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Chitosan coated cotton gauze for antibacterial water filtration

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ABSTRACT

Communicable diseases can be transmitted by contaminated water. Water decontamination process is fundamental to eliminate microorganisms. In this work, cotton gauzes were coated with chitosan using an UV-curing process or cationized by introduction of quaternary ammonium groups and tested, in static and dynamic conditions, as water filter for biological disinfection against both Gram-negative and Gram-positive bacteria. Both materials showed good antibacterial activity, in static assessment, instead in dynamic conditions, chitosan treated gauze showed a high antimicrobial efficiency in few seconds of contact time. This composite could be a good candidate for application as biological filter.

1. Introduction

Cotton gauzes coated with chitosan or cationized by introduction of quaternary ammonium groups have been tested as water filter for biological disinfection against Gram-negative and Gram-positive bacteria. The choice of the support was due to its low cost and easy availability; moreover it is composed by cellulose fibers which can be easily functionalized by radical grafting (Roy, Semsarilar, Guthrie, & Perrier, 2009) and the open structure of the gauze is suitable to limit the pressure drop in continuous filtration system. Chitosan, in acetic acid solution, was applied on the gauze by padding, and grafted by ultraviolet radiation, in consequence of radical reactions promoted by a photoinitiator.

Chitosan, the cationic (1-4)-2-amino-2-deoxy-β-D-glucan, with degree of acetylation in the range 0.10–0.30 is industrially produced from marine chitin (Muzzarelli, 1993; Muzzarelli et al., 2012). Chitosan and its partially depolymerized derivatives and oligomers have a wide spectrum of biological activities, among which the bactericidal and bacteriostatic actions (Didenko et al., 2005; Goy, de Britto, & Assis, 2009; Muzzarelli et al., 1990; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Chitosan promotes aggregation of bacterial cells and disorganization of the bacterial cell wall and cytoplasmic membrane, thus giving place to the loss of cellular fluids. These structural changes may result in bacterial death depending on the strains examined therefore chitosan has been proposed as a textile finishing agent.

In our previous studies chitosan was applied on cotton, polyester, polyamide, silk and wool fabrics by radical UV-curing and high values of antimicrobial activity were found (Ferrero & Periolatto, 2011; Periolatto, Ferrero, & Vineis, 2012; Periolatto, Ferrero, Vineis, & Rombaldoni, 2013). Moreover the same chitosan functionalized gauze was tested as adsorbent medium for the removal of dyes from wastewater, with good performance also in continuous flow assessment (Periolatto & Ferrero, 2013). The choice of UV-curing, rather than thermal polymerization, was focused on an economical and environmental friendly process to obtain an effective treatment with good durability of the composite.

On the other hand the efficiency of quaternary ammonium groups as antibacterial agents is well known (Ladhari, Baouab, Ben Dekhil, Bakhrouf, & Niquette, 2007). For this reason chitosan treated gauze was compared with the same gauze subjected to cationization process by 3-chloro-2-hydroxypropyl-trimethylammonium chloride. Cationization was already applied on cotton fabric to improve its affinity toward different dye classes. Moreover, as for chitosan treated, the cationized gauze was previously applied to dye removal, revealing good efficiency, better than activated carbon, even in continuous flow assessment tests (Ferrero & Periolatto, 2012).

At first a deep chemical and morphological characterization of treated gauzes was carried out. Then their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* was tested both in static and dynamic conditions to evaluate the possibility of a real application in continuous filtration. Since

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the protonation of amino groups is widely influenced by the presence of hydrogen ions in solution, the behavior of both chitosan and quaternary ammonium groups may be pH sensitive. For this reason, the antibacterial activity was investigated even on treated gauzes previously conditioned at different pH values.

2. Materials and methods

2.1. Materials

In a previous study about functionalized fibrous materials for the removal of dyes (Ferrero & Periolatto, 2012), a screening test involving different fibrous materials was carried out. The best results in terms of performance, even in continuous assessment, were found with a pure cotton gauze fabric, with hexagonal holes 2 mm opening, 49 g/m^2, so the same material was chosen in this study.

