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Enhancement of the antimicrobial property of cotton fabric using plasma and enzyme pre-treatments

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ABSTRACT

The synergetic effect of low temperature plasma and enzyme treatments on the physico-chemical properties of cotton fabrics was investigated. The fabrics were treated with DC air plasma (P), cellulase enzyme (E), enzyme preceded by plasma (PE) and plasma preceded by enzyme (EP). The anti-microbial activity of the treated cotton fabrics was assessed after processing with neem leaf extract. The physical and chemical changes on the surface of fabrics due to the treatments were investigated using SEM and UATR-FTIR analyses, respectively. The carboxyl content was estimated quantitatively and the presence of aldehyde was determined. The EP treated sample was found to have higher concentration of C=O group when compared to the other samples. EP treated fabrics showed 100% bacterial reduction against *Staphylococcus aureus* and 98% against *Escherichia coli* organism. These finished fabrics were durable with anti-microbial activity around 95% up to 30 washing cycles. Air permeability of the fabrics was determined before and after anti-microbial finish and the results are discussed.

1. Introduction

Cellulose is the major natural component present in cotton fabrics along with non cellulosic compounds such as waxes, pectins, proteins, ashes, etc. (Karahan & Özdogan, 2008; Wang, Fan, Gao, & Chen, 2006). These natural cellulosic fibers due to their hydrophilic nature support the growth of micro-organisms such as bacteria and fungi. In order to inhibit the growth of microbes on cellulosic fabrics, several eco-friendly bioactive agents are effectively used for antimicrobial finish (Joshi, Wazed Ali, Purwar, & Rajendran, 2009). The need for eco-friendly anti-microbial textiles is increasing in recent times, to protect healthcare workers and the patients mainly in the medical sectors (Gawish, Matthews, Wafa, Breidt, & Bourham, 2007). Though there are several technologies to functionalize or synthesize biocidal cotton fabrics with synthetic anti-microbial agents, those methods are found to be expensive and not eco-friendly (Joshi et al., 2009). Plasma treatment has attracted more attention from the textile industry for incorporating functional properties such as wettability, water repellency, dyeability and anti-microbial finish processes (Cai & Qiu, 2006; Pane, Tedesco, & Greger, 2001). Glow discharge plasma treatment is an effective surface engineering tool for modifying the surface properties of polymers without affecting the bulk properties (Abidi & Hequet, 2004; McCord, Hwang, Qiu, Hughes, & Bourham, 2003; Vohrer, Muller, & Oehr, 1998; Inbakumar et al., 2010). The plasma treatment of cotton fabrics is characterized by the formation of carboxyl groups and free radicals on the fabric surface, making it more reactive by changing the surface chemistry and morphology (Chen, 1996; Ward, Jung, Hinojosa, & Benerito, 1979).

Enzyme treatments are also used extensively in the textile industry due to its effectiveness in improving various properties such as wettability, dyeability, bleachability and finishing processes without fiber devastation (Wang, Fan, Hua, & Chen, 2007; Wang et al., 2006). Cellulase enzyme is found to be very suitable for cellulosic fibers, because of its specific catalytic action on the β-1,4-glycoside bond of cellulose molecule (Cao & Tan, 2002). The cellulase enzyme removes the seed coat fragments and small protruding fibers on the fabric surface facilitating further finishing processes (Csiszar, Urbanszki, & Szakacs, 2001). In addition, combination of plasma and enzyme treatments on textile fabrics is found to improve functional, environmental and economical benefits when compared to traditional wet chemical methods (Park, Venditti, Abrecht, Jameel, Pawalak, & Lee, 2007; Radetic, Jovancic, Jocic, Topalovic, Puac, & Petrovic, 2007). It has been reported that plasma pre-treatments for enzymatic hydrolysis of textile fabrics can further enhance the finishing processes (Radetic et al., 2007b; Wong, Tao, Yuen, & Yeung, 2000; Yoon & Lim, 1996).

