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Bio-approach: Ureolytic bacteria mediated synthesis of ZnO nanocrystals on cotton fabric and evaluation of their antibacterial properties

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ABSTRACT

ZnO nanoparticles (NPs) synthesis on cotton fabric through the formation of biologically activated ammonia from urea broth in the presence of the ureolytic bacterial species *Serratia ureilytica* (HM475278) has been described in the present contribution. The cotton fabric was immersed in biogenic zinc ammonium complex medium and subjected to heat treatment at an optimum temperature of 50 °C for different durations of time (30, 60, 90 min). The crystal growth of ZnO nanoparticles on cotton fabric was characterized by analytical techniques such as SEM-EDAX, XRD, TGA, CHNS and UV–visible spectra, and evaluation of antibacterial activity was carried out against *Escherichia coli* and *Staphylococcus aureus*. Crystal growth and morphological studies confirmed the attachment of ZnO NPs on the cotton fabric. Spherical to nanoflower shaped particles were obtained with increasing time duration from 30 to 90 min. The antibacterial activity of loaded cotton fabrics was found to be substantially higher than the bare cotton samples. Wet film interfacial contact studies have shown greater antibacterial activity as a result of nanoparticle contact at the bio-interface, as observed by Epi-fluorescent microscopic observations.

1. Introduction

The use of cotton fabric has become increasingly widespread as a result of numerous favourable properties such as regeneration, softness, affinity to the skin; sweat absorbing properties and microbial resistance (Dastjerdi & Montazer, 2010; Fouda, Abdel-Halima, & Al-Deyab, 2013; Kiwi & Pulgarin, 2010). Nevertheless, it is highly prone to microbial attack that can cause a wide variety of contagious diseases. The vulnerability of cotton fabric stems from the fact that it has high surface area, which favours bacterial binding and growth (Fouda et al., 2013). Moreover, the fabric exhibits enhanced decolourization, decomposition and decrease in mechanical strength with time (Dastjerdi & Montazer, 2010; Fouda et al., 2013). These points are of concern to textile delivering industrial products. Recent developments in the field of nanotechnology have brought a vast spectrum of novel prospects in the field of antimicrobial textile finishing. The antimicrobial activity of nanoparticles is believed to arise from their small particle size and high surface area, and is attributed to both metal ions as well as nanoparticles. Metal and metal oxide nanoparticles such as Ag, Au, MgO, Cu, ZnO, and TiO_2 are being considered as antimicrobial agents for textile finishing (Dastjerdi & Montazer, 2010; Fouda et al., 2013; Kiwi & Pulgarin, 2010). They have selective toxicity to bacteria and minimal effects on human beings (Gao & Cranston, 2008; Ma, Williams, & Diamond, 2013). Moreover, nanoparticles possess high affinity to cotton fabric, rendering substantial mechanical strength, self-cleaning, UV-protection capability and custom colour tuning (Dastjerdi & Montazer, 2010; Fouda et al., 2013; Kiwi & Pulgarin, 2010).

Presently, much research emphasis is being laid upon the antimicrobial properties of ZnO nanoparticles and their exploitation as antimicrobial agents for textile finishing against a wide spectrum of bacteria, fungi and yeast (Ates & Unalan, 2012; Espitia et al., 2012; Ma et al., 2013). The unique properties of ZnO nanoparticles such as small size and large surface area render them to exhibit greater antibacterial activity than bulk ZnO (Raghupathi, Koodali, & Manna, 2011). Compared to silver, cotton fabric incorporated with ZnO nanoparticles has numerous advantages such as lower cost, atmospheric stability of material, whiteness, self-cleaning ability, UV-blocking properties and mechanical strength. The common techniques for ZnO NPs growth on cotton fabrics are sonochemical, solvothermal/hydrothermal reactions, sol–gel synthesis, and chemical precipitation (Applerot et al., 2009; Baviskar, Nikam, Gargote, Ennaoui, & Sankapal, 2013; Li, Chen, & Jiang, 2007; Perelshtein et al., 2009, 2010, 2012). Li et al. successfully achieved ZnO nanocrystal growth on cotton fabric through the formation of zinc–ammonium complexes by the solution method at optimum conditions of pH, temperature and reaction time (Li et al., 2007).

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Chemical methods for synthesis of ZnO nanoparticles use amine derivatives such as hexamethylenetetramine (HMTA) for ensuring controlled growth and particle size, which are highly detrimental to the environment (Baviskar et al., 2013; Mende & MacManus-Driscoll, 2007; Xur, Wang, Zhang, Jia, & Tian, 2010).