3-Chloro-2-hydroxypropyl-trimethylammonium chloride (CHPTAC) 65 wt% in water (Fluka) was used as reagent for cotton gauze cationization. NaOH of laboratory grade (Sigma Aldrich) was the reagent for the alkalinization of cotton while the non-ionic surfactant Tergitol NP 14 (Union Carbide) was used as wetting agent.

Low viscosity chitosan, 75–85% deacetylation degree (Fluka) was used for gauze coating. It was dissolved in aqueous solution of 2% (v/v) glacial acetic acid, by ripening for 24 h followed by magnetic stirring at ambient temperature for further 24 h. A chitosan concentration of 5% wt was used while 2% on chitosan weight of 2-hydroxy-2-methylphenylpropane-1-one (Darocur 1173, Ciba Specialty Chemicals) was added as photoinitiator.

2.2. Gauze modification

The gauze cationization was studied in a previous work (Ferrero & Periolatto, 2012) and the best procedure found is summarized here. The cotton gauze was first alkalinized with a solution of 250 g/L of NaOH and 0.03 g/L of Tergitol NP 14, 1:20 (v/v) ratio. As the reaction was exothermic, temperature rise was monitored and when it came back to 25 °C, denouncing the complete cotton alkalinization, CHPTAC (400% on weight fibers) was added, and the cationization was carried out for 12 h. Then the cationized gauze was rinsed and dried.

The gauzes coated with chitosan were prepared by spreading of a proper amount of the chitosan-photoinitiator acetic solution on the fabrics with 12 h impregnation time, followed by drying for about 20 min at 80–100 °C. Then the coated fabrics were exposed to UV radiation using a medium pressure mercury lamp, with irradiance on the fabric of about 60 mW/cm^2, in a small box equipped with a quartz window under nitrogen atmosphere (oxygen content under 20 ppm). The required radiation dose was obtained adjusting the distance of textiles from the lamp at about 20 cm and the exposure time at 60 s. To assure the complete curing of the chitosan on the fabrics, they were radiated on both the sides.

The weight gain of fabrics was calculated as in Eq. (1):

$$\text{Weight gain}\quad(\%) = \frac{w - w_0}{w_0} \times 100 \tag{1}$$

where w is the weight of grafted fabric and w_0 that of the original fabric.

Treated samples with 10%, 25% and 40% weight gain were considered for characterization and indicated as 10CH, 25CH and 40CH respectively. These high percentages affect the hand properties of the fabric, nevertheless a soft hand is not required for the final application proposed. Moreover, chitosan should not form a continuous film coating on the support but it should cover the net structure maintaining the inter-yarn holes well open; in this way, if the gauze is crossed by a water flux, the pressure drop should be limited.

2.3. Gauze characterization

Cationized and chitosan-coated cotton surfaces were characterized by FTIR-ATR analysis, using a Nicolet FTIR 5700 spectrophotometer equipped with a Smart Orbit ATR single bounce accessory mounting a diamond crystal. Each spectrum was collected both on treated and untreated cotton samples by cumulating 128 scans, with 4 cm^{-1} resolution and gain 8, in the wavelength range of 4000–600 cm^{-1}.

The surface morphology of chitosan treated fabrics was examined by SEM with a Leica (Cambridge, UK) Electron Optics 435 VP scanning electron microscope with an acceleration voltage of 15 kV, a current probe of 400 pA, and a working distance of 20 mm. The samples were mounted on aluminum specimen stubs with double-sided adhesive tape and sputter-coated with gold in rarefied Argon using an Emitech K550 Sputter Coater with a current of 20 mA for 180 s.

The chemical composition of both cationized and chitosan treated samples was determined by CHNS-O elemental analysis carried out on Thermo Fisher Flash 2000 Analyzer EA 1112. XPS analyses were performed with a PHI 5000 Versa Probe system (Physical Electronics, MN) using a monochromatic Al radiation at 1486.6 eV, 25.6 W power, with an X-ray beam diameter of 100 μm. The energy resolution was about 0.5 eV. XPS measurements were performed at a pressure of 1×10^{-6} Pa. The pass energy of the hemisphere analyzer was maintained at 187.85 eV for survey scan. Since the samples are insulators, an additional electron gun and an Ar^+ ion gun for surface neutralization were used during the measurements.