The objective of the present study is to investigate the improvement of anti-microbial activity of cotton fabrics by surface modification induced by plasma and enzyme treatments. In this work, cotton fabrics were treated with DC air plasma (P) or cellulase enzyme (E) or their combinations (PE & EP). Plasma

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treatment has been applied to cotton fabrics before and after enzymatic treatment; hereafter in this paper, hereafter referred to as PE and EP treatments respectively. Properties such as surface morphology, chemical nature, air permeability, anti-microbial activity and wash durability were investigated and the results are reported.

2. Materials

The fabric sample used in the present study was plain weave, 20 s count, 100% pure, finished cotton fabric with an EPI/PPI count of 72/54. The fabrics used for the treatment were cut to dimensions of 10 cm × 10 cm. Cotton fabrics were kept immersed in water at 90 °C for 15 min and then dried before treatment. For enzyme treatment, commercially available acidic Cellulase, Bio-Lish 2.0 L (Avensa Chemicals, India) with an activity of 0.2 IU/ml was used.

3. Methods

3.1. Plasma treatment

Plasma treatment of cotton fabrics was carried out with air plasma in a 12″ DC plasma chamber. The fabric sample was placed onto the anode and then the chamber was evacuated to a base pressure of 10^{-3} mbar using a rotary pump. Process parameters such as gas pressure, inter-electrode distance, current and exposure time were varied to optimize for the best treatment conditions to attain maximum hydrophilicity. The optimized process parameters in this study are: gas pressure – 0.2 mbar, inter-electrode distance – 3 cm, exposure time – 5 min and current – 60 mA.

3.2. Enzyme treatment

Cellulase hydrolysis of cotton fabrics was performed in an incubator shaker with a material to liquid ratio (MLR) of 1:20 and temperature 55 °C. For the enzymatic hydrolysis, the samples were treated for 30 min at pH of 5.5 using an enzyme dosage of 2% OWF (on weight of fiber). After the treatment, cellulase enzyme was de-activated by washing it thoroughly with hot water (95 °C) for 10 min duration and then dried in air.

3.3. Anti-microbial finish treatment

The methanolic extract of neem leaf prepared as an anti-microbial finish for the cotton fabric was applied onto the fabrics using pad-dry cure method. The fabric samples were immersed in the extract with a material to liquid ratio of 1:20 for 10 min. 8% citric acid was added as a cross linking agent to fix the anti-microbial agent and increase the durability of the finish. Subsequently the fabric samples were passed through a padding mangle running at a speed of 10 m/min with a pressure 1 kgf/cm^2 to remove excess solution. After padding, the fabrics were air-dried and then cured at 140 °C for 3 min.

3.4. UATR-FTIR analysis

The chemical changes that occur during the DC air plasma and Cellulase enzyme treatments were analyzed using UATR-FTIR (Universal Attenuated Total Internal Reflection Fourier Transformation Infrared) spectroscopy. The fabric samples of dimensions 10 mm × 10 mm were placed onto the Zn–Se single crystal and pressure was applied to ensure a good contact between the sample and crystal to prevent the loss of IR radiation. The spectra was recorded using a Perkin Elmer (Spectrum100) FTIR spectrometer in the range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1} for 32 scans.

3.5. Estimation of carboxyl content

The carboxyl content in the treated and untreated samples was estimated using sodium bicarbonate–sodium chloride test (TAPPI Standard Test Method, 1977). The samples were extracted with 0.1 N hydrochloric acid, washed with distilled water, then treated with sodium bicarbonate–sodium chloride solution and then filtered. The filtrate was titrated with 0.01 N HCl in the presence of methyl orange indicator to the pink end point. The titration value for the untreated and treated samples was recorded. The carboxyl content in terms of milli equivalents per 100 g of the bone dried samples were calculated using the following formula:

$$\text{Carboxyl content (meq/100 g)} = \left[B - \left(\frac{A + AxC}{50}\right)\right] \times M \times \frac{200}{W} \quad (1)$$

where A is the volume of 0.01 N HCl (mL) consumed in titration of 25 mL of the filtrate solution; B is the volume of 0.01 N HCl (mL) consumed in titration of 25 mL of $NaHCO_3$–NaCl solution; C is weight in grams (g) of water absorbed in the test specimen; M is the normality of HCl used in the titration (0.01 N); and W is the weight in grams (g) of oven-dried test specimen.

