Antimicrobial Activity of Cotton Fabrics Containing Immobilized Enzymes

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Received 22 August 2006; revised 5 November 2006; accepted 8 November 2006
DOI 10.1002/app.25821
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Immobilization of α-amylase, alkaline pectinase, and laccase enzymes onto ester-crosslinked as well as Cu-chelated cotton fabrics were carried out. Factors affecting the extent of enzyme-loading and retention activities of immobilized enzymes were studied. Proper conditions for attaining higher extent of fixation along with better retained activity were studied. The degree of antimicrobial activity of treated fabric samples against gram-negative and gram-positive bacteria, filamentous, and nonfilamentous fungi were evaluated. The antimicrobial activity is determined by the type of substrate, i.e., Cu-chelated > ester-crosslinked and activated cotton substrate, and the nature of immobilized enzyme, i.e., alkaline pectinase > α-amylase > laccase, irrespective of the used microorganism. The antimicrobial activities of the treated fabrics are completely maintained after laundering at least ten consecutive wash cycles. Further consecutive wash cycles, i.e., 20 or 30 cycles, has practically negative impact on the retained antimicrobial efficacy. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1754–1761, 2007

Key words: antimicrobial; cotton; crosslinking; enzymes; immobilization

INTRODUCTION

Polysaccharide-based fibers have been extensively used in many medical applications because of their unique/advantageous properties, such as biocompatibility, high surface area, absorbency, nontoxicity, potential bioactivity, as well as ease of fabrication into many textile products.[1,2] Medical applications demand different levels of protection and different kinds of protective finishes that will perform quickly, against a broad rang of microbial threats, to help maintain sterile environments. The finish would be required to be durable to care and compatible to humans as well as for ecology.[3,4]

On the other hand, enzymatic applications are of growing importance especially to textile fields ranging from fabric pretreatments to fabric destruction.[5–7] Immobilization of various industrial enzymes onto or within the textile matrix can be achieved via adsorption, covalent bonding, and entrapment; to get increased activity and stability in various applications as well as to build new functionalized textile products.[7–11]

With the above in mind, the main task of the present work is to find out the proper treatments for immobilizing of α-amylase, alkaline pectinase, as well as laccase enzymes onto or within the cotton fabric as well as to study the impact of immobilization on the antimicrobial properties of the modified cotton products.

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Journal of Applied Polymer Science, Vol. 104, 1754–1761 (2007)

EXPERIMENTAL

Materials

Mill-scoured and bleached cotton fabric of 125 g/m^2 was used. Three commercial grade enzymes namely Aquazyme® 240L (an α-amylase, having an activity of 240 KNU/g), Bioprep® 3000L (an alkaline pectinase, having an activity of 3000 APSU/g), as well as Dnilite® IIS laccase (having an activity of 120 LAMU/g) were kindly supplied by Novo Nordisk. 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodi-imide-metho-*p*-toluen-sulfonate (CMC-Sigma), 1,2,3,4-butantetracarboxylic acid (BTCA), Na-hypophosphite, copper acetate, glutaraldehyde, and polyethylenimime (50% - Aldrich) were used.

Methods

Crosslinking cotton fabric containing carboxyl groups[12,13]

Cotton fabric samples were padded twice through a finishing bath containing certain amounts (50, 100, 150, and 200 g/L) of BTCA, respectively, as a reactive crosslinking agent, Na-hypophosphite (80% owBTCA) along with a nonionic wetting agent to a wet pick up

of 100%, followed by drying at 85°C for 10 min, and curing at 180°C for 2 min. The crosslinked cotton fabric samples were washed at 50°C for 30 min, thoroughly rinsed with distilled water to remove excess and unfixed reactants along with by products, and finally dried at 85°C in a vacuum oven. The carboxyl contents of the treated fabric samples were 76, 160, 213, and 249 mequiv./100 g sample respectively, according to the reported method.[14]

Activation of the ester-crosslinked cotton fabrics

Ester-crosslinked cotton fabric samples, containing different carboxyl groups, were activated by immersing in a solution containing a specific concentration of the CMC (mg/g fabric) at 45°C for 19 h followed by thoroughly washing.[14]

Cotton fabric bearing basic groups[15]

Cotton fabric samples were immersed in an alkaline polyethyleneimime (PEI) solutions (5.5%), at pH range (9.5–11), using 0.01*N* NaOH solution for 24 h at room temperature, followed by thoroughly rinsing to remove excess PEI, and postdipping in the glutaraldehyde solution (2%) for 3 h, as a fixing agent. The treated fabric samples were thoroughly washed with distilled water, rinsed, and air-dried. The extent of modification, expressed as %*N*, was evaluated using the Kjeldahl method (ASTM-E258-67). The nitrogen content of the prepared fabric samples were 1.0, 1.5, 2.0, and 2.5%, respectively.