2.4. Antimicrobial activity

The antimicrobial activity was evaluated on differently treated samples, about 1 g, according to ASTM E 2149-01 method (ASTM, 2001). This is designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions. The bacteria were *E. coli* ATCC 11229 (Gram negative), *S. aureus* ATCC 6538 (Gram positive) and *K. pneumoniae* ATCC 4352 (Gram negative). Yeast extract agar and peptone water were supplied by Liofilchem (Italy).

The incubated test culture in a nutrient broth was diluted to give a concentration of $1.5–3.0 \times 10^5$ CFU/mL (working dilution). Each fabric was transferred to flask containing 50 mL of the working solution. All flasks were shaken for 1 h at 190 rpm. After a series of dilutions, 1 mL of the solution was plated in nutrient agar. The inoculated plates were incubated at 37 °C for 24 h and surviving cells were counted. The antimicrobial activity was expressed in percent reduction of the organisms after contact with the test specimen compared to the number of bacterial cells surviving after contact with the control, according to the Eq. (2).

$$\text{Reduction}\quad(\%) = \frac{B - A}{B} \times 100 \tag{2}$$

where A is CFU/mL after contact (end test) and B is CFU/mL at zero contact time.

To check the influence of the protonation grade of both types of functional groups, antimicrobial test was carried out also on treated samples previously conditioned for 24 h at pH 4 or 8, provided by immersion in acetic acid or sodium bicarbonate solutions respectively.

2.5. Antibacterial filtration

The functionalized gauzes were tested in dynamic conditions with bacteria inoculum continuously flowed through the filter several times. A scheme of the system is reported in Fig. 1. It consists of a Pellicon® peristaltic pump (by Millipore), sterile plastic filter

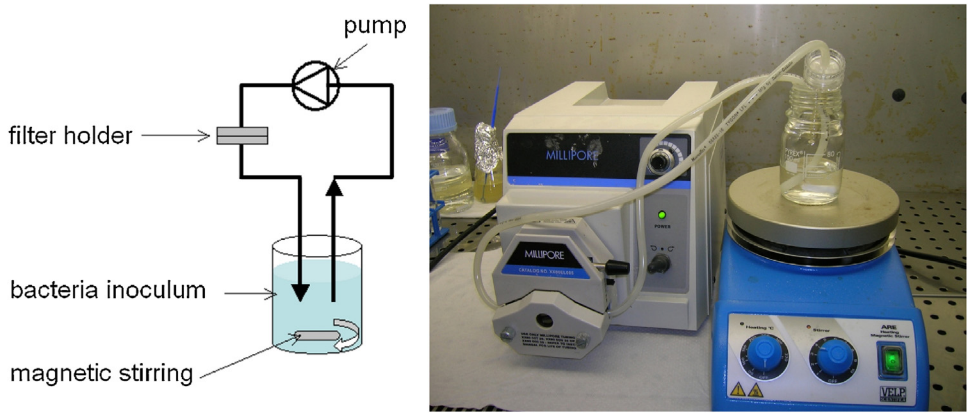

Fig. 1. Continuous filtration system: scheme (left) and operative system (right) of the experiment setup.

holder (25 mm internal diameter) and Materflex® Tygon® LFL autoclavable tubing. The gauzes were cut in 25 mm disks. Three layers of the same fabric were placed in the filter holder. The test culture was incubated at 37 °C in a nutrient broth (peptone water) and diluted in a sterile buffer to give a concentration of 1.5–3.0 × 10^5 CFU/mL obtaining the bacteria inoculum. 50 mL of bacteria inoculum in a reservoir were magnetically stirred and pumped at 4.8 mL/min flow rate in the system. In this way, the volume of bacteria inoculum was pumped in about 10 min and the contact time between the inoculum solution and the fabric was 4 s in each passage. The contact time was calculated as a ratio between the flow rate and the void volume of the gauze placed in the filter holder. The inoculum was cycled for about 50 min in the system, therefore the entire volume of bacteria inoculum passed through the filter 5 times. Every about 10 min (precisely at 0, 10.2, 20.2, 30.0, 39.6 and 49.0 min), 1 mL of bacteria inoculum was taken from the reservoir and pleated in yeast extract agar.