Bio-mediated synthesis of nanoparticle provides solutions to many of the challenges posed by the chemical route. The design and production of nanoparticles for efficient antibacterial behaviour can be achieved using microorganisms (Dickerson, Sandhage, & Naik, 2008; Mandal, Bolander, Mukhopadhyay, Sarkar, & Mukherjee, 2006; Mohanpuria, Rana, & Yadav, 2008). Present research emphasis is on ureolytic bacteria/urease mediated nanoparticle synthesis, due to the possibility of synthesizing a wide range of nanoparticles at low temperatures (Chen et al., 2009; Johnson, Kinsinger, Sun, Li, & Kisailus, 2012; Prywer & Torzewska, 2009; Rica & Matsui, 2008; Unuma, Matsushima, & Kawai, 2011). Urea is hydrolysed by ureolytic bacteria via the urease enzyme, which splits urea into ammonia and CO_2 as a resultant increased pH of the medium and the precipitation of metal ions such as $MgNH_4PO_4{\cdot}6H_2O$, $Ca_{10}(PO_4)_6(CH_2)$, $CaCO_3$ (Chen et al., 2009; Prywer & Torzewska, 2009). The urease enzyme mediated synthesis of semiconductor nanoparticles has also been an area of intensified scientific interest in the recent years. Rica & Matsui, 2008 succeeded in growing crystalline ZnO nanoshells at room temperature exploiting urease as a nanoreactor (Rica & Matsui, 2008). The urease mediated synthesis of nanocrystalline TiO_2 at room temperature has been reported (Johnson et al., 2012). The synthesis of semiconductor nanocrystals mediated by urease enzyme catalyzed biogenic ammonia generation may pave way for unique structural morphologies, which can be tailored as required for specific functional applications (Johnson et al., 2012; Rica & Matsui, 2008). To the best of our knowledge, the synthesis of ZnO nanoparticles mediated by ureolytic bacteria has rarely been reported in scientific literature. In the present study, ureolytic bacterium of *Serratia ureilytica* (HM475278) has been selected for the production of biogenic ammonia in appreciable quantities from urea broth at optimal conditions. Crystal growth and morphological characteristics of ZnO nanoparticles of the cotton fabric were investigated by XRD, SEM-EDAX, UV–visible spectra, TGA and CHNS elemental analyzer. The antibacterial activity of ZnO NPs loaded cotton fabric was evaluated against *Staphylococcus aureus* and *Escherichia coli*. Epi-fluorescent microscopic studies reveal the effectiveness of nanoparticle-bacterial species interfacial contact with regard to bacterial killing.

2. Materials and methods

2.1. Materials

Zinc acetate (99% pure) was purchased from Sigma Aldrich, India. Urea base broth, nutrient broth, nutrient agar plate, fluorescein isothiocyanate (FITC) and propidium iodide (PI) were supplied by Hi-Media, India, and cotton fabric (woven) was obtained from a certified local dealer. The specification of woven cotton fabric is 100% cotton, natural colour, sort: 40's × 40's/132 × 72–63 in. Plain, 60's × 60's/175 × 56/2–120 in. reverse satin, 60's × 60's/175 × 56/2–128 in. reverse satin.

2.2. Selection of bacterial species

In the present study, ureolytic bacterium *Serratia ureilytica* (HM475278) isolated from railway track faecal contamination was identified by molecular techniques and used for the production of ammonia from urea broth. The composition of urea base broth (g/l) is as follows: peptic digested from animal tissue – 1.0, dextrose – 1.0, sodium chloride – 5.0, disodium phosphate – 1.2, monopotassium phosphate – 0.8, and urea 40%. The medium was kept in a bacterial incubator at room temperature for 5 days and bacterial culture enrichment was monitored for the production of ammonia and pH of the medium.

2.3. Estimation of ammonia from ureolytic bacterial culture

The estimation of biogenic ammonia from bacterial culture had been described in our previous contribution (Maruthamuthu et al., 2009). Samples were withdrawn from the enrichment of bacterial culture at different time intervals and centrifuged at 10,000 rpm for 20 min in order to obtain the supernatant solution, which was then filtered through a cellulose acetate membrane having a pore size of 0.45 μm. The filtrate solution was tested for the presence of ammonia by indophenols method. Incubation of the mixture was carried out for 30 min at room temperature. The absorption value was measured at 625 nm by UV–visible spectroscopy and ammonia concentrations were estimated.

2.4. Bio-mediated synthesis of ZnO nanoparticles on cotton fabric

Zinc acetate and biologically activated ammonia were used as the base materials for ZnO nanoparticle synthesis on the cotton fabric surface. In the typical procedure, 0.02 M zinc acetate was dissolved in 300 ml of deionised water. This mixture was stirred at 300 rpm for 30 min, followed by the sudden addition of 25 ml biologically activated ammonia solution. The cotton fabric was immersed in this medium, which was vigorously stirred to facilitate binding of zinc ammonium complexes on the fabric surface. Heating of this medium was carried out at 50 °C for different time durations such as 30, 60 and 90 min. The crystal growth and morphology of ZnO nanoparticles in each fabric sample were characterized, and its antibacterial activity was evaluated against bacterial species *E. coli* and *S. aureus*.

2.5. Characterization of ZnO loaded cotton fabric

The surface morphology of the pure cotton fabric and ZnO particles loaded fabrics were observed by scanning electron microscopy (SEM-Tescan-VEGA3 SB). The control cotton was gold sputtered to render electrical conductivity. The nature of the elements was identified by energy dispersive X-ray absorption spectroscopy (EDAX-Bruker). A computer controlled XRD system, JEOL, JPX-8030 with Cu $K\alpha$ radiation (Ni filter = 13,418 Å) at the range of 40 kV, 20 A was used for the recording of XRD patterns. Identification of peaks was done employing the 'peak search' and 'search match' programmes built in software (syn master 7935). The UV–visible spectrum of the ZnO coated cotton fabric was recorded at 250–600 nm using Spectra 50 ANALYTIKJENA spectrophotometer. The CHNS elemental analyzer was employed for determination of nitrogen content in cotton fabric. The mechanical properties of fabrics were measured on a universal testing machine, KIC-1-100-C, capacity up to 1KN, KALPAK-Tech. Surface roughness of the cotton fabric were measured by Zeta Instruments (ZETA-300: Precision Surface Metrology).