3.6. Colorimetric test for aldehyde groups

The presence of aldehyde groups on the treated and untreated fabrics was found using colorimetric test (Malek & Holme, 2003). Aldehyde groups reduce Fehling's solution to give precipitate of cuprous oxide. Small pieces of fabric samples were boiled in 10 ml of Fehling's solution for 10 min. The samples were then removed, washed in water and examined under optical microscope to detect red coloration if any on the fabric surface. The remaining solution was filtered and checked for red color precipitate.

3.7. Surface morphology studies – SEM analysis

The surface morphology of the untreated and treated cotton samples was studied using Scanning Electron Microscope (SEM) (JEOL-JSAL 6360). Platinum was presputtered onto the fabric before analysis.

3.8. Anti-microbial efficacy

3.8.1. Qualitative assessment of antimicrobial activity (AATCC 147)

The anti-microbial efficacy of the fabric was assessed using parallel streak test (AATCC Technical Manual, 2001). The test organisms used were *E. coli* and *S. aureus*.

One loop full of culture was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks covering the central area of the petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with the agar surface. The plates were incubated at 37 °C for 18–24 h; a clear area of interrupted growth along the sides of sample indicated the anti-bacterial activity of fabric. The average width of the zone of inhibition at the sides of the test specimen was calculated in mm using the formula:

$$\text{Zone of inhibition (mm)} = \frac{T - I}{2} \quad (2)$$

where T is the width of clear zone with the specimen and I is the width of test specimen.

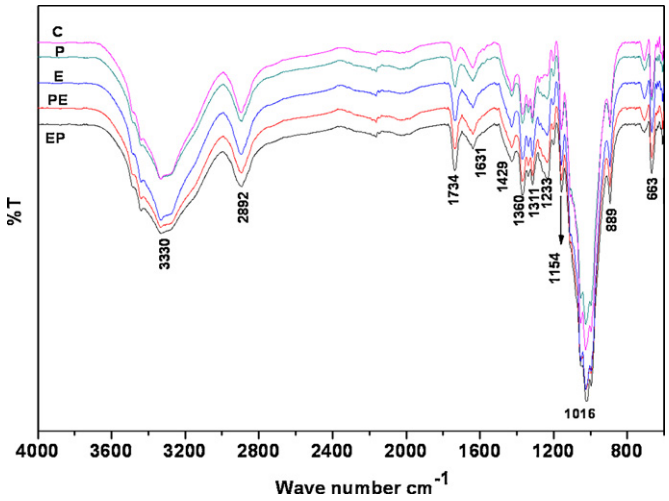

Fig. 1. UATR-FTIR spectra of control (C), DC air plasma (P), cellulase enzyme (E), PE, EP treated cotton fabrics.

3.8.2. Quantitative assessment of anti-microbial activity (AATCC 100)

The anti-microbial activity of the fabric samples was ascertained quantitatively by AATCC 100 method (AATCC Technical Manual, 2001). 1.0 ml of the test inocula (*S. aureus*, *E. coli)* were loaded on the swatches (treated and untreated) of 4.8 ± 0.1 cm diameter. They were then transferred to the sterile AATCC bacteriostasis broth. After an incubation of 24 h, up to 10^{-7} serial dilutions were made for all the samples. 0.1 ml sample from each dilution were spread plated onto the sterile AATCC bacteriostasis agar plates and incubated at 37 °C for 24 h. The percentage reduction of bacteria by the treatment was then calculated using the following formula:

$$(B - A) \times \frac{100}{B} = R \quad (3)$$

where R is the % reduction and A and B are the number of bacteria recovered from the inoculated treated and untreated swatch respectively.

3.8.3. Wash durability test (IS: 687-1979)

Washing test was carried out as per IS: 687-1979 by using a neutral soap (5 gpl) at 40 ± 2 °C for 30 min, keeping the material to liquid ratio at 1:50, followed by rinsing, washing and drying. After drying, the test samples were assessed for anti-microbial activity using AATCC 100 procedure up to 30 washing cycles.

