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Antibacterial Water Filtration by Cationized or Chitosan Coated Cotton Gauze

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Communicable diseases can be transmitted by contaminated water and water decontamination process is fundamental to eliminate micro-organisms. 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. With both treatments 100% microorganisms reduction was reached in static assessment, while in dynamic conditions better results were obtained by chitosan treated gauze, reaching 100% S.Aureus and K. Pneumoniae reduction with 4 s and 8 s contact time respectively. This substrate could be a good candidate for application as biological filter.

1. Introduction

Contaminated water can be a mechanism to transmit communicable diseases such as diarrhoea, cholera, dysentery and typhus. Moreover millions of people are exposed to dangerous levels of biological contaminants in their drinking-water due to inadequate management of urban, industrial or agricultural wastewater. The main micro-organisms that cause water-associated diseases are bacteria such as *Escherichia coli*. When fecal and coliform bacteria are encountered in a water sample, pathogenic micro-organisms must be removed or inactivated.
The water decontamination process is fundamental to eliminate micro-organisms and can be done accomplished by chemical or physical means. The chemical substances used are sodium hypochlorite, gaseous chlorine, calcium hypochlorite, chlorine dioxide, chlorinated lime. Gaseous chlorine or sodium hypochlorite processes are based on the formation of hypochlorous acid and hypochlorite ion in water, which have a biocidal effect. The inconvenience of chlorination is that chlorinated compounds can be formed in harmful concentrations to human health as already stated by Condie in 1990.
Conventional physical water treatments include sedimentation, coagulation, flocculation and filtration. These processes are used for the removal of colloidal particles from raw water. The increasing water demand, deterioration of raw water quality and rigorous regulations on drinking water require new treatments with better performance, higher energy efficiency, lower operating cost and highly reliability. Recently, water treatment system utilizing floating media filter and microfiltration membrane units was developed by Chiemchaisri et al. (2011). Other water treatments studied are ultraviolet irradiation, gamma irradiation or a combination of both (Taghipour 2004), ozonation and chlorination.
In this work, a new approach for the inactivation of micro-organisms has been developed. 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 substrate was due to its low cost and easy availability; moreover it is a natural cellulosic substrate, that can also be involved in radical grafting 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,2-amino-2-deoxy-(1→4)-β-D-glucopyranan, is a biopolymer derived from the deacetylation of the chitin component of the shells of crustaceans, widely available as by-product of the food industry (Goy et

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al. 2009) and used also as food additive. It is proposed as textile finishing agent for its antimicrobial activity. The mechanism of the biocidal action of chitin, chitosan, and their derivatives is still unknown, but several mechanisms have been proposed by Rabea et al. (2003). Antibacterial effect of chitosan on the morphofunctional organization of clinical strains of *Klebsiella pneumoniae* and *Staphylococcus aureus* are reported by Didenko (2005): chitosan promoted aggregation of bacterial cells coupled to disorganization of bacterial cell wall and cytoplasmic membrane, which leads to the release of bacterial contents into the environment. These structural changes result in bacterial death.

In our previous studies (Ferrero and Periolatto 2011, 2012a; Periolatto et al. 2012, 2013) chitosan was applied on cotton, wool, polyester, polyamide and silk fabrics by radical UV-curing and high values of antimicrobial activity were found. Moreover the same chitosan functionalized gauze was tested as filter substrate for the removal of dyes from wastewater, with good performance also in continuous flow assessment (Periolatto and 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.

In UV curing, radical species are generated by the interaction of UV light with a suitable photoinitiator, which induces grafting reactions of reactive chemical groups at low temperature and quickly, with lower environmental impact and lower process cost than conventional thermal curing process.

The efficiency of quaternary ammonium salts as antibacterial agents is well known. For this reason chitosan treated gauze was compared with the same gauze after a 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 by Ferrero and Periolatto (2012b) to dye removal, revealing good efficiency, better than activated carbon, even in continuous flow assessment test. The novelty of the present research work is the application of processes, previously applied to textiles finishing or removal of dyes from wastewaters, to produce water filtration media active as pathogen microorganisms disinfectant.