2.6. Antibacterial activity

E. coli and *S. aureus* cultures were used for this study. The strains were cultured on a nutrient broth (Hi-media, Mumbai) and incubated aerobically at 37 °C overnight. The antibacterial behaviour of nanomaterials and their interaction with bacterial surface are vital tools that render measurement of antibacterial

activity. In the present study, interactions between ZnO nanoparticle loaded cotton fabric and bacterial surface were investigated by three techniques viz. agar diffusion method, solution suspension and wet interfacial contact condition. Our previous contribution (Babu, Dhandapani, Maruthamuthu, & Kulandainathan, 2012) had reported the use of agar diffusion plate test for antibacterial activity assessment of nanoparticle incorporated cotton fabric. Reduction in bacterial viability was monitored by measuring total viable counts at different intervals of time (2, 4, 6, 8, 10 and 12 h). Percentage reduction in bacterial count was calculated using Eq. (1), where A and B are the viable count at 0, 2, 4, 6, 8, 10 and 12 h, respectively.

$$\frac{A-B}{A} \times 100\% \quad (1)$$

Wet interfacial contact method involves consistent direct contact of each bacterial species under study (10^6 CFU/ml in phosphate buffer solution) on the ZnO NPs-cotton fabric interface. The disruption of bacterial cell membrane with respect to incubation time was visualized by epi-fluorescence microscopy. Fluorescein isothiocyanate (FITC) and propidium iodide (PI) dual stains were used for identifying living/dead cells on bacterial. Propidium iodide (PI) penetrates only damaged cells and binds to the DNA emitting red colour, whereas FITC remains exterior to undamaged cell walls giving raise to green emission. About 0.5 μl of dual stain (FITC–PI; 1:1%) was added to bacterial samples, which were then incubated for a 15 min time duration. The excess stain was rinsed with sterile distilled water and examined under Epi-fluorescence microscope (E200 Coolpix; Nikon, Tokyo, Japan).

3. Results and discussions

3.1. Selection of ureolytic bacterial strain

Ureolytic bacterial communities have been reserved in toilet dropping area of Indian railway track. Urea from human urine can be utilized for bacterial proliferation and metabolic activity (Mobley & Hausinger, 1989). The major role of this bacterial species is to convert ammonia to active urease. *Serratia ureilytica* (HM 475278) was used for the production of ammonia from urea broth. This bacterium has the capability to hydrolyze urea to ammonia and to utilize urea as a sole nitrogen source for its growth (Maruthamuthu et al., 2011). Fig. 1(a) depicts the pH of medium at regular intervals of time in the presence of bacterial species, as compared with control urea broth under identical conditions. The pH of the urea broth was 6.7 and 9.4 for the control and bacterial systems, respectively, at the end of the 5th day. In bacterial system, the pH of the medium increases gradually, reaching a maximum value of 9.4 on the 3rd day. The reasoning behind this concept can well be co-related by the production of biogenic ammonia in solution (estimated by indophenols method) shown in Fig. 1(b). It can be seen that the ammonia content in the medium rises to its maximum of 7500 ppm on the 3rd day, corresponding to the maximum increase in medium pH in the presence of bacterial system. The pH and ammonia content falls slightly post attaining their maximum values. This decline may be due to loss of bacterial activity as a consequence of ammonia saturation in the medium. On the other hand, one can clearly observe from Fig. 1(a) that the pH of the medium remains almost constant under control conditions, without any significant raise. These observations point decisively at the effect of ureolytic bacterial species with regard to the generation of biogenic ammonia for the ZnO nanoparticles synthesis. Most contributions related to the chemical synthesis of ZnO nanoparticles from zinc acetate and ammonia at various concentrations in the range of 0.01–0.2 M (Applerot et al., 2009; Baviskar et al., 2013; Ghule, Ghule, Chen, & Ling, 2006; Li et al., 2007; Perelshtein et al., 2009; Yu, Jin, Liu, Liu, & Fu, 2007; Zhai, Wu, Lu, Wang, & Wang, 2008). Moreover, ammonia is used for pH adjustment in the range of 8–9 for nanoparticle synthesis (Baviskar et al., 2013; Perelshtein et al., 2012). Previous investigators had generated ammonia from urea by enzyme method (urease), which had resulted in the production of ammonia at low quantities (Johnson et al., 2012; Rica & Matsui, 2008). However, the employment of microbes has rendered larger quantities of ammonia production. In the present contribution, ureolytic bacterial species were used to generate ammonia in the range of 7500 ppm at 3rd day. This concentration of biogenic ammonia would be sufficient for the synthesis of ZnO nanoparticles.

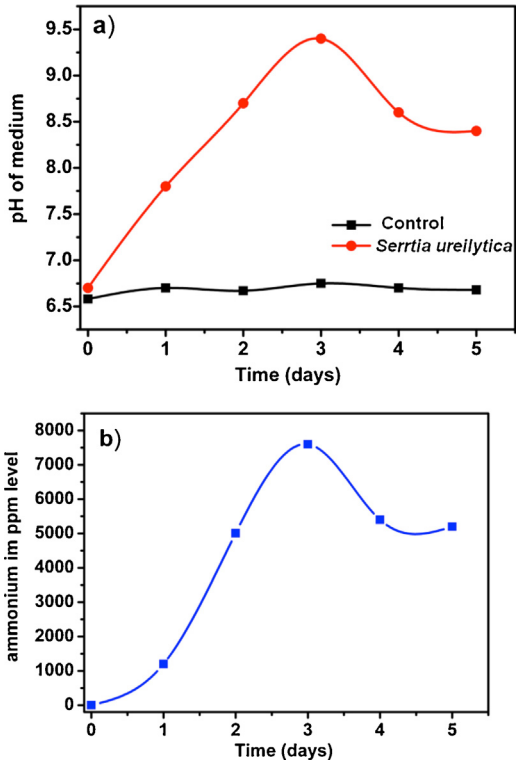

Fig. 1. (a) pH and (b) ammonia estimation plots of the medium at different durations of time under identical conditions with and without bacterial inoculation.