Post-treatment with copper chloride[16]

PEI-treated cotton fabric samples, having different nitrogen contents, were immersed in 1*M* NaOH solution at room temperature for 2 h, followed by thoroughly washing and rinsing to remove the excess NaOH. The alkali-free fabric samples were then soaked in 500 mL of 1*M* copper chloride/ethanol solution at room temperature for 2 h, followed by thoroughly washing with ethanol to remove the excess/unreacted copper chloride, and drying at 80°C for 1 h. Copper content was evaluated by using atomic absorption spectroscopy.

Immobilization of enzymes[17]

The activated-estercrosslinked and copper-chelated cotton fabric samples were added to 10 mL of buffer solution containing 30 mg of: the α-amylase at pH 6.9, the laccase at pH 6.5, or the alkaline pectinase at pH 8. After shaking the components for certain periods at specific temperatures, the fabric samples were filtered and washed. The filtrate and washings were made up to 100 mL. The enzymes content as well as activity were determined according to the reported methods.[18–20]

Fabric evaluation

Biocidal properties of untreated and treated cotton fabric samples were evaluated against gram-negative bacteria (*Escherichia coli*), gram-positive bacteria (*Staphylococcus aureus*), as well as filamentous (*Candida albicanes*), and nonfilamentous (*Asparigillus niger*) fungi according to AATCC Test method 147.

The durability to wash was determined according to AATCC method 124.

RESULTS AND DISCUSSION

To examine the emerging technology of immobilized enzymes and to explore how it can be used as a novel tool for building of new functionalized textiles, the present work focused on immobilizing α-amylase, laccase, and alkaline pectinase enzymes on cotton fabric for imparting antimicrobial properties. The obtained results along with the appropriate discussion follows.

Ester-crosslinked cotton fabric

Table I shows the effect of enzymatic treatment conditions, time, and temperature, on the extent of immobilization of α-amylase, laccase, and alkaline pectinase individually onto the estercrosslinked cotton fabric samples (Cell.COOH) as well as on their activities. For a given set of treatment conditions, it is clear that: (i) prolonging the reaction time from 5 to 30 min or raising the fixation temperature from 70 to 90°C results in a gradual increase in the extent of immobilization, irrespective of the used enzyme (H_2N-Enz.), (ii) the positive impact of both reaction time and temperature on the extent of immobilization is a direct consequence of enhancing the swellability of the cellulose structure and availability of its reactive anionic sites, —COOH groups, as well as the mobility and activity of the used enzymes thereby accelerating and improving the extent of enzyme fixation [eq. (1)].[7]

$$\text{Cell.COOH} + \text{H}_2\text{N-Enz} \longrightarrow \text{Cell.COO.NH.ENZ} + \text{H}_2\text{O} \quad (1)$$

(iii) the extent of picking up the used enzymes follows the decreasing order alkaline pectinase > α-amylase > laccase reflecting the differences among these enzymes in molecular weight, performance, and its specific activity, active sites, stability, extent of diffusion to and into the substrate, and substrate specitivity1,[21,22] (iv) increasing the time of reaction up to

TABLE I
Effect of Enzymatic Treatment Conditions on the Extent of Binding of the Used Enzymes Onto Ester-Crosslinked Cotton Fabric and on Their Activities

Reaction time (min)	α-Amylase						Laccase						Alkaline pectinase					
	Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)			Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)			Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)		
	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C
5	12	16	19	77	70	60	10	13	17	66	50	37	15	19	22	90	80	70
10	15	18	22	80	76	70	14	16	20	70	60	46	18	23	26	95	86	75
15	19	23	26	75	70	65	17	19	23	73	64	50	23	28	31	91	82	70
20	21	25	31	70	67	60	19	22	27	60	55	43	26	32	36	83	73	66
25	25	30	34	64	60	50	22	26	31	53	46	37	30	37	40	77	67	60
30	27	33	37	55	48	40	25	29	34	47	36	27	33	40	43	70	60	52

Carboxyl content, 213 mequiv./100 g sample; Enzyme concentration, 20 mg/100 g sample; pH, 6.9 for α-Amylase, 6.5 for laccase, and 9.0 for alkaline pectinase; CMC activating agent concentration, 20 mg/g fabric.