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 statically and dynamic conditions to evaluate the possibility of a real application in continuous filtration. Since the protonation of amino groups is widely influenced by the presence of hydrogen ions in solution, the behavior of both chitosan and quaternary ammonium salt may be pH sensitive. For this reason, the antibacterial activity was investigated even on gauzes treated at different pH values.

2. Materials and Method

2.1 Gauze preparation

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

For what concern cotton cationization, the reagent was 3-chloro-2-hydroxypropyl-trimethylammonium chloride (CHPTAC) 65% wt in water (Fluka). The non-ionic surfactant Tergitol NP 14 (Union Carbide) was used as wetting agent while NaOH of laboratory grade (Sigma Aldrich) was the causticization reagent. The substrate was first causticized treating it with a solution of 250 g/l of NaOH and 0.03 g/l of Tergitol NP 14, 1:20 liquor ratio. As the reaction was exothermic, temperature rise was monitored and when it came back to 25°C, denouncing the complete substrate causticization, CHPTAC (400% on weight fibers) was added, and the cationization was carried out for 12 h. Then the cationized gauze was rinsed and dried.

Alternatively, chitosan treated gauzes were coated with low viscosity chitosan, 75–85% deacetylation degree (Fluka). 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 24 h. A chitosan concentration of 5% w was used while 2% on chitosan weight of 2-hydroxy-2-methylphenylpropane-1-one (Darocur 1173, Ciba Specialty Chemicals) was added as photoinitiator. Further steps were spreading of the mixture on the fabrics with 12 h impregnation time, drying for about 20 min at 80–100 °C and finally UV-curing.

The surface coated fabrics were exposed to UV radiation using a medium pressure mercury lamp, with a light intensity on the fabric of about 60mW/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 20cm and the exposition time at 60 s. To assure the complete curing on the fabrics, they were radiated on both the sides.

The weight gain of fabrics, that is the polymer add-on, was calculated as in equation (1)

$$Weight\ gain\ (\%) = (w - w_0) \times 100 / w_0 \quad (1)$$

where w is the weight of grafted fabric and w_0 the weight of the original fabric.
Treated samples were prepared with a chitosan add-on of about 25% w: it is an high percentage affecting the hand properties of the fabric, nevertheless a soft hand is not required for the final application proposed. Moreover, chitosan does not form a coating on the substrate but covers the net structure maintaining the inter-fibers holes well open; in this way, if the substrate is crossed by a water flux, the pressure drop is limited.

2.2 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. 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 20mm. 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, 25.6W power, with an X-ray beam diameter of 100µm.

2.3 Antimicrobial test
The antimicrobial activity was evaluated on differently treated samples, about 1 g, according to ASTM E 2149-01 “Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions”. This method is designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions. The bacteria were *Escherichia coli* ATCC 11229 (Gram negative), *Staphylococcus aureus* ATCC 6538 (Gram positive) and *Klebsiella pneumoniae* ATCC 4352 (Gram negative).
The antimicrobial activity was expressed in % reduction of the organisms after contact with the test specimen compared to the number of bacterial cells surviving after contact with the control, according (2).

$$Red,\%\ (CFU/ml) = (B-A) / B\ x100 \quad (2)$$

where A is CFU/ml after contact (end test) and B is CFU/ml at zero contact time.
Antimicrobial test was carried out also on treated samples kept for 24h at pH 4 or 8, provided by immersion in acetic acid or sodium bicarbonate solutions respectively.
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 Figure 1. It consists of a Pellicon® peristaltic pump (by Millipore), sterile plastic filter holder (25 mm internal diameter) and Materflex® Tygon® LFL autoclavable tubing. The gauzes were cut in disks with a 25 mm. Three layers of the same fabric were placed in the filter holder.
50 ml of bacteria inoculum 1.5–3.0 $\times$ 10^5 CFU/ml 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 10 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, due to the finishing processes. Moreover, cationized gauze showed a shrinkage, with the consequent reduction of the dimension of the holes but not affecting the homogeneous flux of water through the filter.
Beyond these clear indications of cotton modification, cationization and chitosan presence were confirmed by FTIR-ATR spectra (Figure 2). Comparing the spectra related to cationized and untreated samples 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.