3.2. Characterization of ZnO loaded cotton fabric

Scanning electron microscopy (SEM) measurements were carried out in order to critically image the growth and morphological characteristics of ZnO nanostructures on the cotton fabric surface. Fig. 2(a) reveals a typical fibril structure with clean and smooth surface of pristine cotton fibres. After a treatment time of 30 min, spot nucleation of ZnO nanoparticles (170–250 nm) is seen, as evident from Fig. 2(b). The continuation of treatment causes agglomeration of nuclei. Hence, aggregated ZnO nanoparticles (300–600 nm for aggregated nanoparticles) are observed in Fig. 2(c), the SEM image of ZnO nanostructures after 60 min. Further heat treatment of cotton fabric for 90 min has resulted in progressive growth of ZnO nanoparticles (185–360 nm for single nanoflower), leading to the formation of flowers, as seen in Fig. 2(d). SEM observations confirm the time dependent growth and change in morphological characteristics of bio-synthesized ZnO nanocrystals on cotton fabric upon heat treatment at 50 °C. ZnO NPs size measurements from SEM are provided in supplementary information Fig. 1. Growth of ZnO nanostructures on fabric is further confirmed by EDAX, provided in the information supporting this contribution (Supplementary information Fig. 2). The EDAX spectrum of control cotton fabric prior to treatment indicates the absence of zinc, whereas loading

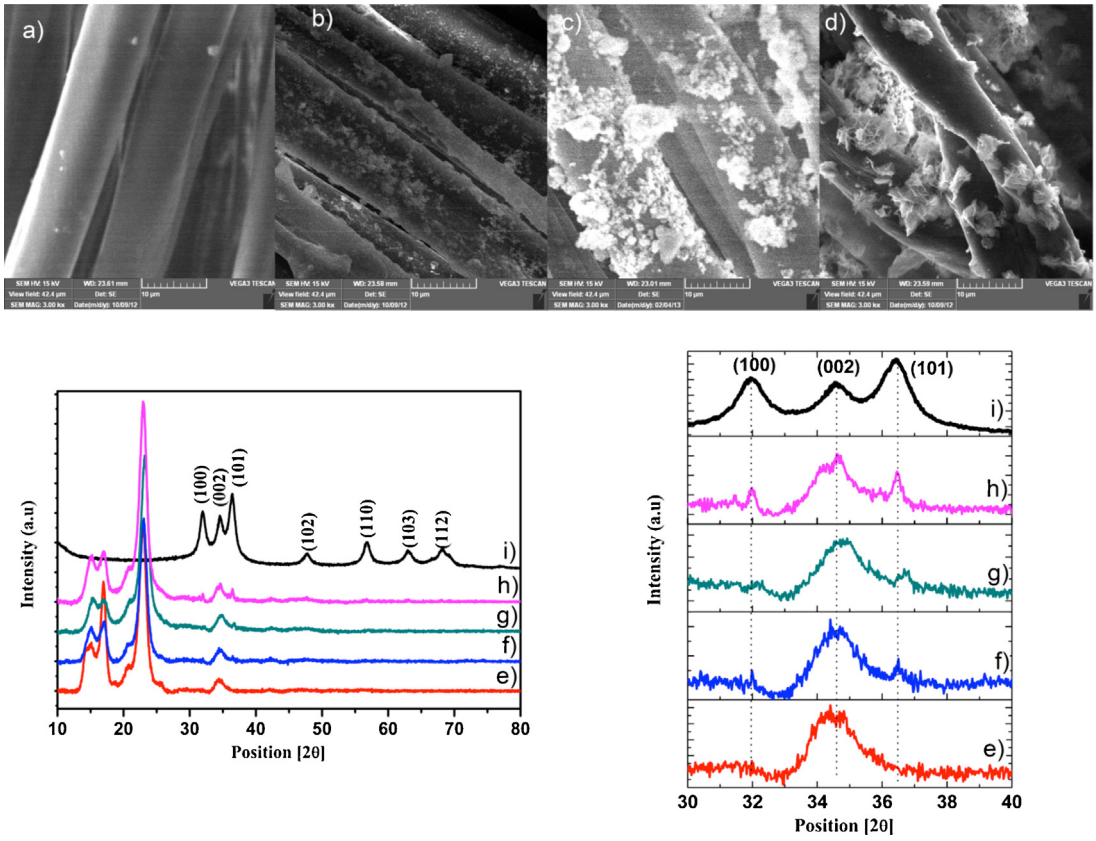

Fig. 2. Scanning electron microscopy (SEM) images of (a) control cotton fabric, (b) ZnO NPs loaded cotton fabric prepared by (b) 30 min, (c) 60 min, (d) 90 min heat treatment at 50 °C, and X-ray diffraction pattern of (e) control cotton fabric, ZnO loaded on cotton fabric surface at different time intervals (f) 30 min, (g) 60 min, (h) 90 min, and (i) bio-synthesized ZnO nanoparticles in the absence of cotton fabric.

of Zn after treatment is clearly evident from the EDAX spectra of cotton fabric recorded post 90 min of heat treatment.