10 min in case of using α-amylase or alkaline pectinase or up to 15 min in case of using laccase enzyme has a positive impact on improving the retained enzyme activities, (v) further increase in reaction time or raising the reaction temperature from 70 to 90°C has practically a negative impact on the retained enzyme activity, (vi) the decrease in the retained activity of the immobilized enzyme can be ascribed to denaturation of the enzymes and unfolding of their catalytic domain along with their lower thermal stability,[5,23] and (vii) the retained activity is determined by the nature of immobilized enzyme and follows the decreasing order:

Alkaline pectinase > α-amylase > laccase,
irrespective of the applied conditions.

Copper-chelated cotton fabric

For a given set of treatment conditions, the results in Table II show that, (i) increasing the enzymatic treatment from 5 to 30 min or raising the treatment temperature from 70 to 90°C results in a significant improvement in the extent of fixation, expressed as the enzyme content, onto the copper-chelated cotton fabric, (ii) the extent of immobilization is governed by the nature of the enzyme and follows the decreasing order: alkaline pectinase > α-amylase > laccase, (iii) the enhancement in the enzyme content reflects the positive impact of both longer treatment time and higher treatment temperature on facilitating the ability of Cu-chelated substrate to bind the enzymatic protein,[24] (iv) prolonging the reaction time up to 15 min as well as decreasing the reaction temperature down to 70°C give rise to higher retention of enzyme activity, regardless of the used enzyme, and (v) further increase in reaction time up to 30 min, or reaction temperature, up to 90°C, reduces the activity of immobilized enzymes most probably due to enzyme denaturation as well as lower thermal stability.[5,23]

CMC as activating agent

The dependence of the extent of fixation of the used enzymes, expressed as enzyme content, onto the

TABLE II
Effect of Enzymatic Treatment Conditions on the Extent of Binding of the Used Enzymes Onto Copper-Chelated Cotton Fabric and on Their Activities

Reaction time (min)	α-Amylase						Laccase						Alkaline pectinase					
	Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)			Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)			Enzyme content (mg/100 g fabric)			Retention of enzyme activity (%)		
	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C	70°C	80°C	90°C
5	23	26	30	80	75	70	18	22	27	70	66	60	25	30	36	94	90	86
10	25	30	35	90	80	75	21	26	30	80	70	65	30	34	40	96	93	90
15	27	34	40	92	83	78	25	30	35	84	75	70	35	40	47	98	96	93
20	30	40	46	86	77	70	28	34	40	75	69	60	40	46	51	90	98	86
25	35	46	52	80	70	63	30	38	44	70	60	55	45	50	56	84	80	82
30	39	50	57	71	63	56	34	43	49	64	55	49	50	54	60	80	75	77

Copper content, 126 mmol/100 g sample; Enzyme concentration, 20 mg/100 g sample; pH, 6.9 for α-Amylase, 6.5 for laccase, and 9.0 for alkaline pectinase; CMC activating agent concentration, 20 mg/g fabric.

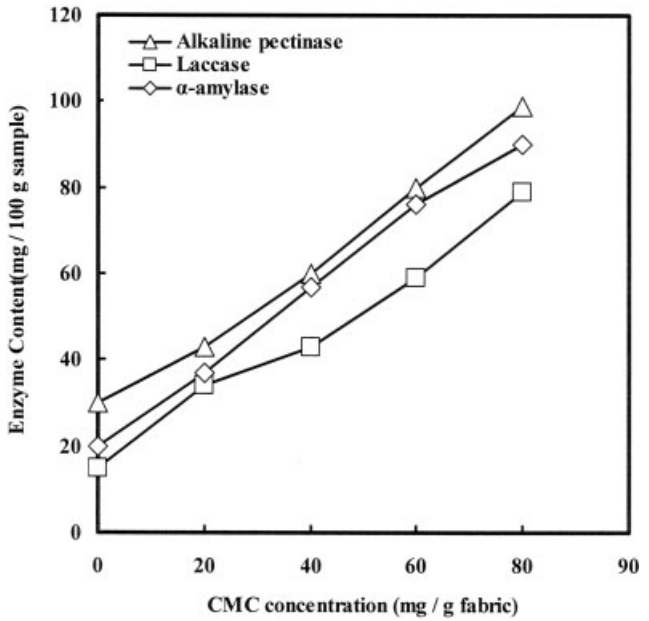

Figure 1 Effect of using CMC as activating agent on the extent of immobilization of enzymes. Carboxyl content, 213 mequiv./100 g fabric; enzyme concentration, 20 mg/g fabric; pH, 6.9 for amylase, 6.5 for laccase, and 9 for alkaline pectinase, and activation at 45°C for 19 h.

