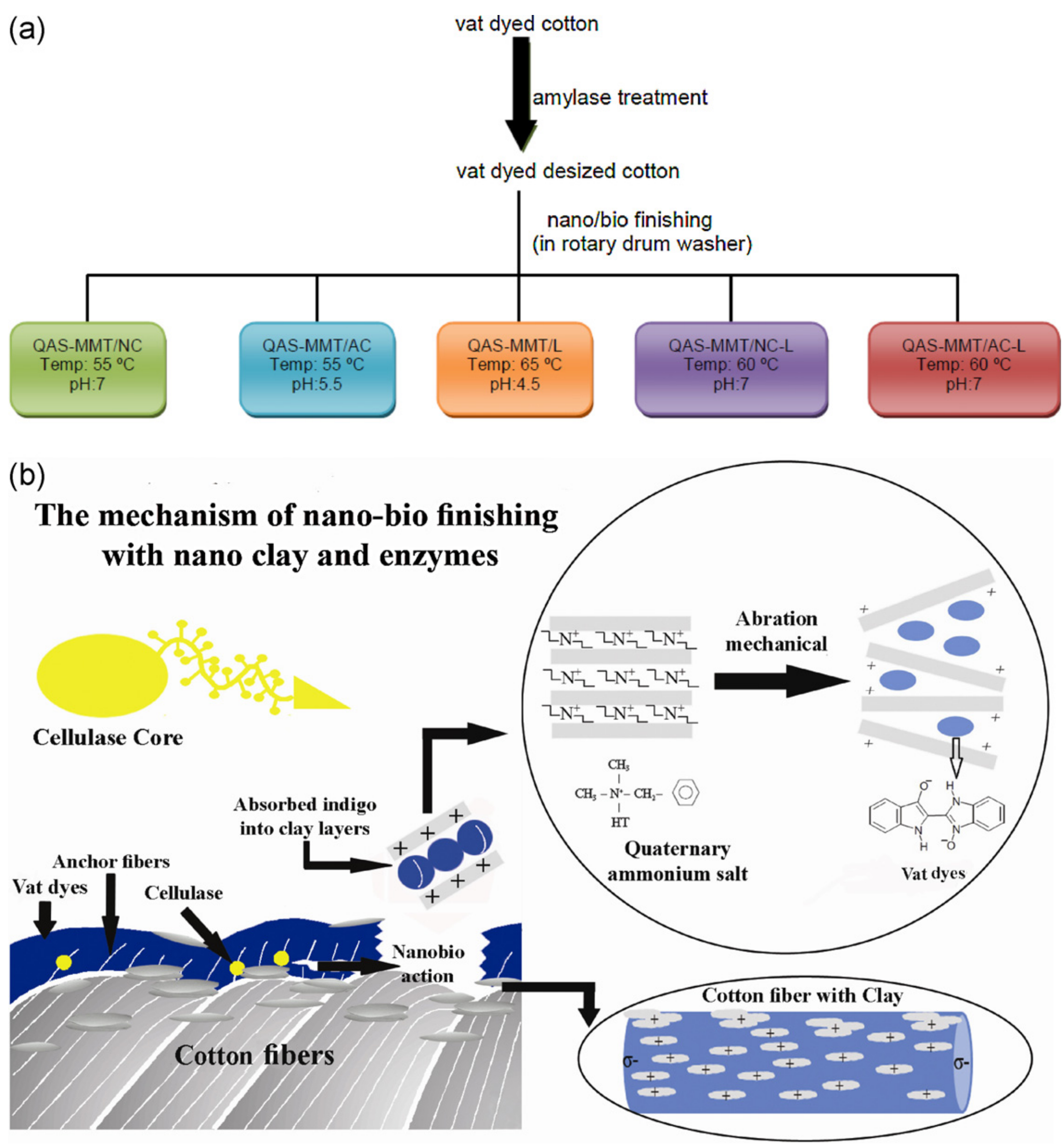


Fig. 1. (a) Schematic diagram of fabric treatment, QAS-MMT: 30% (on weight of fabric), Enzymes: 10 g/L. QAS-MMT: modified montmorillonite with quaternary ammonium salt, NC: neutral cellulase, AC: acid cellulase, L: laccase. (b) Schematic diagram of QAS-MMT/enzyme cotton treated fabric.

X-ray studies were performed using a Siemense D5000 X-ray diffractometer (Cu Ka, 1.5418 A°, operation voltage 40 kV).

The Fourier transform infrared (FTIR) spectra were obtained by Nicolet 670 USA.

The distribution range of clay particles in washing effluent was measured using a Dynamic Light Scattering (DLS), Malvern Co., England.

L^* (Lightness), a^*(redness-greenness) and b^* (yellowness-blueness) color values of treated samples were obtained by datacolor spectrophotometer (Microflash 200d) and CIELAB ΔE, color difference values under illuminant D65 for 1964 standard observer were quantified according to Eq. (1).

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* are differences between color values of samples before and after treatment.

2.2.3. Anti-bacterial and anti-fungal activity

The antimicrobial properties of cotton treated fabrics were evaluated by quantitative experiments according to AATCC test method 100-2004. *Staphylococcus aureus*, a Gram-positive bacterium, and *Escherichia coli*, a Gram-negative bacterium, were used as the test organisms. For evaluation of the antifungal effect, the diploid fungi *Candida albicans* was used. The antimicrobial and antifungal activities were expressed in terms of the percentage reduction of the organism after contact with the test specimen compared to the number of colonies of bacteria surviving after contact with control. The results were reported as percent reduction of bacteria (C) by Eq. (2).

$$C = \left[\frac{M_1 - M_2}{M_1}\right] \times 100 \quad (2)$$

where M_1 is the number of colonies in control microbial suspension and M_2 is the number of colonies existing in the suspension after contact with treated samples.

2.2.4. Cytotoxicity test

Normal primary human skin fibroblast was used for the cytotoxicity test. Fibroblasts were cultivated in Dulbecco's modified Eagles medium (DMEM, Biochrom, Berlin, Germany) and 10% fetal calf serum (FCS), containing 2 mM L-glutamine and incubated in a humidified atmosphere at 37 °C and 5% CO_2. Cells from passage number 3 were used and seeded in a 96-well plate and incubated for 48 h. Treated cotton fabrics cut into 1 inch × 1 inch pieces were soaked in 2 mL culture medium for 24 h. The cultured medium with leaching substance was then serial-diluted and the sample in each dilution was added to the cells and incubated for 24 h. The treated fabrics were removed from the cell cultures, and the cells were re-incubated for a further 24 h in fresh medium and then tested with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Kangwansupamonkon, Lauruengtana, Surassmo, & Ruktanonchai, 2009). A Tecan Sunrise^{TM} microplate reader at 492 nm was used to measure the absorbance. Experiments were performed at least five times and the obtained results were recorded as percentage absorbance relative to untreated control cells.