The X-ray diffraction patterns depicted in Fig. 2(e–i) demonstrate the crystalline nature of bio-synthesized ZnO nanoparticles and ZnO loaded on the cotton fabric surface at different time intervals (30, 60, and 90 min). Fig. 2(i) is the XRD pattern of biosynthesized ZnO nanoparticles in the absence of cotton fabric. The peak signals at (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3) and (1 1 2) reflection lines confirm that the formation of wurtzite phase hexagonal structure of ZnO, which coincides with JCPDS Card No. 89-1397. Decreased prominence of obtaining peaks may be attributed to the loading of cotton in trace amounts. Hence, an enlarged pattern elucidating the additional peaks is provided. The diffraction peaks obtained are found to be in accordance with the hexagonal phase of ZnO on the cotton fabric. Peaks at $2\theta = 31.77$, 34.42 and 36.25 are assigned to the (1 0 0), (0 0 2) and (1 0 1) crystal planes of hexagonal ZnO particles, respectively. These peaks are found to be absent in the control cotton fabric (Fig. 2(e)), indicating the loading of ZnO nanoparticles upon treatment. It can also be observed from the diffraction pattern that the prominence of ZnO diffraction peaks increases with treatment time. Fig. 2(f) and (g) shows the diffraction patterns of ZnO loaded cotton fabrics synthesized by treatment for 30 and 60 min, respectively. For a treatment time of 90 min (Fig. 2(h)), the diffraction peak intensity is found to be at a maximum, with an additional hexagonal ZnO peak corresponding to the (0 0 2) crystal plane at 2θ at 34.42, just adjacent to the cotton peak. This observation confirms the growth of hexagonal phase ZnO on cotton fabric. Thus, the biogenic ammonia mediated route is an efficient method for the synthesis of ZnO nanocrystals.

The UV–vis spectra of control cotton fabric as compared to that coated with ZnO nanoparticles at different treatment times is provided in the information supporting this contribution (Supplementary information Fig. 3). The spectra were recorded in absorption mode along a wavelength range of 250–600 nm. It can be seen that the control cotton fabric exhibits its characteristic absorption peak at a threshold wavelength of 346 nm. Upon the incorporation of hexagonal ZnO nanoparticles, this peak shifts along the range of 350–410 nm at different treatment times. Absorption peaks at wavelengths of 360 and 390 nm are observed in the samples treated for 30 and 60 min, respectively. The sample treated for 90 min exhibits maximum shift in absorption wavelength at 410 nm. From these observations, it can be construed that the wavelength of absorption increases with treatment time due to increased crystal growth ZnO on cotton fabric.

3.3. Mechanical properties

Tensile strength measurements were performed on a double folded fabric of dimensions 25 mm × 75 mm. The elongation–force plots of control and ZnO NPs loaded cotton fabric produced by heat treatment for 90 min are provided in Fig. 4 of supplementary information. It can be seen from the plots that the tensile force was marginally greater for the ZnO NPs coated cotton fabrics (force 75 N), than that of the control fabric (72 N). It can also be seen that the maximum elongation at breakdown (27.24 mm) is greater for the ZnO NPs loaded cotton fabric than that of the control (24.10 mm). These findings from tensile strength studies point at the fact that the mechanical properties of the cotton fabric do not deteriorate upon ZnO NPs coating. The surface roughness plots of bare and ZnO NPs loaded cotton fabrics prepared by heat treatment for 90 min are provided in Fig. 5 of supplementary information. By comparing the plots Fig. 5(a) and (b), it can be noticed

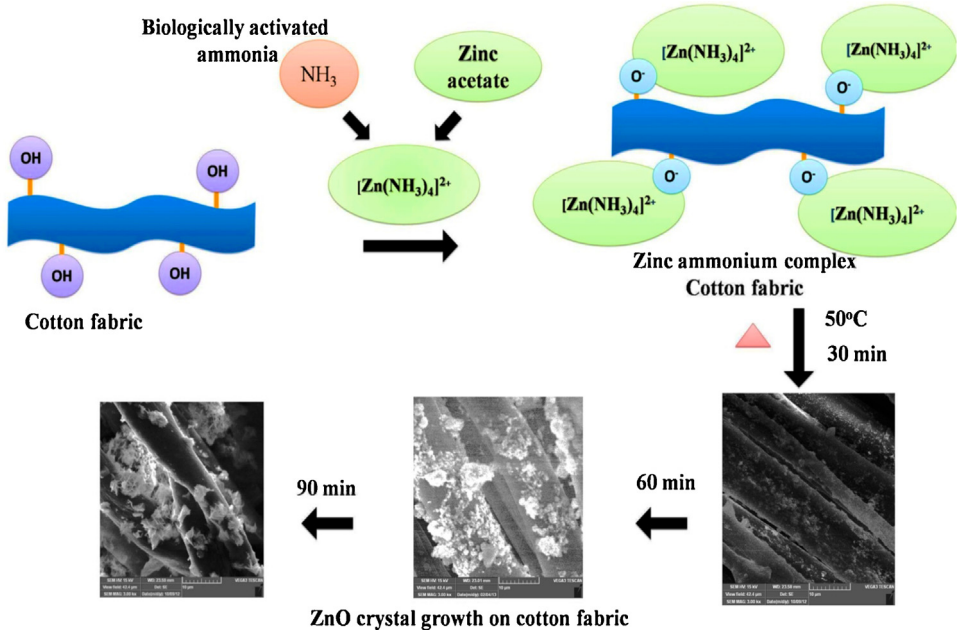

Fig. 3. Mechanism of the ureolytic bacteria mediated synthesis of ZnO nanoparticles and in situ generation on cotton fabric.

that the surface roughness of ZnO loaded cotton fabric (standard deviation (SD) = 21.59 μm) is much higher than the pristine control (SD = 1.897 μm). Hence, the nanoparticle loaded fabrics may be conveniently used for practical applications in textile and biomedical industry.