3. Results and discussion

3.1. Gauze characterization

Cotton in both cationized or chitosan treated samples presented an evident stiffness increase with respect to untreated cotton gauze, due to the treatment processes. Moreover, cationized gauze showed a shrinkage, with the consequent reduction of the dimension of the holes (Fig. 2). This is due to the structural change that the cotton fibers undergo by treatment with concentrated alkali in the finishing process known as mercerization. Nevertheless the effect is not detrimental since the mercerization increases the hydrophilicity of the fibers with a consequent improvement of the absorption performance.

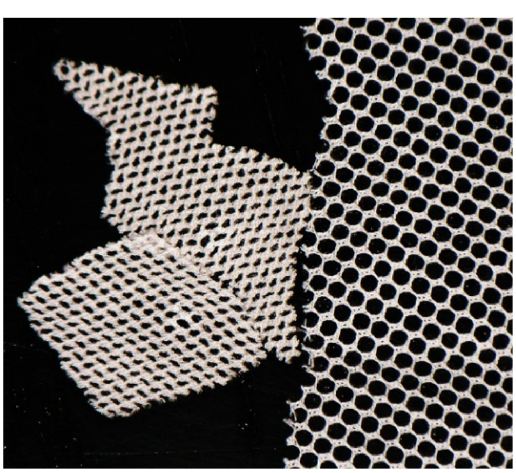

Fig. 2. Gauze shrinkage after cationization process: untreated (right) compared to treated sample (left).

Comparing the FTIR-ATR spectra related to cationized and untreated samples some differences were observed. In particular, in the spectrum of treated fabric, an increased absorbance of distinct bands can be observed between 1430 and 1373 cm^{-1} corresponding to CH stretching, bending deformations and rocking vibrations of methylene groups. However the comparison between FTIR-ATR spectra of untreated and 25CH sample shows only the presence, in the spectrum of the treated fabric, of two typical peaks of chitosan at 1648 cm^{-1} (C=O) and 1560 cm^{-1} (NH bending in amide group) since the other peaks are overlapped by those of cellulose (Fig. 3).

Comparing SEM images related to untreated and chitosan weighted samples, reported in Fig. 4, the presence of the added polymer is clear, in particular at lower magnifications. On treated samples, in fact, chitosan is visible on cotton fibers as a coating; nevertheless, the opening of holes are maintained quite similar to the untreated gauze showing just a light shrinkage.

Chitosan is spread on the fabric in a homogeneous way, covering meshes without occluding them. It is clear till magnification of 600×, without substantial differences between the structure of treated and untreated samples. However, on treated samples no agglomerated chitosan is present on the surface, denoting the good quality of chitosan solution and the effectiveness of the impregnation. Moreover no morphological change, ascribable to UV radiation, was revealed on fibers of treated cotton; it means that the curing radiation had no bad effects on the fiber structure.

Elemental analysis revealed the presence of chitosan on treated fabrics since nitrogen percentages increased with chitosan weight gain (Table 1). Comparing the measured chitosan percentage with the theoretical value, about 82%, 87% and 65% yields are found for 10CH, 25CH and 40CH samples respectively. It suggests some saturation weight gain, corresponding to 25–30%, over which the gauze cannot fix further chitosan, so it can be indicated as the best in

Table 1
Elemental analysis of cotton gauze.

Gauze	Theoretical N (%)	N (%)	C (%)	H (%)
Untreated	0	0.12	43.38	6.34
10CH	0.87	0.72	42.84	6.36
25CH	2.175	1.89	42.16	6.40
40CH	3.48	2.27	42.06	6.36
Cationized	6 max	0.18	41.55	–