The cytotoxicity results were used to calculate percentage relative cell viability after incubation with the samples using Eq. (3):

$$\text{Cell-viability}\,(\%) = \left[\frac{abs_{sample}}{abs_{control}}\right] \times 100 \tag{3}$$

where abs_{sample} is the absorbance in a well containing sample and $abs_{control}$ is the absorbance of untreated control cells.

2.2.5. Softness property of treated fabrics

Fabric softness was evaluated by measuring the bending rigidity of treated samples using Shirley's Bending Length apparatus (Shirley Development Limited, England), according to BS 3356/9073-7 in which the fabric specimen was allowed to bend under its weight. The free length, which bended under its weight sufficiently to make its leading edge intersected a plane of 41.5° inclination, was called as bending length of the fabric C (cm). Bending rigidity of samples G (mg/cm) was calculated based on Eq. (4).

$$G = 0.1 \times M \times C^3 \tag{4}$$

where M (g/cm^2) is the weight per area of each sample.

3. Results and discussion

3.1. SEM image analysis

The morphologies of untreated cotton fabric (a–c), cotton treated fabric with QAS-MMT/NC (d–f) and cotton treated fabric with QAS-MMT/L (g–i) are shown in Fig. 2. It can be seen that the surface of the untreated sample is covered by anchor fibers (Fig. 1a and b). Also, SEM picture of untreated fabric at higher magnification (7500×) indicates that there are no nano particles on the surface (Fig. 2c).

According to Fig. 2d, it can be seen that bio-polishing by cellulase removes protruding fibers dotted over the surface, giving it a smoother appearance. Where cleavage of cellulose chain occurs, microfibrils, which are loose fibers break off under the influence of bio-catalytic degradation and modify the surface of the fabric. Some broken and damaged fibers are seen in Fig. 2e, indicating that the successful treatment of the samples with cellulase is limited to the surface of fabric. The ability of cellulase in removing the protruding fiber ends from the yarns, leading to less pilling tendency has been reported by many researchers (Ibrahim et al., 2011; Saravanan et al., 2009).

SEM image of QAS-MMT/L treated fabric (Fig. 2g) compared to the QAS-MMT/NC (Fig. 2d), demonstrated the disability of laccase in weakening, rupture and removal of loose surface microfibrils. It is known that laccase, capable to act on chromophore compounds, has many biotechnical and environmental applications, among which color removal from both liquors and materials (bleaching) is of particular interest (Montazer & Sadeghian, 2008, 2010; Murugesan et al., 2007). Therefore, using laccase will result in removal of dye from the treated fabric, leading to brighter color.

SEM micrograph of the samples treated with QAS-MMT/NC and QAS-MMT/L (Fig. 2f and i) in comparison with the untreated sample indicated that nano clays loaded on the fiber surface.

In order to estimate the average particle size of nano clays distributed on the fiber surface, SEM images were taken at (30,000×) magnification that are shown in Fig. 3a and b. It is revealed that nano clay particles of nearly 60 nm are uniformly distributed on the fiber surface during bio-finishing with QAS-MMT/NC (Fig. 3a) and QAS-MMT/L (Fig. 3b).

3.2. EDX analysis

EDX spectrum of cotton treated sample with QAS-MMT/NC was examined to understand the elemental specification of the treated fabric (Fig. 3c). Photoelectron peaks at around 200 eV were observed, depicting the presence of Si, Al and Mg which is another evidence of nano clay particles on the fiber surface. As the treated sample was coated by gold before the EDX experiment, Au peaks are also included in the spectrum.

3.3. DLS analysis

The size distribution of clay particles (opened layer silicate) after bio-finishing with QAS-MMR/NC-L was determined by dynamic light scattering (DLS) (Fig. 3d). The results indicate that nano clay particles smaller than 100 nm presented in the bio-finishing effluent.

3.4. XRD analysis

Fig. 4a shows XRD reflection pattern of QAS-MMT after bio-finishing treatment. The XRD characteristic of nano clay particles before bio-finishing is also given in Fig. 4a for comparison. XRD patterns of untreated cotton fabric and cotton fabric treated with QAS-MMT/NC are also demonstrated in Fig. 4b.

The reflections at 2θ = 22.78°, 37.24° and 63.32° in the diffractogram of QAS-MMT before bio-finishing showed slight shift in the spectra of QAS-MMT after bio-finishing. The changes in the higher order reflections of QAS-MMT after bio-finishing, compared to those obtained before bio-finishing, may be due to delamination and disorientation of clay platelets (Li, Gao, Yue, & Hu, 2009; Sarier, Onder, Ozay, & Ozkilic, 2011).

The X-ray diffraction pattern of QAS-MMT/NC treated fabric resembles that of untreated sample, except for a group of reflections occurred for 2θ values greater than 65° (Fig. 4b). Two prominent peaks appeared at 2θ = 66.4° and 70.2°, indicating the presence of nano clay particles on fabric surface which has been also confirmed by SEM and EDX analyses. Therefore, results of X-ray diffractometer analysis reveal that loading of the organoclays on fiber surfaces was successfully achieved.

3.5. FTIR analysis

The FTIR spectra of QAS-MMT before and after bio-finishing treatment are illustrated in Fig. 5. The characteristic bands of QAS-MMT before bio-finishing are seen bellow 1100 cm^{-1}. A peak at 1020 cm^{-1} belongs to the stretching vibrations of Si—O bonds of the tetrahedral silica layers. The peaks at 719, 619, 519 and 451 cm^{-1}