3.4. Mechanism of the ureolytic bacteria mediated synthesis of ZnO nanoparticles and in situ generation on cotton fabric

Fig. 3 illustrates the mechanism of the ureolytic bacteria mediated synthesis of ZnO nanocrystals on cotton fabric. Optimum values of pH and ammonia bio-production were obtained on the 3rd day. The culture medium was centrifuged at the end of the 3 days of bacterial cell exclusion, and the supernatant solution was used for synthesis of ZnO nanocrystals on cotton fabric. In our study, optimum concentration of zinc ions (0.02 M) and biologically activated ammonia were used under a controlled pH of 9.0, and medium temperature was maintained at 50 °C. The ZnO nanoparticle loaded cotton fabric samples were subjected to morphology and crystal growth analysis at different treatment time durations (30, 60, 90 min). The first step is urea hydrolysis to produce biogenic ammonia by ureolytic bacterial species *S. ureilytica*. Biogenic ammonia reacts with zinc ions to produce $Zn(OH)_2$ and $Zn(NH_3)_4^{2+}$ (Eq. (6)), which can be dehydrated and crystallized to form ZnO nanocrystals at 50 °C (Baviskar et al., 2013; Ghule et al., 2006; Yu et al., 2007; Zhai et al., 2008). The biochemical processes are summarized in the following equations:

Serratiaureilytica(HM475278)

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad (2)$$

$$NH_3 + H_2O \leftrightarrow NH_3{\cdot}H_2O \leftrightarrow NH_4^+ + OH^- \quad (3)$$

$$Zn^{2+} + 2OH^- \leftrightarrow Zn(OH)_2 \quad (4)$$

$$2Zn^{2+} + 4NH_3 + 4H_2O \rightarrow 2Zn(OH)_2 + 4NH_4^+ \quad (5)$$

$$Zn(OH)_2 + 4NH_3 \rightarrow [Zn(NH_3)_4]^{2+} + 2OH^- \quad (6)$$

$$[Zn(NH_3)_4]^{2+} + 2OH^- \leftrightarrow ZnO + 4NH_3 + H_2O \quad (7)$$

Thermo gravimetric analysis (TGA) was conducted across the temperature range of 30–500 °C at a constant heating rate of 5 °C/min to confirm the mechanism of biogenic ammonia mediated synthesis of ZnO nanocrystal growth on cotton fabric (Fig. 4). The TGA plot of control cotton fabric shows an initial weight loss of 3.34% at 84.94 °C due to the removal of physically adsorbed components on the fabric surface. Between the temperatures 311.73 °C and 498.00 °C, 95.58% weight loss was observed due to the complete decomposition of cotton fabric. For ZnO NPs loaded cotton fabric, the initial 9.65% loss in weight loss at 49.35 °C may be attributed to the release of ammonia from zinc–ammonium complexes. It is an evidence of ZnO nanoparticle growth of the cotton fabric from zinc ammonium complex anchor on fabric surface (Ghule et al., 2006). The 86.48% weight reduction between 283.25 °C and 497.96 °C is due to comprehensive cotton fabric decomposition. This may be due to the presence of adsorbed impurities such as bio-organic in ZnO NPs loaded cotton fabric. However, the final weight loss after cotton fabric decomposition was found to be 13.52% greater in the control fabric than the ZnO NPs loaded cotton fabric. This inversion

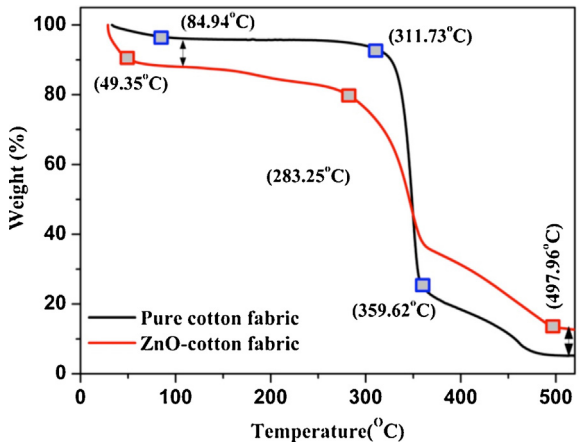

Fig. 4. TGA spectrum of control cotton fabric as compared to that coated with ZnO nanoparticles.

Table 1
Antibacterial inhibition zone for pure and ZnO NPs loaded cotton fabrics against *E. coli* and *S. aureus*.

S. no.	Fabric sample	Antibacterial inhibition zone (mm)	
		E. coli	*S. aureus*
1	Pure cotton	–	–
2	CT-30	2.0	1.6
3	CT-60	2.1	1.9
4	CT-90	2.4	2.0

in weight loss trends is due to the residual presence of ZnO nanoparticles in ZnO NPs loaded cotton fabric. The observations from TGA point at the growth and crystallization of ZnO nanoparticles on cotton fabric mediated by zinc–ammonium complexes. CHNS elemental analysis was employed to determine the nitrogen content in cotton fabric. The amounts of nitrogen (%) in the cotton fabric were found to be 0.88, 0.48, and 0.05 for 30, 60, and 90 min of heat treatment, respectively. This observation is a clear indication of gradual reduction in nitrogen content with respect to time, which is due to the depletion of zinc-ammonium complexes because of ZnO nanoparticle growth on the cotton fabric surface during the process of heating. These results support observations from TGA data. SEM images suggest reaction time dependent morphology of ZnO nanocrystals on cotton fabric.