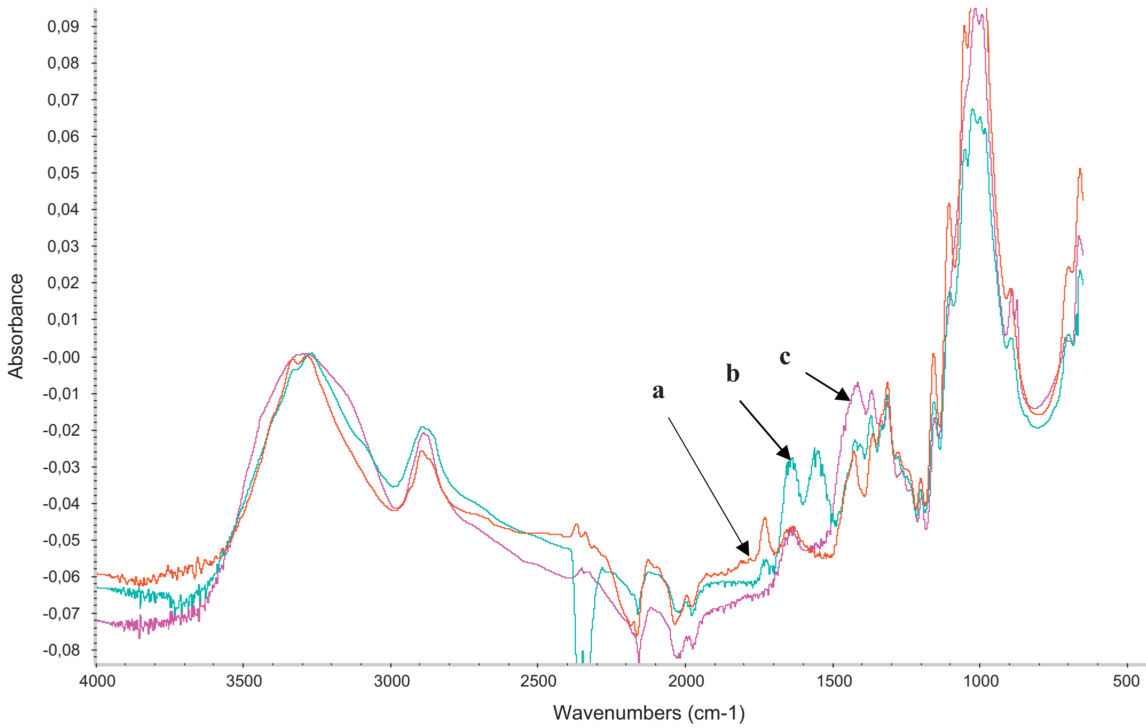

Fig. 3. FTIR-ATR spectra. (a) Untreated cotton, (b) chitosan coated sample (25CH) and (c) cationized cotton.

terms of process yield. Therefore the 25CH sample was chosen for the evaluation of antimicrobial activity. On the other hand, data related to cationized sample are quite similar to untreated gauze, due to a minimal supply of nitrogen by the cationization agent.

Some results of surface analysis obtained by XPS are reported in Table 2. The high amount of nitrogen detected on chitosan treated sample, in comparison with elemental analysis results, confirmed that almost all the chitosan fixed on the gauze was on the surface