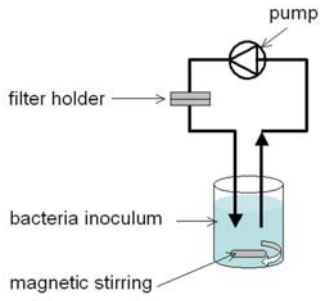

Figure 1: scheme of continuous filtration system

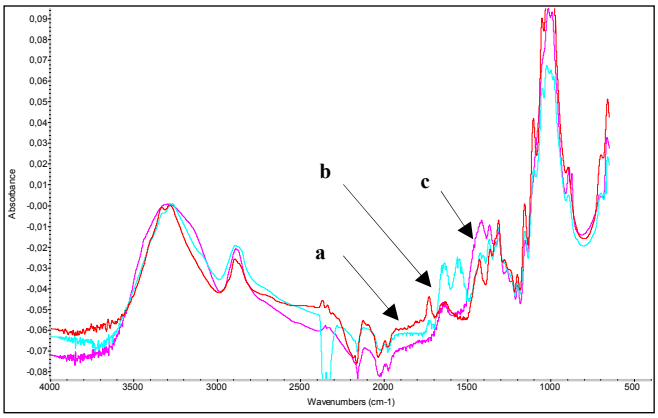

Figure 2: ATR spectra of untreated (a), chitosan 25% weighted (b) and cationized (c) cotton gauze

Moreover the comparison of FTIR-ATR spectra of untreated and 25% chitosan treated cotton shows the evidence, in the spectrum of the treated fabric, of typical peaks of chitosan at 3290 cm^{-1} and 2900 cm^{-1} due to NH and CH stretching respectively. Other typical peaks at 3360 cm^{-1} (OH groups), 1648 cm^{-1} (C=O), 1560 and 1075 cm^{-1} (NH bending in amide group) are not clearly visible because partially overlapped by those of cellulose.

Comparing SEM images related to untreated and chitosan weighted samples, reported in Figure 3, the presence of the finishing 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 an homogeneous way, covering meshes without gluing them. It is clear till magnification of 600X, 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 damage, ascribable to UV radiation, was revealed on fibers of treated cotton; it means that the curing radiation had no bad effects on the fibers structure, so even the mechanical characteristics of the original fabric should not be affected.

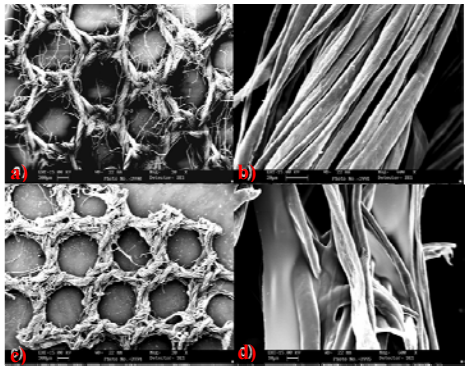

Figure 3: SEM microphotographs. Untreated sample: (a) magnification 20X, (b) magnification 600X. Gauze 25% chitosan weighted: (c) magnification 20X, (d) magnification 600X.