3.5. Antibacterial activity

The nanomaterials incorporated on the cotton fabric act as a barrier and serve to control the rate of bacterial proliferation and attachment. ZnO NPs loaded into cotton fabric proved to be of great interest in terms of antimicrobial activity against a variety of pathogenic microorganisms (Dastjerdi & Montazer, 2010; Fouda et al., 2013; Kiwi & Pulgarin, 2010). ZnO nanoparticles are located at the centre of cotton fabric, which generate reactive oxygen species on the bacteria–nanoparticle interface, leading to bacterial cell wall destruction and resultant decrease in the bacterial population (Raghupathi et al., 2011; Sivakumar et al., 2010). In the present study, the antibacterial activity of ZnO NPs loaded cotton fabrics was studied against *E. coli* and *S. aureus* bacterial species by agar diffusion, bacterial solution suspension, and wet interfacial contact. For agar diffusion tests, loaded fabrics were placed on the bacterial culture medium to measure the diameter of inhibition zone. The killing of bacterial species in solution was monitored by bacterial solution suspension studies. Interfacial contact investigations were conducted in order to gain an insight on the interactions occurring at the wet ZnO NPs–bacteria interface, and had provided substantial results with regard to bacterial killing as a result of consistent interfacial contact binding. The efficacy of ZnO NPs loaded cotton fabrics in inhibiting bacterial colonization and growth was studied by agar diffusion tests. Antibacterial activity of loaded fabrics is evident from a clear zone of inhibition around the fabric (Table 1), where no bacterial growth is observed. The diameter of this inhibition zone was found to be approximately in the range of 1.6–2.4 mm for both *E. coli* and *S. aureus*, with *E. coli* exhibiting marginally higher inhibition diameters than *S. aureus* due to tolerance of cell membrane against ZnO NPs. Results from agar diffusion studies prove that ZnO NPs play a vital role in the prevention of bacterial growth on cotton fabric.

Bacterial killing efficiency for *E. coli* by solution suspension method was found to be greater when compared to that of *S. aureus*, as seen from Fig. 5(a) and (b). It can also be seen that *E. coli* survival after 6 h of solution suspension is lower for ZnO NPs loaded cotton fabric prepared by heat treatment for 90 min is 0%, whereas than that of fabric treated for 30 min shows 20% bacterial survival. A similar trend is observed for *S. aureus*, in which the % bacterial survival

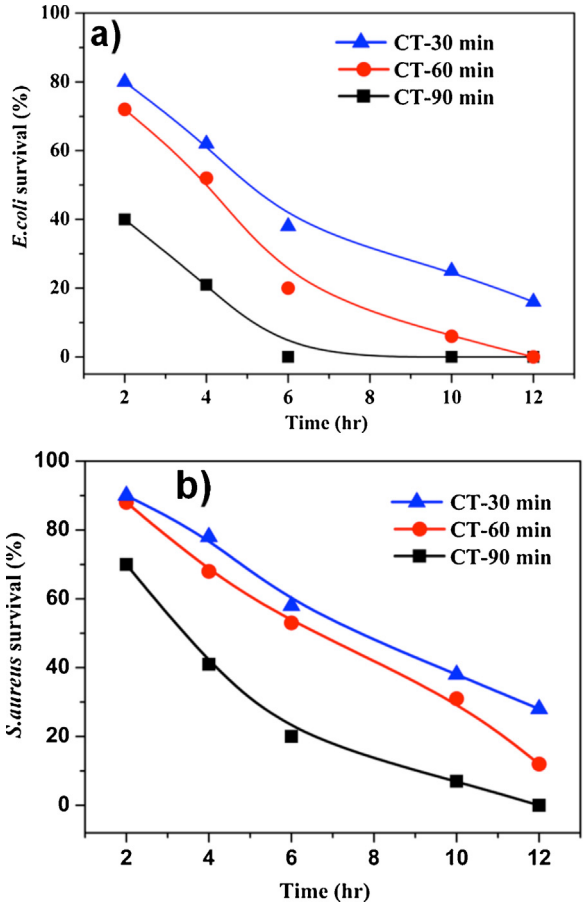

Fig. 5. Comparative solution suspension antibacterial activity assessments of ZnO coated fabric in terms of bacterial killing efficiency vs time against (a) *E. coli* and (b) *S. aureus*.

at the end of 12 hr was found to be greater for ZnO NPs loaded cotton fabric prepared by 30 min heat treatment (37%) than the fabric sample prepared by heat treatment for 90 min (0%). The observed increase in antibacterial activity with treatment time may be due to greater crystal growth of ZnO NPs on fabric surface at higher treatment times, as shown in the SEM images.

Figs. 6 and 7 are the Epi-fluorescent microscopic images of bacterial species *E. coli* and *S. aureus* under wet conditions in the presence of nanoparticle loaded cotton fabrics. The studies were performed using ZnO NPs loaded cotton fabric fabricated by treatment for 90 min, which exhibited greater antibacterial activity in solution suspension studies. The loaded fabric samples were placed in the 4.5×10^6 CFU/ml of bacterial culture at different time durations to render monitoring of both dead and living cells. They provide certain vital insights on the contact interactions occurring in the wet bacteria–ZnO NPs–cotton interface. Living cells are stained green in colour, while the dead cells are coloured red. The Epi-fluorescent microscopic images of *E. coli* and *S. aureus* bacterial species were recorded at definite time intervals during bacterial killing by direct interfacial contact method. Results from solution suspension studies point at greater tolerance of *S. aureus* against ZnO NPs than *E. coli*. Hence data for interfacial contact studies were recorded at 2 h intervals for *S. aureus*, whereas a 1 h time interval was chosen for *E. coli*. Fig. 6(a) is Epi-fluorescent microscopic image of control *E. coli* species at prior to the initiation of wet fabric exposure. It depicts live bacterial cells stained in green. Initiation of cell membrane is indicated by reddish defect regions around the bacterial species, as illustrated in Fig. 6(b), the Epi-fluorescent image of bacterial cells post 2 h of wet NPs loaded fabric exposure.