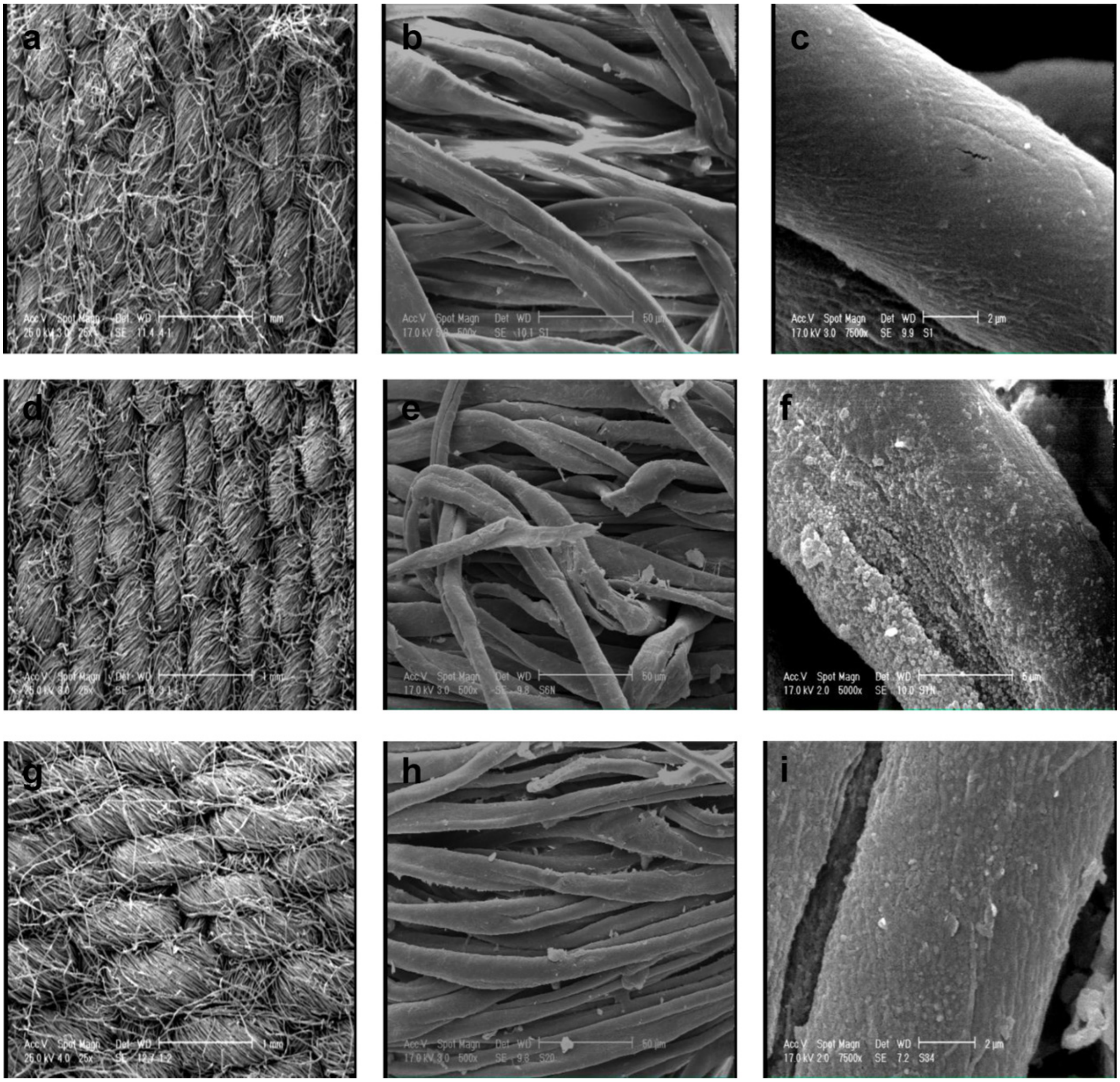


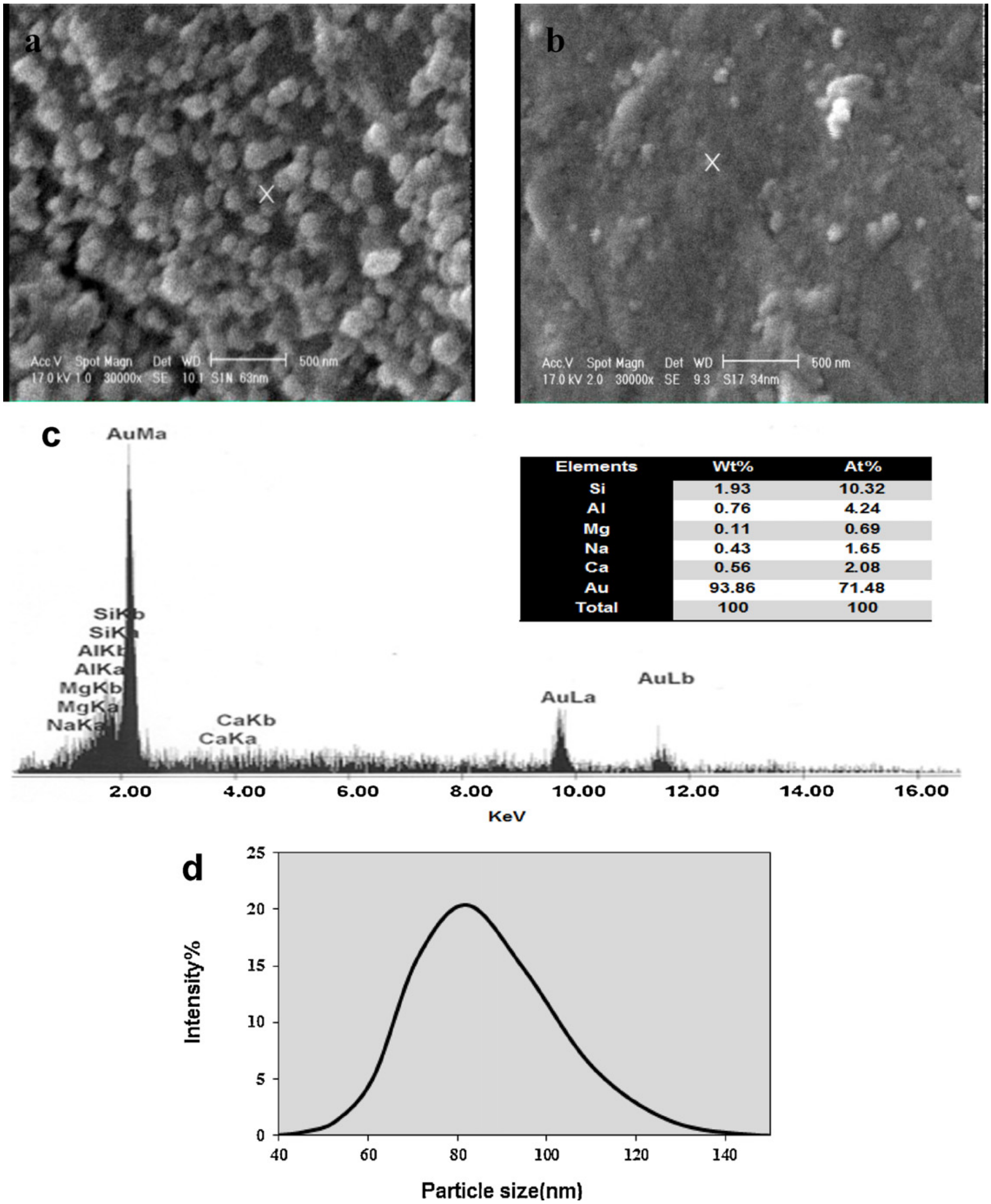
Fig. 2. SEM images of untreated cotton fabric (a)–(c), cotton fabric treated with QAS-MMT/NC (d)–(f) and cotton fabric treated with QAS-MMT/L (g)–(i). Micrographs were taken at 50× (a, d and g), 500× (b, e and h) and 7500× (c, f and i).