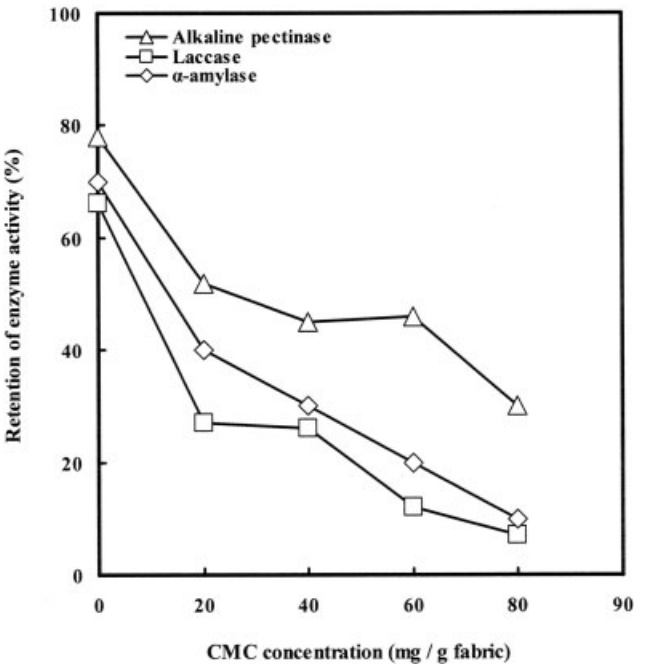

Figure 2 Effect of using CMC as activating agent on the retention of the used enzymes activities. Carboxyl content, 213 mequiv./100 g fabric; enzyme concentration, 20 mg/g fabric; pH, 6.9 for amylase, 6.5 for laccase, and 9 for alkaline pectinase, activation at 45°C for 19 h.

ester-crosslinked-activated cotton fabric samples using different amounts, 0–80 mg/g fabric, of CMC [1,cyclohexyl-3-(2-morpholinoethyl)-carbodiimide-metho-*p*-toluene sulfonate] as an activator is shown in Figure 1. For a given set of conditions, it is clear that, increasing the CMC amount from 0 to 80 mg/g fabric results in a remarkable increase in the enzyme content, regardless of the used enzyme. The improvement in the extent of immobilization of the used enzymes by increasing the amount of CMC in the activating bath is a direct consequence of enhancing the availability and number of the supporter active sites along with offering the proper hydrophilic environment for the binding of these enzymes.[10] On the other hand, the extent of immobilization is governed by the type of enzyme, i.e., alkaline pectinase > α-amylase > laccase.

As is evident, Figure 2 shows that, increasing CMC amount in the activating bath has a negative impact on the retention of the used enzymes activities, regardless of the used enzymes. The decrease in the percentage retention of the used enzymes activities could be discussed in terms of: (i) coupling of each enzyme molecule to more than one carboxyl group on the ester-crosslinked/activated cellulose support, (ii) self-condensation of enzyme molecules, and (iii) distortion of the enzyme–protein structure via interaction with its carboxyl groups residue, thereby adversely affecting its reactivity.[10]

Enzyme type and concentration

Figures 3 and 4 show the effect of type and concentration of the used enzyme on the extent of immobi-

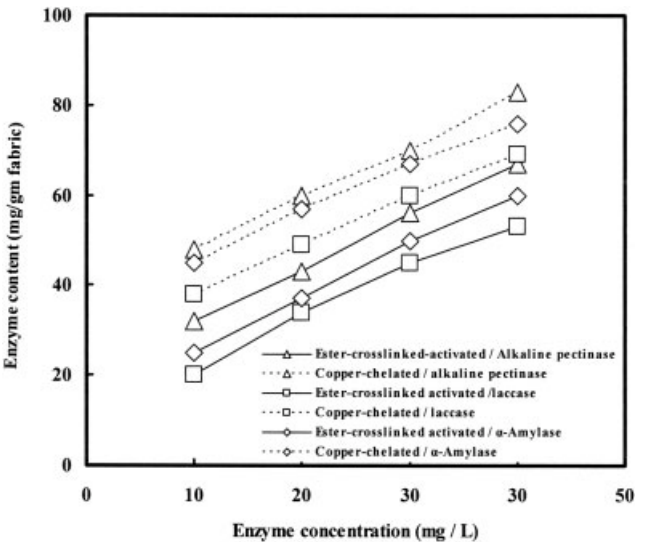

Figure 3 Effect of enzyme type and concentration on the extent of immobilization onto the ester-crosslinked-activated and copper-chelated fabric samples. Carboxyl content of ester-crosslinked activated samples (213 mequiv./100 g sample), reaction temperature, 90°C; reaction time, 15 min; copper content of copper-chelated samples (126 mol/100 g sample); pH, 6.9 for amylase, 6.5 for laccase, and 9 for alkaline pectinase.