4. Results and discussion

4.1. Physico-chemical properties of DC air plasma and cellulase enzyme treated cotton fabric

4.1.1. Chemical analysis – UATR-FTIR analysis

UATR-FTIR spectra of the control (C), DC air plasma (P), cellulase enzyme (E), PE and EP treated cotton fabrics are shown in Fig. 1. The spectra reveals the presence of all the peaks corresponding to various functional groups in cellulose for both untreated (control) and treated fabrics as reported and discussed in our previous work (Nithya, Radhai, Rajendran, Shalini, Rajendran, & Jayakumar, 2011).

Fig. 2 shows the proposed reaction mechanism of cotton cellulose during plasma and enzyme treatments. In plasma treated fabrics, dehydrogenation takes place at C_6 carbon followed by oxidation resulting in the formation of carboxylic acid. During the

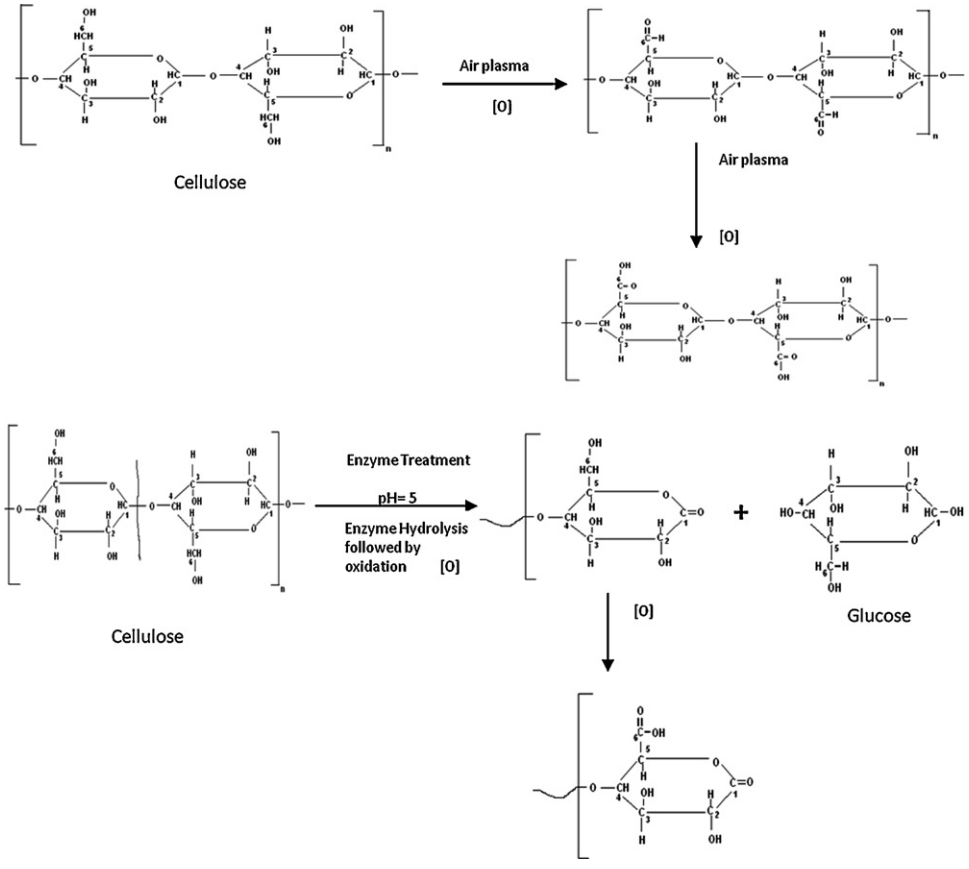

Fig. 2. Reaction mechanism for plasma and enzyme treatment on cotton fabrics.

Table 1
Chemical (carboxyl and aldehyde group) and antimicrobial analysis (AATCC 100) of the treated fabric samples.