Table 1: Elemental analysis on cotton gauze

Sample	Theoretical N [%]	N [%]	C [%]	H [%]
Untreated	0	0.12	43.38	6.34
10% chitosan weight on	0.87	0.72	42.84	6.36
25% chitosan weight on	2.175	1.89	42.16	6.40
40% chitosan weight on	3.48	2.27	42.06	6.36
Cationized	Max 6	0.18	41.55	-

Elemental analysis revealed the presence of chitosan on treated fabrics since nitrogen percentages increased with chitosan weight on. (Table 1) Comparing the measured chitosan percentage with the theoretical value, about 82%, 87% and 65% yields are found for 10%, 25% and 40% weighted samples respectively. It suggests some saturation weight on, corresponding to 25-30%, over which the gauze cannot fix further chitosan, so it can be indicated as the best in terms of process yield. 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.
Obtained results were confirmed by XPS, measuring 1 and 6.9% of nitrogen on the surface for cationized and chitosan treated samples respectively. The high amount of nitrogen detected on the surface of chitosan treated sample, related to elemental analysis results, confirmed that almost all the chitosan fixed on the gauze was on the surface 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.

3.2 Evaluation of antimicrobial activity

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*.
The influence of the pH on the antibacterial activity of treated samples was investigated with regard to *E. coli* and *S. aureus* (Table 2). Concerning chitosan weighted samples just a light decrease of antibacterial activity was noted keeping the substrate for 24h at pH 8 before the contact with the microorganisms, while the acid solution did not affect the substrate performance. This is in agreement with Chatterjee et al. (2005) literature data referring chitosan as a positively charged surface with a pH value of 6.5 as zero potential. Reducing the pH, 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 by Kong et al. (2010). On the contrary, at higher pH the amount of anions present in solution limits the chitosan protonation and, as consequence, its antibacterial activity.
Cationized cotton showed instead a further improved antibacterial activity in both cases, reaching 100% and 99% of microorganisms reduction.
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; moreover on *E. coli* and *S. aureus* the effect was negligible or caused an improvement of performance, so the same was assumed also for *K. pneumoniae*.
Results of continuous flow assessment test highlighted the potentiality of chitosan treated gauze for a real application as biologic filter. (Figure 4) 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 seconds 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* show 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.

4. Conclusions

A cotton gauze was cationized or chitosan weighted by UV-curing and then tested as antibacterial substrate for water filtration. In both cases the antimicrobial activity was due to protonable sites provided by the functionalization. In particular, the presence of nitrogen, specifically amino groups on chitosan treated gauze, was revealed by FTIR-ATR, XPS and elemental analysis.
Macroscopically, treated gauze showed a certain shrinkage, in the case of cationization, or partial occlusion of the interfibral holes, in case of chitosan treatment. Nevertheless, it does not compromise the possible use for continuous filtration since the pressure drop is low.

Table 2: Influence of sample maintainance at controlled pH on antimicrobial activity

Sample	pH	E. coli [% red]	S.aureus [% red]
Chitosan	As prepared	100	100
Chitosan	4	100	100
Chitosan	8	97	95
Cationized	As prepared	89	79
Cationized	4	97	99
Cationized	8	97	100

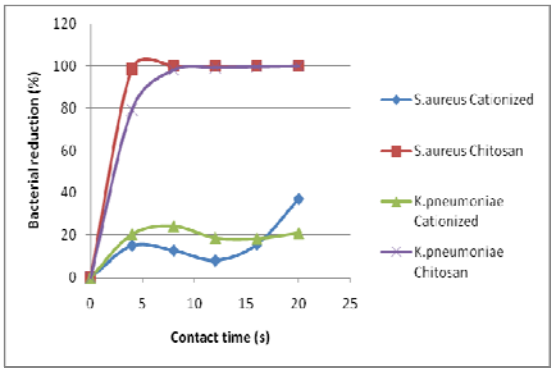

Figure 4: Continuous flow test

Both substrates showed good antibacterial activity, in static assessment, against Gram positive and Gram negative microorganisms. A certain pH sensitivity was found, but in all cases microorganisms reduction never fall under 80%.

Finally, from continuous assessment test, chitosan treated gauze showed an high antimicrobial efficiency in a very fast way, that is with high flow rates. It makes this substrate a good candidate for its real use as biological filter. On the contrary, results not so good were obtained with cationized gauze, which requires prolonged contact times, so its application should be limited to static filtration systems.

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