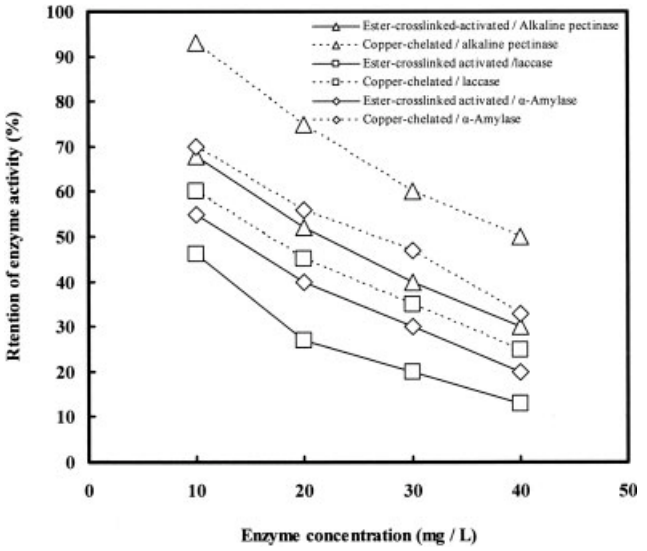

Figure 4 Effect of enzyme type and concentration on the retention of the activity of immobilized enzymes onto ester-crosslinkes activated and copper-chelated cotton fabric. Carboxyl content of ester-crosslinked activated samples (213 mequiv./100 g sample); reaction temperature, 90°C; reaction time, 15 min; copper content of copper-chelated samples (126 mol/100 g sample); pH, 6.9 for amylase, 6.5 for laccase, and 9 for alkaline pectinase.

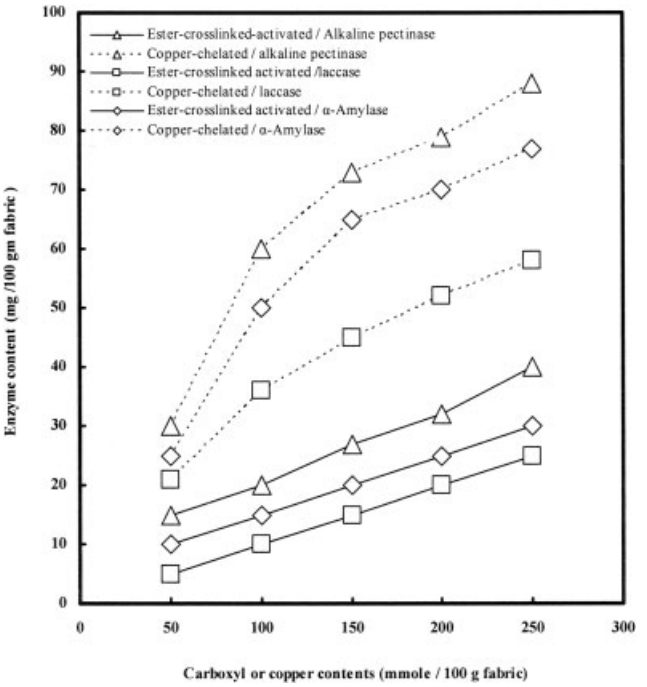

Figure 5 Effect of modification degree, expressed as carboxyl or copper content, on the extent of enzymes immobilization.

lization expressed as enzyme content, as well as the retention of enzyme activity, respectively. For a given set of enzymatic treatment conditions, it is clear that:, (i) increasing the enzyme concentration up to 30 mg/L brings about a significant gradual increase in the enzyme content, Figure 3, regardless of the used enzyme, (ii) this increase in the enzyme content is a direct consequence of increasing the enzyme concentration in the vicinity of the treated substrate-binding sites thereby enhancing its fixation, regardless of the support used, and (iii) the extent of immobilization is determined by the type of enzyme, i.e., alkaline pectinase > α-amylase > laccase, as well as the nature of support, i.e., Cu-chelated substrate > ester-crosslinked and activated substrate, Figure 3, reflecting the difference between the two substrates in chemical structure, number, availability and accessibility of active sites, and the ability and capacity of these sites to bind and immobilize the used enzymes onto or within the treated substrates.

As far as the variation in retention of the immobilized enzymes activities as a function of enzyme type and concentration, Figure 4 shows that increasing the enzyme concentration up to 40 mg/L results in a gradual decrease in retention of the immobilized enzyme activity most probably due to distortion of the protein molecules as a direct consequence of steric hindrance of the enzyme.[10] The extent of retention is governed by both the type of enzyme as well as the number of the carrier as discussed earlier.