Test sample	Concentration of carboxyl group (meq/100 g)	Calorimetric test (aldehyde group) inference	Bacterial reduction (%)	
			S. aureus	*E. coli*
Control	10.86	No red precipitate	–	–
Plasma	11.35	Red precipitate	100	97.6
Enzyme	12.11	Red precipitate	100	98.1
PE	14.83	Red precipitate	100	98.5
EP	17.21	Red precipitate	100	100

cellulase enzyme treatment, the enzyme hydrolyzes the cellulose fibers into shorter segments and cleaves glucose from the molecular chain followed by oxidation at C_1 and C_6 resulting in the formation of carbonyl group as shown in Fig. 2. When the fabrics were treated with plasma as well as enzyme (PE and EP), the number of carboxyl groups was found to increase in cellulose molecular chain, as a result there was an increase in hydrophilicity of the fabric samples. The characteristic peak (C=O group) along 1734 cm^{-1} may be due to the presence of aldehyde as well as carboxylic acid group on the fabric surface. To further quantify the carboxyl content, sodium bicarbonate–sodium chloride test was done and the results are reported in Table 1. From the table it is inferred that the carboxyl content increased in the order of C < P < E < PE < EP. This result is in accordance with our previously published results. The presence of aldehyde groups were also identified using the colorimetric test as shown in Table 1. It is evident that the plasma and enzyme treatments enhanced the oxidation of primary alcohol resulting in the formation of aldehyde group which further oxidizes to carboxylic acid as shown in Fig. 2. These functional groups are responsible for the increase in hydrophilicity because of its polar nature, facilitating more absorption of neem leaf extract thereby increasing the anti-microbial activity.

4.1.2. SEM and air permeability

Figs. 3 and 4 show the SEM micrographs of untreated and EP treated cotton fabric, respectively. The SEM micrograph of the EP treated fabrics confirm that enzyme treatment has smoothened the fabric surface by removing the surface impurities and the DC air plasma treatment has etched the hair-like protrudants present on the surface of the fabric which resulted in the increase of interstitial pore size, facilitating air permeability in the fabric.

The air permeability of the fabric samples was ascertained by Kawabata evaluation system (KES) and is reported in Fig. 5. It is evident from the figure that the air permeability is found to be more for the PE and EP treated samples when compared with the cotton fabric before anti-microbial finish. The air permeability of plasma treated samples without anti-microbial finish was found to be higher when compared to other fabrics, which may be due to the plasma surface etching as evident from SEM micrographs. When the fabrics were given anti-microbial finish and checked for its air permeability, it was found to decrease for all the treated fabrics. It is also observed that, the air permeability of the neem leaf extract treated fabric was found to vary nominally without affecting the comfort property.

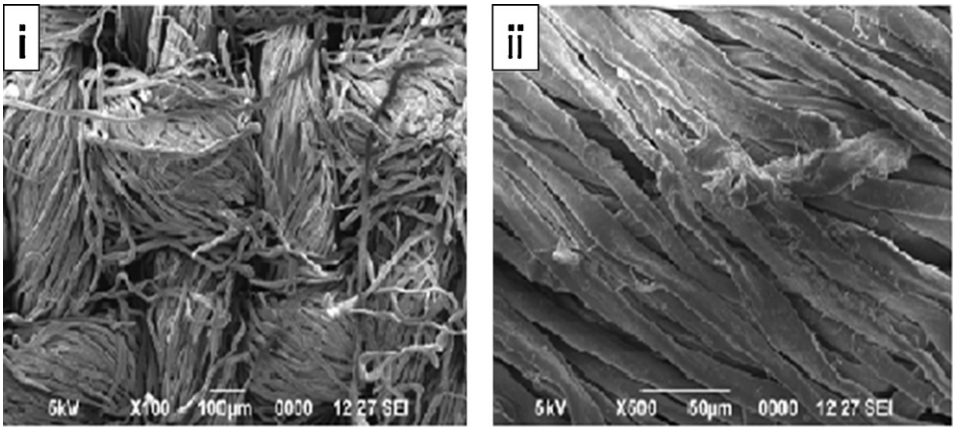

Fig. 3. SEM images of untreated cotton fabric (i) warp and weft (ii) fibers.