correspond to the Si—O and Al—O bending vibrations of the octahedral and tetrahedral silica-alumina layers. The bands appeared at 2845 and 2920 cm^{-1} are specific to stretching vibrations of the $-CH_2$ and $-CH_3$ of alkylammonium cation, respectively. The —CH bending vibration of $-CH_3$ is also observed at 1465 cm^{-1}. The stretching vibration of the —OH groups are characterized by a band at 3622 cm^{-1}, which is in accord with literature (Kozak & Domk, 2004). It had a slight shift toward the smaller frequencies comparing to the MMT, implying the removal of some hydroxyl groups from the Si—OH and Al—OH sites and some H_2O from the interlayer space when the QAS entered.

In the FTIR analysis of QAS-MMT after bio-finishing, the characteristic QAS-MMT bands below 1100 cm^{-1} are broadened, weakened and shifted to the lower wave numbers. These changes may be arisen from the adsorption of vat dye presented in the effluent by QAS-MMT. Compared with the IR spectra of QAS-MMT before bio-finishing (before dye adsorption), the new absorption bands (between 1580 and 1710 cm^{-1}), corresponding to the stretching vibrations of C=O of the dye, are observed in QAS-MMT after dye adsorption.These results indicate that dye adsorption from the effluent has been successfully carried out by QAS-MMT particles. There have been many studies reporting the high adsorption capacity of organoclay minerals and their promising role as sorbents for environmental and purification purposes (Liu & Zhang, 2007; Wang & Wang, 2007, 2008; Wang et al., 2004).

3.6. Antimicrobial and antifungal activity of treated samples

The percentage reduction of bacteria and fungi in all treated samples is summarized in Fig. 6a. Textile goods especially natural fabrics without antimicrobial modifications can provide an excellent environment for microorganisms to grow (Fig. 6a (untreated fabric)). The results indicated that QAS-MMT has an excellent antibacterial and antifungal activity and is completely effective in inhibitory and killing of microorganisms (Sajomsang et al., 2009; Wu et al., 2011). As can be seen in Fig. 6a, fabrics treated by different types of enzymes did not have any antimicrobial activity. Therefore, enzymes made no contributions to imparting antibacterial property into cotton fabrics and QAS played the prominent role.



Elements	Wt%	At%
Si	1.93	10.32
Al	0.76	4.24
Mg	0.11	0.69
Na	0.43	1.65
Ca	0.56	2.08
Au	93.86	71.48
Total	100	100

Fig. 3. SEM images of cotton fabric treated with (a) QAS-MMT/NC and (b) QAS-MMT/L. EDX spectrum of cotton sample treated with QAS-MMT/NC (c). Size distribution analysis of clay particles after bio-finishing with QAS-MMR/NC-L by means of dynamic light scattering (DLS) (d).

In particular, QASs have been extensively used as cationic disinfectant and biocidal coating to prevent the growth of microorganisms on the surface of textile materials (Kim, Kim, & Rhee, 2010). The QASs have been proved to be an appropriate antimicrobial agent for nylon66 and cotton fabrics (Son, Kim, Ravikumar, & Lee, 2006). QAS-MMT particles are capable of killing the microorganisms by adsorbing on to the microbial cell surface, releasing of QAS, diffusion of the released QAS through the cell wall and coagulation and disruption of the cytoplasmic membrane, interfering in the normal activity of the cell and consequently cell death (Kim et al., 2010).

In order to evaluate the washing durability of the imparted antibacterial property, QAS-MMT treated fabric was scoured according to AATCC 61(2A)-1996. The ability of the treated sample in killing the bacteria and fungi after number of washing cycles is shown in Fig. 6b.

It can be concluded that even after 50 home washing cycles, the treated sample still had the inhibitory effect on bacteria and resulted in 69.39%, 64.90% and 61.99% reduction of *E. Coli*, *S. aureus* and *C. Albicans*, respectively. Therefore, the proposed method was effective in imparting durable antibacterial and antifungal property into dyed cotton fabric.

3.7. Cytotoxicity test

Information about the toxicity of textile materials is important especially for people who are very sensitive and have adverse