Extent of modification

Figures 5 and 6 show the effect of the extent of modification of the used cotton fabric, expressed as carboxyl or Cu-content (mmol/100 g fabric), on degree of immobilization as well as retention of the activity of immobilized enzymes, respectively. As is evident from Figure 5, increasing the extent of modification of the treated cotton fabric samples, i.e., ester-cross-

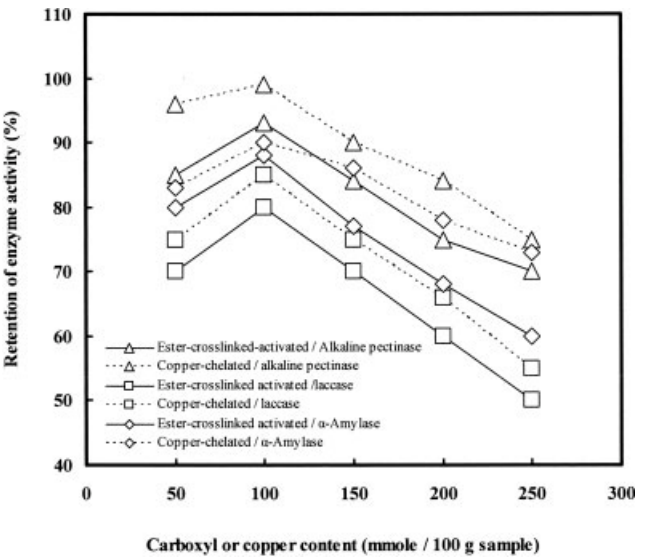

Figure 6 Effect of modification degree on the retained activity of immobilized enzymes.

TABLE III
Antimicrobial Activities of α-Amylase, Laccase, and Alkaline Pectinase Immobilized Onto Ester-Crosslinked and Activated Cotton Fabrics Against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicanis*, and *Aspergilluss niger* Using Different Enzyme Contents

Enzyme content (mg/100 g fabric)	Clear inhibition zone diameter (mm)											
	α-Amylase				Laccase				Alkaline pectinase			
	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.
Crude enzyme	14.0	12.0	9.7	5.8	9.0	5.0	6.8	5.8	17.0	14.0	9.8	5.8
Untreated cotton	–	–	–	–	–	–	–	–	–	–	–	–
30	10	8.0	7.0	5.0	6.3	4.5	5.5	4.6	15	11	9.0	4.0
40	8.0	6.2	6.0	4.0	5.0	3.7	4.6	3.7	13	9.0	8.6	3.3
50	6.0	5.0	4.3	3.3	4.0	3.0	3.5	3.0	10	7.5	6.6	3.0
60	4.0	3.5	2.5	2.5	3.0	2.0	3.0	2.5	8,0	5.0	4.5	2.0
70	2.6	2.0	1.6	1.5	2.0	1.0	1.5	1.9	5,0	3.5	4.0	1.0

S. A., *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; C. A., *Candida albicanis*; A. N., *Aspergilluss niger*.

linked and activated as well as Cu-chelated substrates, from 50 to 250 mmol/100 g fabric is accompanied by a significant increase in the enzyme load in case of using Cu-chelated substrate or a reasonable increase in the enzyme content in case of using ester-crosslinked and activated substrate irrespective of the used enzyme, most probably due to the greater accessibility and availability of the Cu-chelated substrate active sites i.e., carrier, to bind and immobilize the used enzymes. Nevertheless, the extent of enzyme fixation, expressed as enzyme content, is determined by the nature of enzyme, i.e., alkaline pectinase > α-amylase > laccase regardless of the used substrate.

Figure 6 shows the effect of degree of cotton modification, expressed as carboxyl or Cu-content, on the retained activity of the immobilized enzymes. It is evident that, within the range examined, increasing the carboxyl or the Cu-content of modified substrate up to 100 mmol/100 g sample result in an enhancement in the retention value of the activity regardless of the immobilized enzyme most probably through offering a proper hydrophilic environment for maintaining the immobilized enzymatic proteins conformation.[10] Further increase in the extent of modification, i.e., beyond 100 mmol/100 g, has practically a negative impact on retention of the used enzymes activities as a direct consequence of increasing the extent of coupling, crosslinking, and steric hindrance of the enzyme, thereby leading to the distortion of the enzyme protein. Figure 6 also shows that the retained activity of immobilized enzymes depends on the type of the substrate, i.e., Cu-chelated > ester-crosslinked and activated substrate, and the nature of the immobilized enzyme, i.e., alkaline pectinase > α-amylase > laccase.