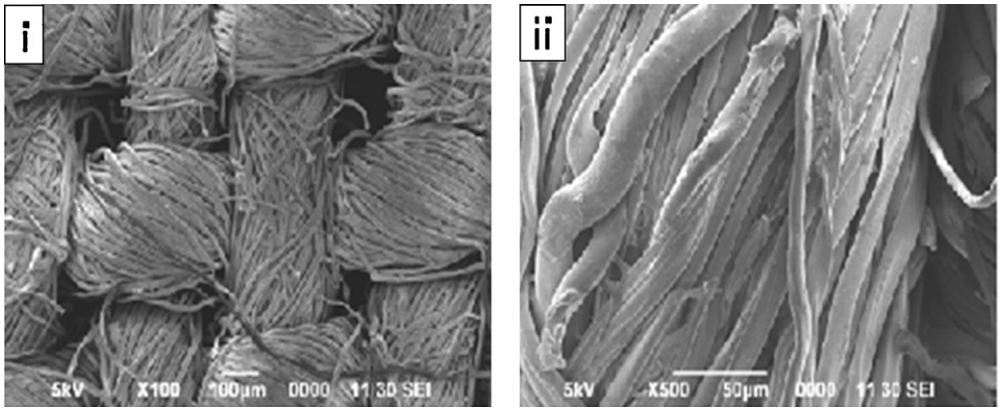

Fig. 4. SEM images of EP treated cotton fabric (i) warp and weft (ii) fibers.

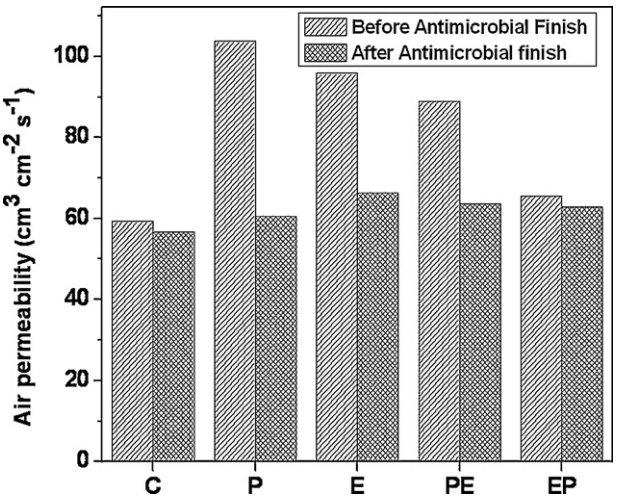

Fig. 5. Air permeability of plasma (P), enzyme (E), PE, and EP treated cotton fabrics before and after anti-microbial finish.

4.2. Effect of type and subsequent treatment on anti-microbial property of cotton fabrics

Before evaluation, Plasma, Enzyme, PE and EP treated fabric samples were finished with neem leaf extract by pad-dry-cure method. The extent of their anti-microbial activity was measured in terms of their zone of inhibition in mm and the results are shown in Fig. 6a. The photographs of the plates are shown in Fig. 6b for untreated and EP treated fabrics against *S. aureus* and *E. coli* organisms.

Plasma, enzyme, PE and EP treated fabrics show an increase in anti-microbial activity for both the organisms; this is attributed to the increase in hydrophilicity. Increase in the hydrophilicity due to plasma and enzyme treatments has enhanced the uptake of anti-microbial finish, thereby increasing the anti-microbial efficacy of the fabric. The maximum zone of inhibition was found to be 9 mm against *S. aureus* and 7 mm against *E. coli* (Fig. 6a) for EP treated fabrics. This is due to the synergetic effect of enzyme and plasma on the cotton fabrics. The enzyme pre-treatment has facilitated the effective impingement of plasma species on the fabric surface thereby increasing reactive sites on it.