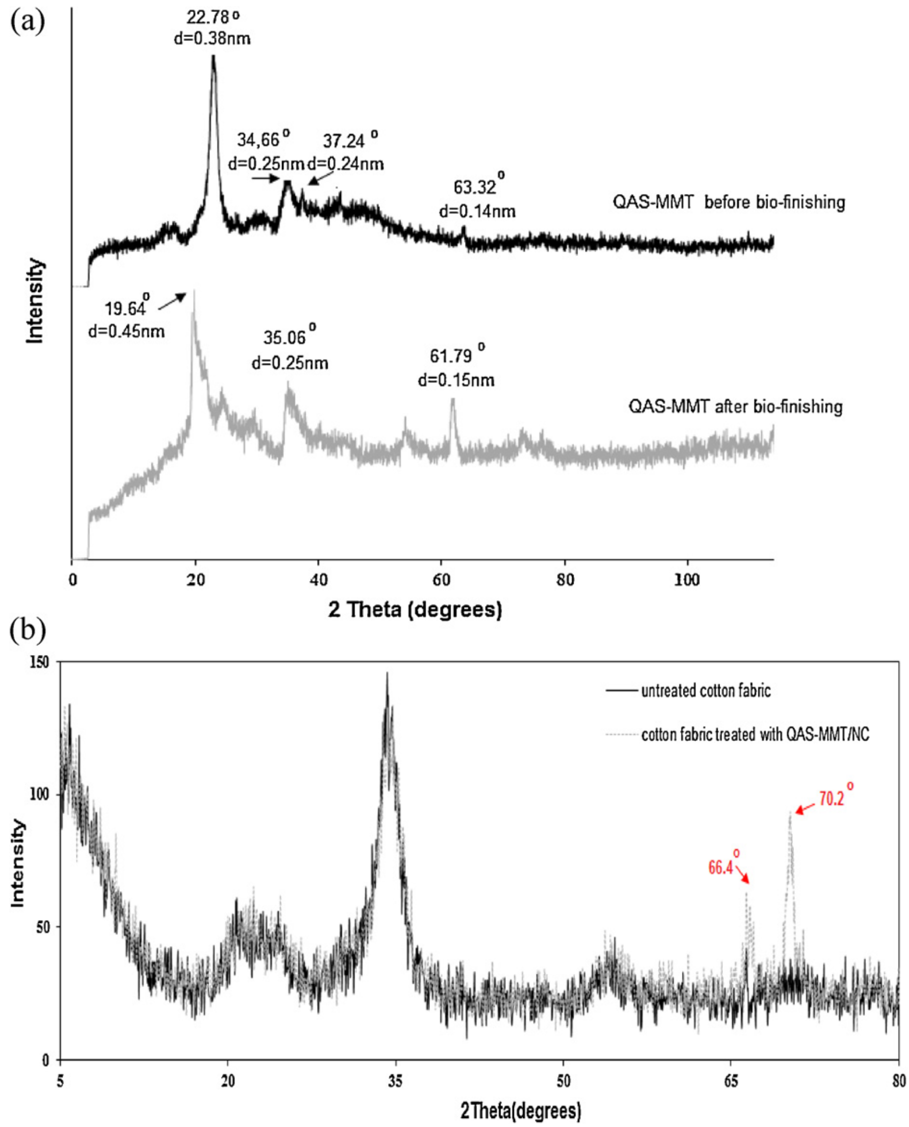


Fig. 4. (a) XRD reflection patterns of QAS-MMT before and after bio-finishing treatment, (b) XRD reflection patterns of untreated cotton fabric and cotton fabric treated with QAS-MMT/NC.

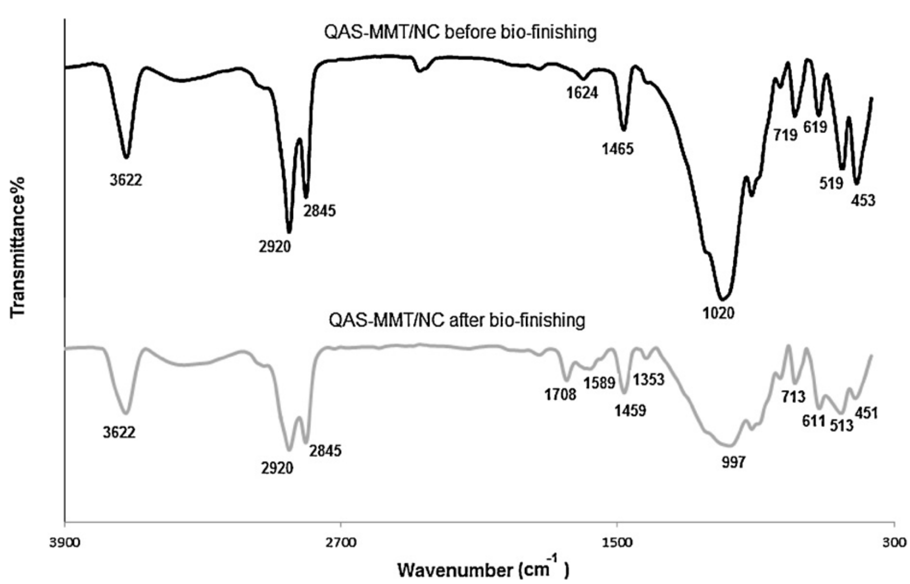


Fig. 5. FTIR spectra of QAS-MMT before and after bio-finishing treatment.

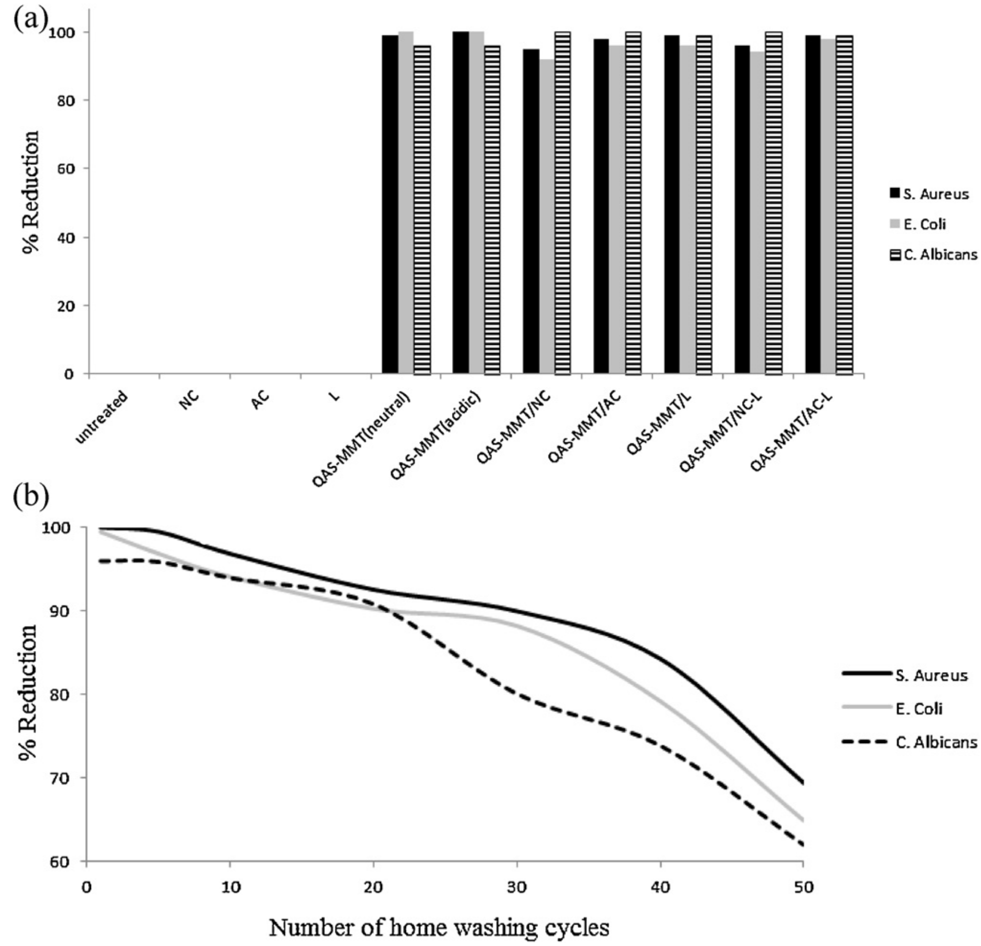


Fig. 6. (a) Antibacterial test results of untreated and treated cotton fabrics, (b) washing durability of the imparted antibacterial and antifungal properties into QAS-MMT treated fabric.

reactions to different chemical components. Although QASs have been reported to impart antibacterial properties to fabric, the cytotoxic effects of these compounds on human cells/tissues must be also considered (Klemola, Pearson, Liesivuori, & Lindström-Seppä, 2009).