Antimicrobial activities of immobilized enzymes

As far as the changes in the antimicrobial activities expresses as inhibition zone diameter of modified cotton fabrics containing immobilized enzymes with different enzyme loads or contents, and for a given set of treatment conditions, the data in Tables III and IV reveal that: (i) increasing enzyme load from zero up to 30 mg/100 g fabric, in case of using ester-crosslinked and activated cotton fabric as a carrier (Table III) or up to 40 mg/100 g fabric in case of

TABLE IV
Antimicrobial Activity of α-Amylase, Laccase, and Alkaline Pectinase Immobilized Onto Copper-Chelated Cotton Fabrics Against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicanis*, and *Asparigallus niger* Using Different Enzyme Contents

Enzyme content (mg/100 g fabric)	Clear inhibition zone diameter (mm)											
	α-Amylase				Laccase				Alkaline pectinase			
	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.
Crude enzyme	14.0	12.0	9.7	5.8	9.0	5.0	6.8	5.8	17.0	14.0	9.8	5.8
Untreated cotton	–	–	–	–	–	–	–	–	–	–	–	–
30	19	16	10	8.0	14	10	12	10	23	19	15	10
40	22	18	13	11	16	7.0	9.0	8.0	26	22	12	9.0
50	17	14	9.0	7.0	11	5.0	7.0	6.0	20	17	10	6.0
60	15	12	7.0	5.0	9.0	3.0	5.0	5.0	17	15	8.0	4.0
70	12	9	4.0	3.0	6.0	2.0	3.0	2.5	14	12	6.0	2.0

S. A., *Staphylococcus aureus*; E. coli, *Escherichia coli*; C. A., *Candida albicanis*; A. N., *Aspergilluss niger*.

TABLE V
Antimicrobial Activity of Ester-Crosslinked-Activated As Well As Copper-Chelated Cotton Fabrics Containing Immobilized Alkaline Pectinase After Laundering

	Clear inhibition zone diameter (mm)							
	Ester-crosslinked-activated cotton fabric				Copper-chelated cotton fabric			
Laundering cycles	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.
0	15 (100%)*	11 (100%)	9 (100%)	4 (100%)	23 (100%)	17 (100%)	15 (100%)	10 (100%)
10	15 (100%)	11 (100%)	9 (100%)	4 (100%)	23 (100%)	17 (100%)	15 (100%)	10 (100%)
20	10 (66.7%)	9 (81.8%)	6 (66.7%)	2 (50%)	20 (87%)	13 (76.5%)	12 (80%)	7 (70%)
30	7 (46.7%)	4 (36.4%)	3 (33.3%)	1 (25%)	15 (65.2%)	10 (58.8%)	8 (53.3%)	3 (30%)

S. A., *Staphylococcus aureus*; E. coli, *Escherichia coli*; C. A., *Candida albicanis*; A. S., Asparigallus niger.
* Retained activity (%).

using Cu-chelated cotton fabric (Table IV), results in a remarkable improvement in antimicrobial activities of the used carriers, regardless of the microorganism used, (ii) the enhancement in antimicrobial activity of treated substrate is a direct consequence of utilizing an electrochemical mode of action to penetrate cell wall of microorganism, thereby causing leakage of essential metabolites and physically disrupting other key cell functions on contact to kill it and thoroughly leaching antimicrobial moieties to enter or react chemically with the microorganisms acting as a poison or inhibitor,[25,26] (iii) further increase the enzyme content has a negative impacts an antimicrobial activities of the used substrates, most probably due to the decrease in the activity of the immobilized enzymes as mentioned earlier, (iv) the antimicrobial activity of crude enzymes follow the descending order: alkaline pectinase > α-amylase > laccase, irrespective of the microorganisms used, (v) the antimicrobial activity of crude enzymes against the microorganisms used are determined by the nature and structure of the microorganism and can be ranked as follows[27]: *Staphylococcus aureus* > *Escherichia coli* > *Candida albicanis* > *Aspergilluss niger*, in case of using alkaline pectinase or α-amylase, but in case of using laccase enzyme, the antimicrobial activity follows the decreasing order *Staphylococcus aureus* > *Candida albicanis* > *Aspergilluss niger* > *Escherichia coli*, (vi) the change in antimicrobial activity of the used enzymes reflects the difference among them in, enzyme specificity, activity against microorganism, mode of action, molecular size, active sites, and biochemical properties,[21,22] and (vii) the data in Table III shows that, the antimicrobial activity follows the decreasing order: free-crude enzyme > immobilized enzyme ≫ untreated cotton fabric, while the data in Table IV show the following order: immobilized enzymes onto Cu-chelated fabric matrix (to certain enzyme content 30–40 mg/100 g fabric) > free crude enzymes ≫ untreated cotton, reflecting the positive impact of chelated copper on enhancing the extent of interactions between the substrate and immobilized enzymes or increasing spin states of the adsorbed enzyme thereby improving its antimicrobial activity in addition to the antimicrobial activity of copper it self.[28–30]