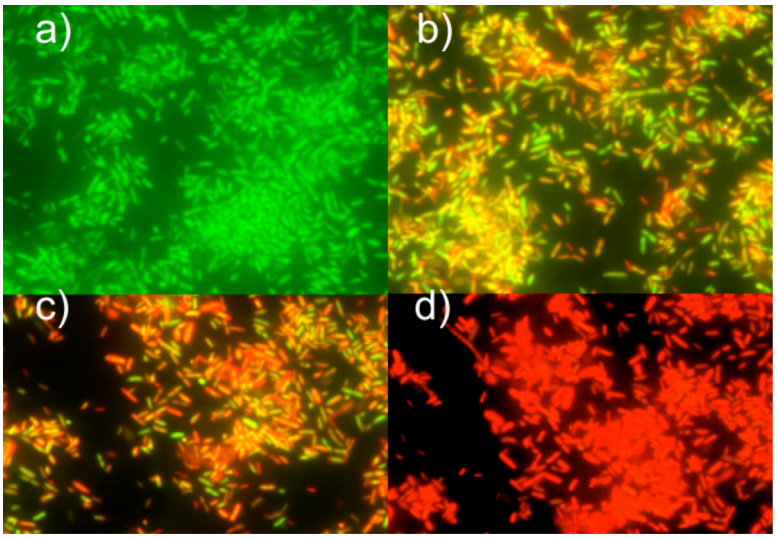

Fig. 6. Epi-fluorescent microscopic images of bacterial species *E. coli* (a) initial, (b) 2 h, (c) 3 h, (d) 4 h during interfacial contact studies in the presence of nanoparticle loaded cotton fabrics prepared by heat treatment for 90 min.

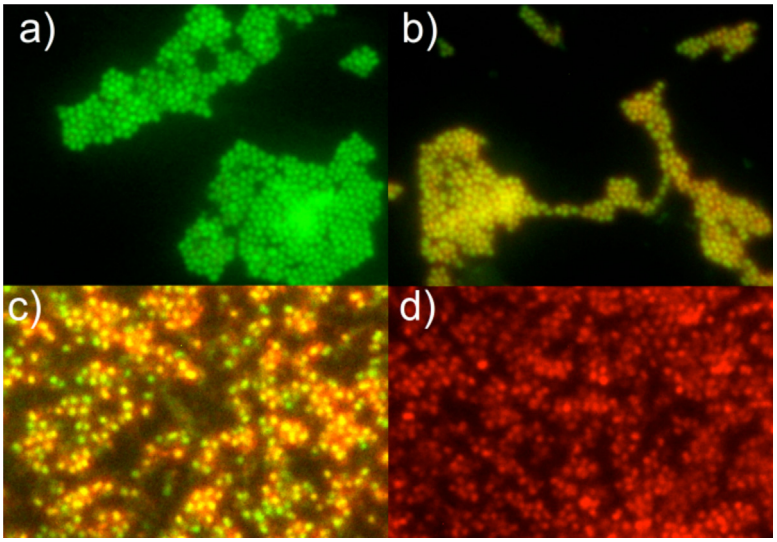

Fig. 7. Epi-fluorescent microscopic images of *S. aureus* (a) initial, (b) 2 h, (c) 4 h, (d) 6 h during interfacial contact studies in the presence of nanoparticle loaded cotton fabrics prepared by heat treatment for 90 min.

Further deterioration of bacterial cells can observe with increasing contact time, as depicted in Fig. 6(c), recorded after 3 h. Most cells of *E. coli* are completely destroyed after 4 h, as elucidated in Fig. 6(d). A similar trend is noticed with regard to the neutralization of *S. aureus*, shown in Fig. 7(a)–(d). However, the destruction of *S. aureus* takes a longer time (6 h) due to increased cell wall stability. When the ZnO NPs loaded cotton fabric samples were immersed in a solution containing bacterial species, it was found that complete nullification of *E. coli* and *S. aureus* cells occurred after time periods of 8 and 12 h respectively, which is significantly higher when compared with those observed in wet fabric exposure studies. This observation may be due to the fact that effective destruction of bacterial cells by reactive oxygen radical species is a function of contact distance between ZnO NPs and bacterial cells. Hence, subdued bacterial killing rate in bacterial solution can be attributed to varying distances and inconsistent contact between the ZnO NPs and individual bacterial species in solution. Under wet ZnO loaded fabric exposure, the bacterial species remain in perennial contact with water insoluble ZnO nanoparticles, rendering the efficient antimicrobial activity at the ZnO NPs–bacteria interface. The destruction of bacterial species initiates at the cell membrane and rapidly proceeds along the interior of the cell, as depicted in the Epi-fluorescent microscopic images. Similar results were observed by previous investigators for ZnO nanoparticles (Raghupathi et al., 2011; Sivakumar et al., 2010). This antimicrobial system is closed with consistent interfacial contact, and may prove to be significantly efficient when compared to other systems used for rendering antibacterial activity in textile fabrics.

4. Conclusion

This contribution has reported the fabrication of antimicrobial textile fabrics loaded with ZnO nanoparticles synthesized by ureolytic bacteria mediated biochemical precipitation process. The generation of biogenic ammonia of the bacterial species and its subsequent precipitation to form zinc ammonium complexes has been monitored by pH and ammonia content estimation. The loading of ZnO nanoparticles on cotton fabric has been characterized by analytical techniques. XRD has confirmed the wurtzite type crystallographic orientation on ZnO nanoparticles. SEM-EDAX

observations depict the binding of ZnO nanoparticles on cotton fabric. The structural morphology of ZnO nanoparticles is seen to vary between spherical to flower-like with increasing treatment times. The tensile force and maximum elongation at breakdown were greater for the nanoparticle loaded fabric, indicating its mechanical strength and suitability for practical textile and biomedical applications. Antibacterial activity studies on the ZnO NPs loaded cotton fabric by wet interfacial contact studies reveal substantial killing efficiency against *E. coli* and *S. aureus* because of consistent interfacial contact between the bacterial species and nanocrystals on the cotton fabric.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.12.074.

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