4.3. Quantitative determination of anti-bacterial activity (AATCC–100)

The percentage reduction of the test bacteria (*S. aureus* and *E. coli*) were confirmed quantitatively using AATCC 100 method. The results obtained were tabulated in Table 1. It is observed that, there was 100% bacterial reduction of *S. aureus* organism for all the treated samples, but percentage reduction was comparatively lesser for P, E and PE treated samples against *E. coli* organism. However, EP treated samples showed excellent anti-microbial activity having 100% bacterial reduction for both the test organisms. This shows that the EP treatment has facilitated the increase in uptake of the neem leaf extract making it more active against gram positive and gram negative bacteria.

4.4. Wash durability test

Durability of the anti-microbial finish was assessed by wash durability test. The percentage reduction in the anti-bacterial activity up to 30 washing cycles was evaluated using AATCC 100 test method. The fabric samples treated with plasma, enzyme, PE and EP were given neem leaf extract finish before testing. The binding

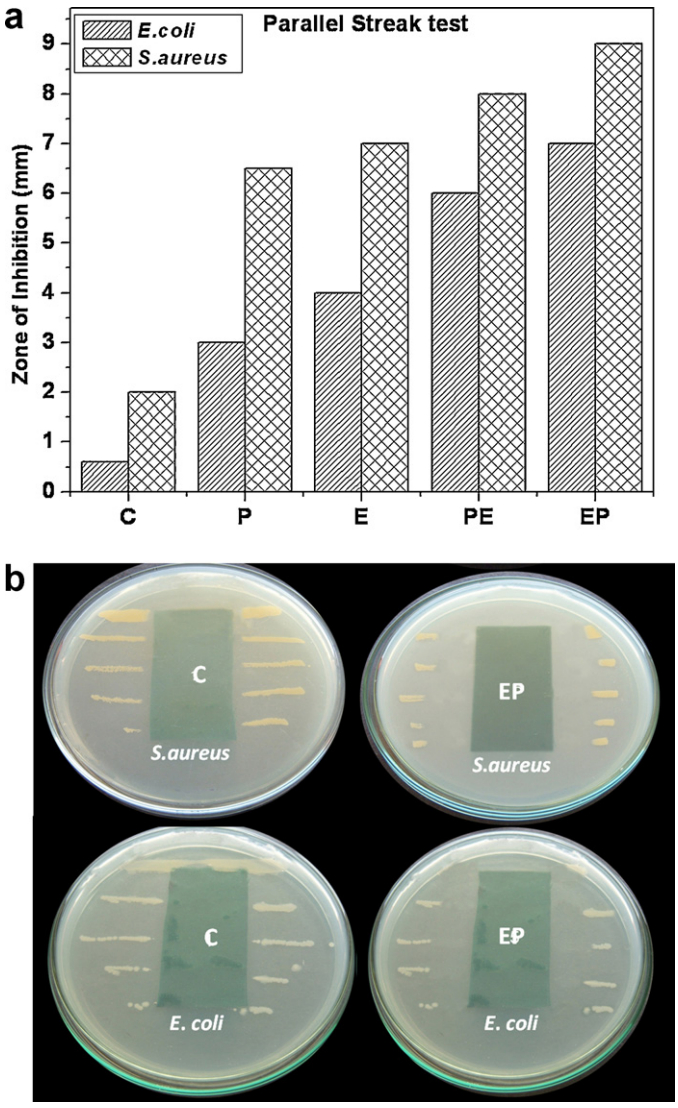

Fig. 6. (a) Antimicrobial activity of the treated (P, E, PE, EP) and untreated (C) cotton fabrics using parallel streak test for *S. aureus* and *E. coli* test organisms. (b) Photographs of the parallel streak plates for control and EP treated cotton fabrics against *S. aureus* and *E. coli* test organisms.

agent, citric acid was added to the extract to increase the durability of the finish. The fabrics were then tested for percentage reduction of the micro-organisms (*S. aureus* and *E. coli)* using AATCC 100. The results obtained are presented in Fig. 7a and b. When all the treated fabrics were subjected to 10 washing cycles it was found that only EP treated samples have 100% bacterial reduction for both the test organisms and thereafter it was found to decrease. Whereas, plasma, enzyme, and PE treated fabrics exhibit 100% bacterial reduction for *S. aureus* up to 10 washes, and there was a reduction in the activity from fifth wash for *E. coli* organism. When the fabrics were subjected to more washing cycles of 15, 20, 25 and 30, there was a decrease in the percentage bacterial reduction for all the treated samples against *S. aureus* and *E. coli*. The durability of anti-microbial finish was found to be more for EP treated fabrics. This increase in wash durability of the fabric is attributed to the increase in uptake capacity and penetration of anti-microbial finish into the fabric. Further the carboxyl groups present in citric acid molecule undergo esterification with hydroxyl groups in cellulose and bio active compounds of neem leaf extract. Thus the citric acid acts as a binding agent between cellulose and the anti-microbial finish.