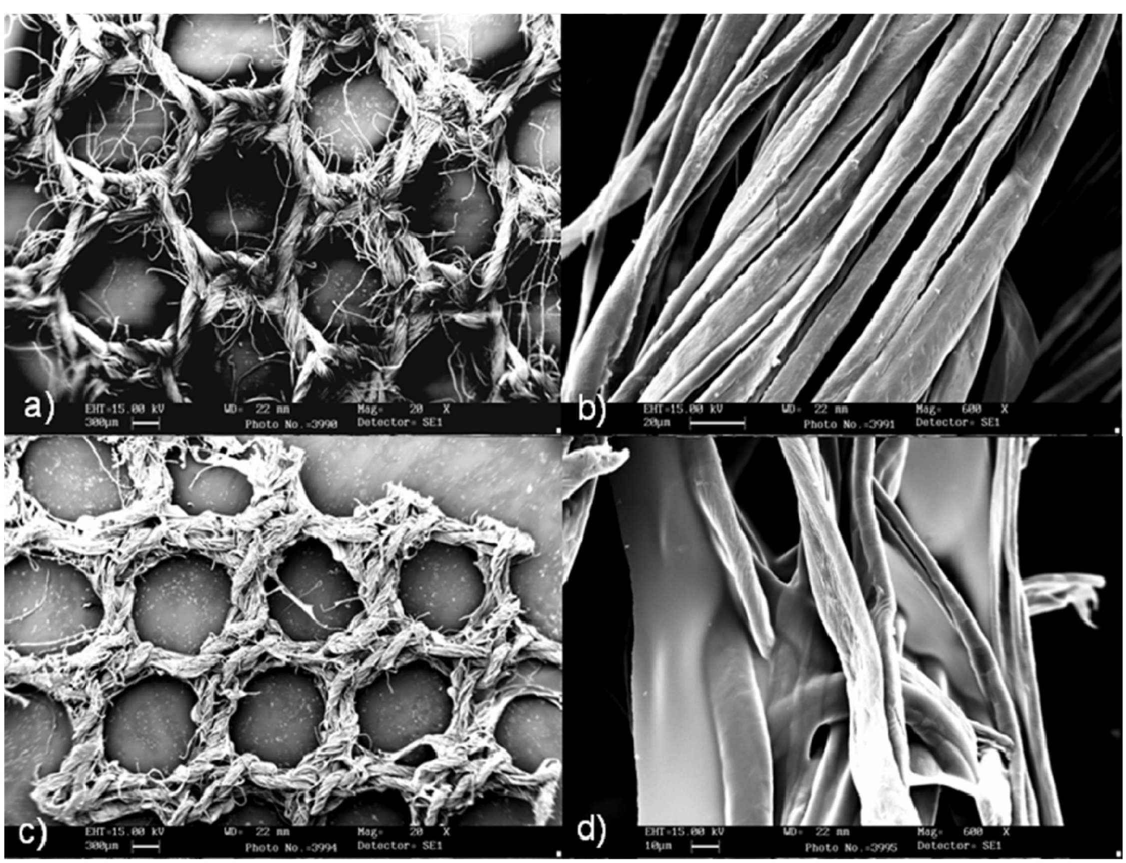

Fig. 4. SEM images. Untreated sample: (a) magnification 20×, and (b) magnification 600×. 25CH: (c) magnification 20×, and (d) magnification 600×.

Table 2
XPS analysis on cotton gauze. Survey scan.

Element (%)	Untreated	Cationized	25CH
C	66.1	65	58.2
O	33.9	31.2	34.9
N	–	1	6.9
Cl	–	–	<0.1
Ca	–	1.6	–
Mg	–	0.9	–
Zn	–	0.3	–

of cotton fibers rather than inside the bulk, as desirable. In this way in fact, the whole amount of chitosan active sites are easily accessible by microorganisms and the treatment efficiency is maximum. Moreover for the cationized gauze, the lower concentration of nitrogen is in agreement with the result of elemental analysis while the low amounts of Ca, Mg and Zn are attributable to impurities introduced by the treatment with high concentration of NaOH solution.

3.2. Evaluation of antimicrobial activity

No antibacterial activity was measured on untreated cotton fabric while results related to treated samples are reported in Table 3. A strong antibacterial activity was evaluated on both chitosan treated and cationized samples; in particular, as prepared chitosan weighted cotton provided the total microorganisms reduction toward all the bacteria investigated. A light decrease of performance was measured on cationized samples, tested as prepared, toward *E. coli* and *S. aureus* while the total reduction was reached against *K. pneumoniae*. A lower antibacterial activity of the cationized gauze can be due to the lower concentration of amino groups in comparison with that of chitosan coated gauze as shown by the results of surface nitrogen obtained by XPS analysis.

Table 3
Antimicrobial activity of treated gauzes as prepared and after maintenance at controlled pH for 24 h before evaluation.

Gauze	Condition	*E. coli* reduction (%)	*S. aureus* reduction (%)	*K. pneumoniae* reduction (%)
25CH	As prepared	100	100	100
	pH 4	100	100	–
	pH 8	97	95	–
Cationized	As prepared	89	79	100
	pH 4	97	99	–
	pH 8	97	100	–

The influence of the pH on the antibacterial activity of treated samples was investigated with regard to *E. coli* and *S. aureus*. Concerning chitosan weighted samples just a light decrease of antibacterial activity was noted keeping the filters for 24 h at pH 8 before the contact with the microorganisms, while the acid solution did not affect the antibacterial performance. This is in agreement with literature data referring chitosan as a positively charged surface with a pH value of 6.5 as zero potential (Chatterjee, Chatterjee, Chatterjee, Das, & Guha, 2005). As the pH is lowered, the positive charge of the surface increases thanks to the protonation of chitosan amino groups, with consequent formation of cationic amines and enhancement of electrostatic interactions between chitosan and the microorganisms.