For this purpose, cytotoxicity test was carried out to investigate any harmful effects of QAS-MMT/enzyme treated fabrics on human dermal fibroblasts. The extraction medium was cultured with normal human dermal fibroblast and its viability was determined by MTT assay. Those cells without a treatment of the sample were considered as a control with a cell viability of 100%. Cell viability over untreated and each treated samples after 24 h of incubation is given in Fig. 7a.

For all the treated samples, the viability values are higher than that of the untreated sample. The average elative cell viability was over 70%, indicating good biocompatibility properties for the treated fabrics.

Cells change their shape in response to toxic agents, prior to strong changes in metabolism or proliferation (Brunner, Wick, & Manser, 2006). Therefore, the photographs of cells were used for observing the alteration in cell shape or morphology due to exposure to toxic materials. Fig. 7b shows normal growing spindle-shaped cells. After incubation with treated fabric (Fig. 7c), although some cells underwent morphological changes, most of them are viable retaining their initial spindle shape. Therefore, no considerable adverse effects on human skin were found using the treated fabrics.

3.8. Color measurements

Enzymatic modification of cotton fabrics with cellulase and laccase has been proved to improve brightness of dyed fabrics and the fabric surface will become more lustrous after bio-finishing (Table 1) (Cavaco-Paulo, 1998; Koo et al., 1994).

L^* a^* b^* color values of untreated and nano/bio treated samples along with their color changes due to finishing treatment are summarized in Table 1. Laccase, as an oxidoreductase enzyme, has the ability to act on chromophore compounds and oxidize dyes. It can remove the dye from the treated fabric and improve the fabric lightness. The mechanism of dye degradation by laccase has been reported in literature (Montazer & Sadeghian, 2010). The product of dye degradation would cause yellow color on the treated fabrics (Table 1).

Cellulase is capable of cellulose hydrolysis, resulting in cellulose bio-polishing, removing of the protruding fibers from fabrics, reducing pilling, softening fabric hand, providing a smooth fabric appearance and improving the fabric lightness.

The results reveal that bio treatment has a positive effect on fabric lightness, producing fabrics with higher lightness (L^*) values. In this regard, incorporation of laccase with QAS-MMT was found to be more efficient, leading to 33% lightness increment.

The cotton fabric treated with QAS-MMT/L was also greener, indicating that laccase turned the fabric hue from red to green and from blue to yellow. The highest ΔE value also belonged to QAS-MMT/L treated fabric.

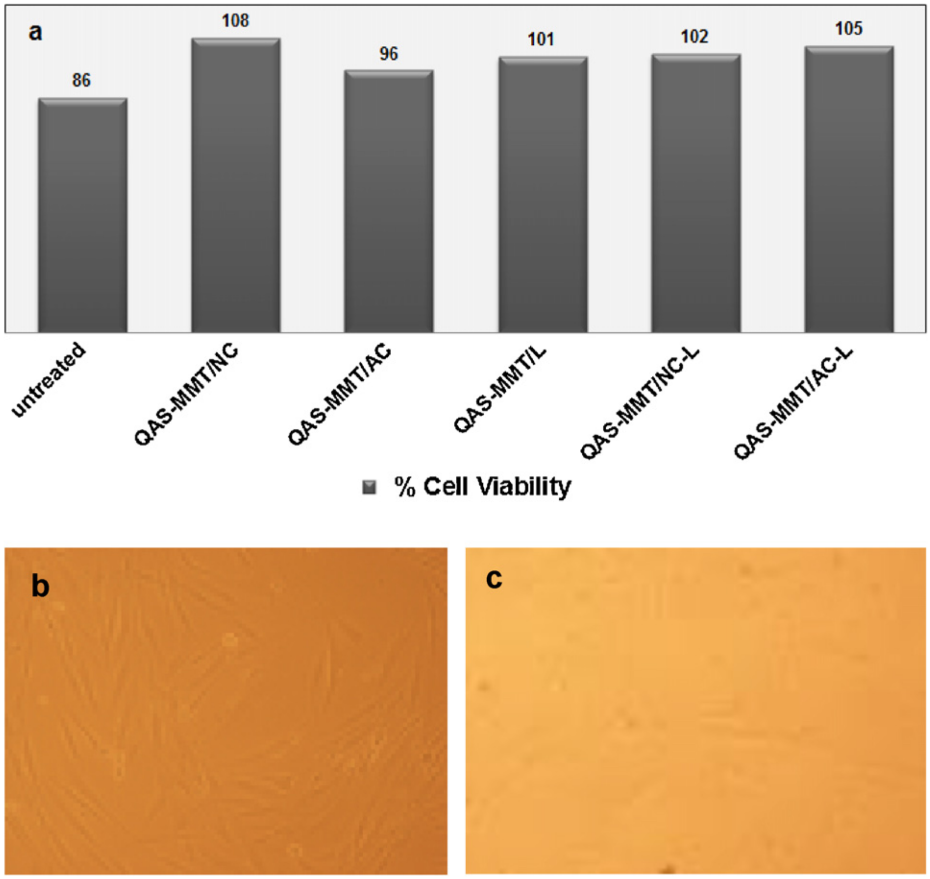


Fig. 7. Cell viability after 24 (h) of incubation with the untreated and QAS-MMT/enzymes treated samples (a), Photograph of dermal fibroblast cells of control (b) and treated samples (c).

Table 1
Colorimetric properties and bending rigidity of untreated and treated samples. L^*, a^*, b^* and ΔE correspond to lightness, redness-greenness, yellowness-blueness and color difference values, respectively.