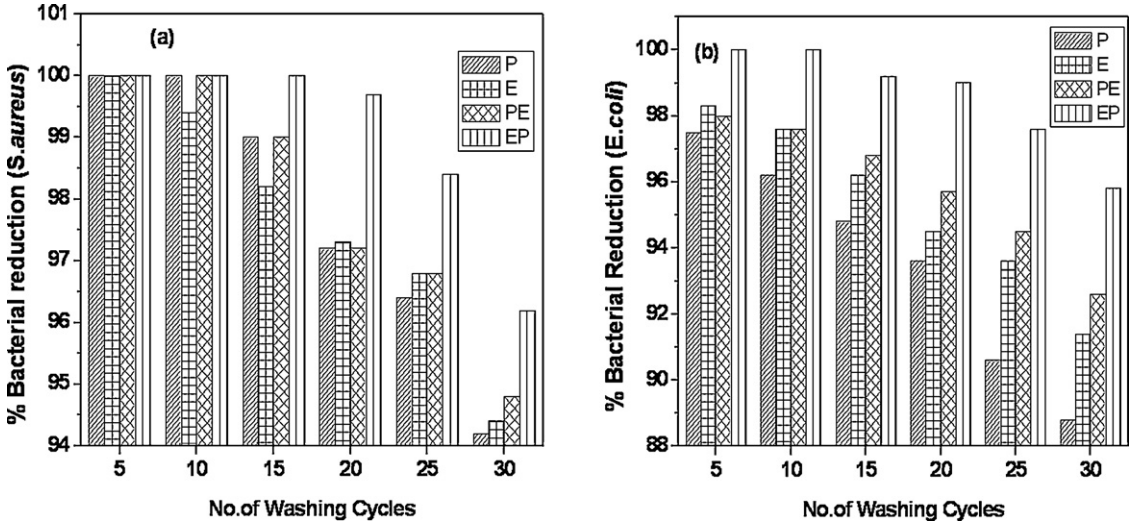

Fig. 7. Percentage bacterial reduction (AATCC 100) (a) *S. aureus* and (b) *E. coli* of the treated fabrics for 30 washing cycles.

5. Conclusions

The effects of plasma and enzyme treatments on the physico-chemical and anti-microbial properties of cotton fabrics were investigated. UATR-FTIR results of treated fabrics are in good correlation with the chemical tests. Sodium bicarbonate–sodium chloride test results proves the formation of more polar groups (C=O) on the fabric surface which is responsible for the increase in neem leaf extract uptake. Based on the FTIR and chemical test results, a reaction mechanism has been proposed for the formation of polar groups on the fabric surface during plasma and enzyme hydrolysis. SEM micrographs reveal the etching effect of plasma and smoothening due to the removal of non cellulosic impurities and surface fibrils during enzyme treatments, increasing the air permeability of the fabric samples. The anti-microbial testing of the EP treated fabric samples revealed 100% bacterial reduction for *S. aureus* and *E. coli* organism. EP treated fabric was found to have better wash durability than other samples up to 30 washing cycles. The air permeability of the samples was also found to have nominal variation after the anti-microbial finish.

Plasma preceded by enzyme treatment was better than the other treatments, as it promotes more oxidation on the fabric surface leading to the formation of polar groups. The increase in carbonyl group has enhanced the uptake of neem leaf extract resulting in better antimicrobial activity. Combination of plasma and enzyme treatment (EP) was found to be suitable for durable finishing of textiles without deterioration of fabric quality and comfort, which can be exploited for industrial applications.

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