At neutral pH, about 50% of free amino groups are protonated and theoretically useful for antibacterial activity, so a pH range between 3 and 6 is reported as optimal. On the contrary, at higher pH the amount of anions present in solution limits the chitosan protonation and, as consequence, its antibacterial activity (Kong, Chen, Xing, & Park, 2010).

Cationized cotton showed instead a further improved antibacterial activity at both pH, reaching a reduction of 100% and 97% of *S. aureus* and *E. coli* respectively. The investigation on pH effect was not carried out on *K. pneumoniae*, in fact in this case the total microorganisms reduction was found already on the as prepared samples even for the cationized sample.

Results of continuous flow assessment test highlighted the potentiality of chitosan treated gauze for a real application as biologic filter (Fig. 5). A contact time of few seconds was in fact enough to reach the total bacterial reduction on both the investigated microorganisms. Best results were related to *S. aureus*, where the total reduction was obtained at the first sampling, corresponding to a contact time of 4 s. At the same contact time, 80% reduction was obtained against *K. pneumoniae*, nevertheless 98% bacterial reduction was reached after 8 s of contact, already a widely interesting time.

Worst results were obtained on cationized samples: reduction percentages not higher than 20% were obtained, in 20 s, against *K. pneumoniae* and about 40% against *S. aureus*. The curve related to *S. aureus* shows a rise in the final part, suggesting that prolonged contact times could improve the filter efficiency. Nevertheless it means that cationized cotton gauze is not suitable for continuous filtration because too prolonged contact times are requested to reach a satisfactory purification of the filtered water.

Besides the higher surface content of amino groups, the different kinetic behavior of chitosan coated gauze in comparison with that cationized can be justified by the different structure

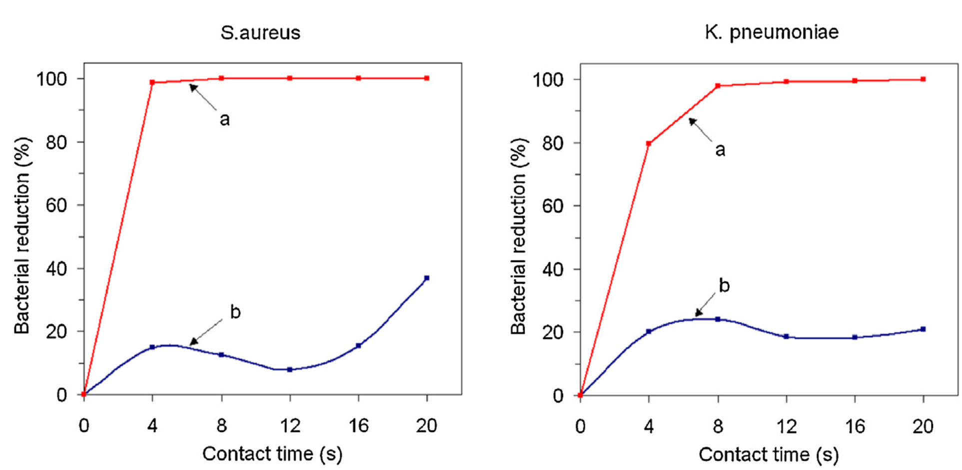

Fig. 5. Continuous flow test against *S. aureus* and *K. pneumoniae*: (a) Chitosan treated gauze (25CH) and (b) cationized gauze.

of the two materials. In fact the chitosan treated gauze unlike the cationized showed at SEM analysis an evident coating surrounding the fibers (Fig. 4). This coating can be more accessible to microorganisms than the quaternary amino groups grafted into the cotton fibers which can be attained with a slower diffusion stage.

4. Conclusions

A cotton gauze was coated with chitosan by UV-curing and tested as antibacterial medium for water filtration in comparison with the same gauze modified by cationization. In both cases the antimicrobial activity was due to the functionalization. Both filters showed good antibacterial activity, in static assessment, against Gram positive and Gram negative microorganisms. A certain pH sensitivity was found, but in all cases microorganism reduction never fell under 80%. Finally, in continuous assessment test, chitosan treated gauze showed high antimicrobial efficiency in a very fast way, that is with high flow rates. It makes this composite a good candidate for its real use as biological filter.

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