Laundering and durability

Treated fabric samples were laundered 10, 20, and 30 cycles in a home laundering machine, and subjected to antimicrobial activity tests as mentioned in the experimental work according to AATCC Test method 124. The results are given in Tables V–VII and reveal that: (i) increasing laundering cycles up to 10 cycles has practically no effect on the antimicrobial activity

TABLE VI
Antimicrobial Activity of Ester-Crosslinked-Activated As Well As Copper-Chelated Cotton Fabrics Containing Immobilized α-Amylase After Laundering

	Clear inhibition zone diameter (mm)							
	Ester-crosslinked-activated cotton fabric				Copper-chelated cotton fabric			
Laundering cycles	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.
0	10 (100%)*	8 (100%)	7 (100%)	5 (100%)	19 (100%)	16 (100%)	10 (100%)	8 (100%)
10	10 (100%)	8 (100%)	7 (100%)	5 (100%)	19 (100%)	16 (100%)	10 (100%)	8 (100%)
20	7 (60%)	6 (75%)	5 (71.4%)	2 (71.4%)	15 (78.9%)	12 (75%)	8 (70%)	4 (50%)
30	4 (40%)	3 (37.5%)	2 (28.6%)	1 (20%)	10 (52.6%)	8 (50%)	4 (40%)	2 (25%)

S. A., *Staphylococcus aureus*; E. coli, *Escherichia coli*; C. A., *Candida albicanis*; A. S., *Asparigallus niger*.
* Retained activity (%).

TABLE VII
Antimicrobial Activity of Ester-Crosslinked-Activated As Well As Copper Chelated Cotton Fabrics Containing Immobilized Laccase After Laundering

	Clear inhibition zone diameter (mm)							
	Ester-crosslinked-activated cotton fabric				Copper chelated cotton fabric			
Laundering cycles	S. A.	*E. coli*	C. A.	A. N.	S. A.	*E. coli*	C. A.	A. N.
0	6.3 (100%)*	4.5 (100%)	5.5 (100%)	4.6 (100%)	16 (100%)	10 (100%)	8 (100%)	7 (100%)
10	6.3 (100%)	4.5 (100%)	5.5 (100%)	4.6 (100%)	16 (100%)	10 (100%)	8 (100%)	7 (100%)
20	4.5 (71.4%)	2.5 (55.6%)	3 (54.5%)	2 (43.5%)	12 (75%)	7 (70%)	4 (50%)	2 (28.6%)
30	2.6 (41.3%)	1.5 (22.2%)	1 (18.2%)	1 (21.7%)	6 (37.5%)	3 (30%)	2 (25%)	1 (14.3%)

S. A., *Staphylococcus aureus*; E. coli, *Escherichia coli*; C. A., *Candida albicanis*; A. S., Asparigallus niger.
* Retained activity (%).

of the treated substrates, regardless of the immobilized enzyme as well as the nature of microorganisms, (ii) further increase in laundering cycle numbers, up to 20 cycles, has a negative impact on the retained antimicrobial activities depending on the type of immobilized enzyme and ranging from 50 to 87% in case of immobilized alkaline pectinase (Table V), 50 to 78.9% in case of immobilized α-amylase (Table VI), and 28.6 to 75% in case of immobilized laccase (Table VII), depending on the type of microorganisms, and (iii) after 30 laundering cycles the antimicrobial property against gram-positive and gram-negative bacteria as well as filamentous and nonfilamentous fungi remains at over 14%.

CONCLUSIONS

In this study α-amylase, alkaline pectinase, and laccase enzymes were ironically immobilized onto both the ester-crosslinked-postactivated cotton as well as coordinated on the preaminated-Cu-chelated cotton fabrics. The potential to use these modified substrates for medical purposes was investigated through evaluation of their antimicrobial activities. Selecting the proper parameters for increasing the extent of immobilization without adversely affecting the retained activity of loaded enzyme could be the key for attaining better antimicrobial properties. Antimicrobial testing of the modified substrates demonstrated their antimicrobial effect against gram-positive and gram-negative bacteria as well as filamentous and nonfilamentous fungi. The antimicrobial activity of the modified cotton fabric samples is maintained after 30 laundering cycles, irrespective of the immobilized enzymes and the type of cotton support.

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