Sample	L^*	a^*	b^*	ΔE	Bending rigidity (mg/cm)
Untreated	21.0	1.1	−8.7	0.0	37.2
Nc	22.5	0.9	−9.1	1.5	19.4
Ac	22.1	1.0	−9.4	1.3	20.1
L	23.5	0.2	−7.5	2.9	31.0
QAS-MMT (Neutral Media)	22.4	0.7	−7.6	1.8	23.0
QAS-MMT (Acidic Media)	22.6	0.6	−7.5	2.0	21.0
QAS-MMT/NC	24.7	0.5	−9.6	3.8	8.8
QAS-MMT/AC	24.4	0.5	−9.1	3.4	9.6
QAS-MMT/L	27.9	−4.0	−2.0	10.8	26.4
QAS-MMT/NC-L	25.0	−0.3	−5.7	5.1	18.3
QAS-MMT/AC-L	24.8	−0.7	−5.7	5.1	17.9

Moreover, no adverse effect on the activity of the enzymes was observed in samples treated by QAS-MMT/enzymes. The presence of QAS-MMT did not reduce the enzyme effects such as improved handle and softness (in case of cellulase) and enhanced lightness (in case of laccase).

3.9. Softness property

Bending property has an important effect on the handle performance. According to the results obtained for bending rigidity (Table 1), decrease in the bending rigidity is observed in all finished fabrics, which could possibly improve handle of the treated fabrics, resulting in loss in stiffness and enhancement of softness.

Bio-finishing has been reported to cause similar effects, imparting softness properties to fabrics and providing them with smoother appearance (Cavaco-Paulo, 1998; Koo et al., 1994; Ibrahim et al., 2011).

4. Conclusions

An ideal anti-bacterial, anti-fungal dyed cotton fabric with improved lightness, handle, softness and surface properties was successfully achieved by applying a facile method using modified nano clay/enzymes. In addition to the remarkable effects of the applied method, treated cotton fabrics were proved to be less toxic to human skin cells. Apart from the promising role of modified nano caly in killing the microorganisms, they also acted as absorbents resulting in less polluted effluents. Therefore, the proposed method can be introduced as a simple and effective route to impart various desirable multifunctional features to cotton fabric.

References

Avalo, F., Ortiz, J., Zitzumbo, R., López-Manchado, M., Verdejo, R., & Arroyo, M. (2009). Phosphonium salt intercalated montmorillonites. *Applied Clay Science*, *43*, 27–32.

Belghith, H., Ellouz-Chaabouni, S., & Gargouri, A. (2001). Biostoning of denims by *Penicillium occitanis* (Pol6) cellulases. *Journal of Biotechnology*, *89*, 257–262.

Brunner, T. J., Wick, P., & Manser, P. (2006). In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environmental Science Technology*, *40*, 4374–4381.

Buschle-Diller, G., & Traore, M. K. (1998). Influence of direct and reactive dyes on the enzymatic hydrolysis of cotton. *Textile Research Journal*, *68*, 185–192.

Cavaco-Paulo, A. (1998). Mechanism of cellulase action in textile processes. *Carbohydrate Polymers*, *37*, 273–277.

Chena, J., Wang, Q., Hua, Z., & Du, G. (2007). Research and application of biotechnology in textile industries in China. *Enzyme and Microbial Technology*, *40*, 1651–1655.

Cortez, J. M., Ellis, J., & Bishop, D. P. (2002). Using cellulases to improve the dimensional stability of cellulosic fabrics. *Textile Research Journal*, *72*, 673–680.

Csisza'r, E., Losonczi, A., Szaka'cs, G., Ruszna'k, I., Bezu'r, L., & Reicher, J. (2001). Enzymes and chelating agent in cotton pretreatment. *Journal of Biotechnology*, *89*, 271–279.

Czilik, M., Paszt, E., Reczey, I., Alta, J., Rusznak, I., & Vigc, E. A. (2002). Effects of reactive dyes on the enzymatic depolymerization of cellulose. *Dyes and Pigments*, *54*, 95–106.

Dastjerdi, R., & Montazer, M. (2010). A review on the application of inorganic nanostructured materials in the modification of textiles: Focus on antimicrobial properties. *Colloids and Surfaces B: Biointerfaces Engineering Aspects*, *79*, 5–18.

Ghosh, A. (2011). Nano-clay particle as textile coating. *International Journal of Engineering and Technology*, *11*, 40–43.

Gusakov, A., Sinitsyn, A. P., Markov, A. V., Sinitsyna, O. A., Ankudimova, N. V., & Berlin, A. G. (2001). Study of protein adsorption on indigo particles confirms the existence of enzyme – Indigo interaction sites in cellulose molecules. *Journal of Biotechnology*, *87*, 83–90.

Huang, G., Gao, J., & Wang, X. (2012). Preparation and characterization of montmorillonite modified by phosphorus–nitrogen containing quaternary ammonium salts. *Applied Surface Science*, *258*, 4054–4062.

Ibrahim, N. A., EL-Badry, K., Eid, B. M., & Hassan, T. M. (2011). A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. *Carbohydrate Polymers*, *83*, 116–121.

Ibrahim, N. A., Fahmy, H. M., Hassan, T. M., & Mohamed, Z. E. (2005). Effect of cellulase treatment on the extent of post-finishing and dyeing of cotton fabrics. *Journal of Materials Processing Technology*, *160*, 99–106.

Jincheng, W., Xiaoyu, Z., Wenli, H., Nan, X., & Xingchen, P. (2012). Synthesis of hyperbranched quaternary ammonium salt and its application into montmorillonite. *Powder Technology*, *221*, 80–89.

Kangwansupamonkon, W., Lauruengtana, V., Surassmo, S., & Ruktanonchai, U. (2009). Antibacterial effect of apatite-coated titanium dioxide for textiles applications. *Nanomedicine: Nanotechnology, Biology and Medicine*, *5*, 240–249.

Kim, H. W., Kim, B. R., & Rhee, Y. H. (2010). Imparting durable antimicrobial properties to cotton fabrics using alginate–quaternary ammonium complex nanoparticles. *Carbohydrate Polymers*, *79*, 1057–1062.

Klemola, K., Pearson, J., Liesivuori, J., & Lindström-Seppä, P. (2009). Evaluating the toxicity of fabric extracts using thehepa-1 cytotoxicity test, the HaCaT cytotoxicity test and the spermatozoa motility inhibition test. *Journal of the Textile Institute*, *100*, 330–337.

Koo, H., Ueda, M., & Wakida, T. (1994). Cellulase treatment of cotton fabrics. *Textile Research Journal*, *64*, 70–74.

Kozak, M., & Domk, L. (2004). Adsorption of the quaternary ammonium salts on montmorillonite. *Journal of Physics and Chemistry of Solids*, *65*, 441–445.

Li, M. K. S., Gao, P., Yue, P., & Hu, X. (2009). Synthesis of exfoliated CNT–metal–clay nanocomposite by chemical vapor deposition. *Separation and Purification Technology*, *67*, 238–243.

Liu, P., & Zhang, L. (2007). Adsorption of dyes from aqueous solutions or suspensions with clay nano-adsorbents. *Separation and Purification Technology*, *58*, 32–39.

Montazer, M., & Morshedi, S. (2012). Nano photo scouring and nano photo bleaching of raw cellulosic fabric using nano TiO_2. *International Journal of Biological Macromolecules*, *50*, 1018–1025.

Montazer, M., & Sadeghian Maryan, A. (2008). Application of laccase with cellulase on denim for clean effluent and repeatable biowashing. *Journal of Applied Polymer Science*, *110*, 3121–3129.

Montazer, M., & Sadeghian Maryan, A. (2010). Influences of different enzymatic treatment on denim garment. *Applied Biochemistry and Biotechnology*, *160*, 2114–2128.

Montazer, M., & Seifollahzadeh, S. (2011a). Enhanced self-cleaning, antibacterial and UV protection properties of nano TiO_2 treated textile through enzymatic pretreatment. *Photochemistry and Photobiology*, *87*, 877–883.

Montazer, M., & Seifollahzadeh, S. (2011b). Pretreatment of wool polyester blended fabrics to enhance titanium dioxide nanoparticle adsorption and self-cleaning properties. *Coloration Technology*, *127*, 1–6.

Mori, R., Haga, T., & Takagishi, T. (1992). Relationship between cellulase treatment and direct dye dyeing for cotton. *Journal of Applied Polymer Science*, *45*, 1869–1872.

Mori, R., Haga, T., & Takagishi, T. (1996). Reactive dye dyeability of cellulose fibers with cellulase treatment. *Journal of Applied Polymer Science*, *59*, 1263–1269.

Murugesan, K., Dhamija, A., Nam, I., Kim, Y., & Chang, Y. (2007). Decolourization of reactive black 5 by laccase: Optimization by response surface methodology. *Dyes and Pigments*, *75*, 176–184.

Nazari, A., Montazer, M., Rashidi, A., Yazdanshenas, M., & Anary-Abbasinejad, M. (2009). Nano TiO_2 photo-catalyst and sodium hypophosphite for cross-linking cotton with poly carboxylic acids under UV and high temperature. *Applied catalysis A: General*, *371*, 2010–2016.

Paiva, L., Morales, A., & Valenzuela Díaz, F. (2008). Organoclays: Properties, preparation and applications. *Applied Clay Science*, *42*, 8–24.

Pereira, L., Bastos, C., Tzanov, T., Cavaco-Paulo, A., & Guebitz, G. M. (2005). Environmentally friendly bleaching of cotton using laccases. *Environmental Chemistry Letters*, *3*, 66–69.

Riva, S. (2006). Laccases: Blue enzymes for green chemistry. *Trends in Biotechnology*, *24*, 219–226.

Rousselle, M., & Howley, P. S. (1998). Molecular weight of cotton cellulose: Effect of treatment with a total cellulase. *Textile Research Journal*, *68*, 606–610.

Sajomsang, W., Gonil, P., & Tantayanon, S. (2009). Antibacterial activity of quaternary ammonium chitosan containing mono or disaccharide moieties: Preparation and characterization. *International Journal of Biological Macromolecules*, *44*, 419–427.

Saravanan, D., Vasanthi, N. S., & Ramachandran, T. (2009). A review on influential behaviour of biopolishing on dyeability and certain physico-mechanical properties of cotton fabrics. *Carbohydrate Polymers*, *76*, 1–7.

Sarier, N., Onder, E., & Ersoy, S. (2010). The modification of Na-montmorillonite by salts of fatty acids: An easy intercalation process. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *371*, 40–49.

Sarier, N., Onder, E., Ozay, S., & Ozkilic, Y. (2011). Preparation of phase change material–montmorillonite composites suitable for thermal energy storage. *Thermochimica Acta*, *524*, 39–46.

Sarkar, A. K., & Etters, J. N. (2004). Enzymatic hydrolysis of cotton fibers: Modeling using an empirical equation. *The Journal of Cotton Science*, *8*, 254–260.

Sen, A. K. (2001). *Coated textiles principles and application*. Pennsylvania: Technomic.

Son, Y., Kim, B., Ravikumar, K., & Lee, S. (2006). Imparting durable antimicrobial properties to cotton fabrics using quaternary ammonium salts through 4-aminobenzenesulfonic acid chloro–triazine adduct. *European Polymer Journal*, *42*, 3059–3067.

Tian, L., White, B., Wang, W., Nie, H., & Zhu, L. (2012). Laccase-mediated system pretreatment to enhance the effect of hydrogen peroxide bleaching of cotton fabric. *International Journal of Biological Macromolecules*, *50*, 782–787.

Tzanov, T., Basto, Cs., Gübitz, G. M., & Cavaco-Paulo, A. (2003). Laccases to improve the whiteness in a conventional bleaching of cotton. *Macromolecular Materials and Engineering*, *288*, 807–810.

Uddin, F. (2008). Clays, nanoclays, and montmorillonite minerals. *Thermochimica Acta*, *39*, 2804–2814.

Wang, C., Juang, L., Hsu, T., Lee, C., Lee, J., & Huang, F. (2004). Adsorption of basic dyes onto montmorillonite. *Journal of Colloid and Interface Science*, *273*, 80–86.

Wang, L., & Wang, A. (2007). Adsorption characteristics of Congo Red onto the chitosan/montmorillonite nanocomposite. *Journal of Hazardous Materials*, *147*, 979–985.

Wang, L., & Wang, A. (2008). Adsorption properties of Congo Red from aqueous solution ontosurfactant-modified montmorillonite. *Journal of Hazardous Materials*, *160*, 173–180.

Wu, T., Xie, A., Tan, S., & Cai, X. (2011). Antimicrobial effects of quaternary phosphonium salt intercalated clay minerals on *Escherichia coli* and *Staphylococci aureus*. *Colloids and Surfaces B: Biointerfaces*, *86*, 232–236.

Yamada, M., Amano, Y., Horikava, E., Nozaki, K., & Kanda, T. (2005). Mode of action of cellulases on dyed cotton with reactive dye. *Bioscience: Biotechnology and Biochemistry*, *69*, 45–50.