Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Xing, Xiaodong , Lu, Diannan , Wang, Xiaogong and Liu, Zheng(2009) 'Preparation and Antibacterial Function of Quaternary Ammonium Salts Grafted Cellulose Fiber Initiated by Fe^{2+}-H_2O_2 Redox', Journal of Macromolecular Science, Part A, 46: 5, 560 — 565
To link to this Article: DOI: 10.1080/10601320902797897
URL: http://dx.doi.org/10.1080/10601320902797897

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2009) **46**, 560–565

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601320902797897

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Preparation and Antibacterial Function of Quaternary Ammonium Salts Grafted Cellulose Fiber Initiated by Fe^{2+}-H_2O_2 Redox

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Received September 2008, Accepted December 2008

The surface contact disinfecting technique is a newly developed method for water sterilization. In this paper, the grafted quaternary ammonium salts (QAS) antibacterial fibers were prepared and designed to apply for the surface contact disinfecting process in water treatment. The antibacterial fibers were directly prepared by grafting methacryloxylethyl benzyl dimethyl ammonium chloride (DMAE-BC) onto cellulose fiber using thiocarbonate-H_2O_2 redox system. All kinds of factors in the grafting reactions, such as reaction time, reaction temperature, monomer concentration, initiator concentration, which influence the percentage of grafting, were studied and optimized. The modified cellulose fibers were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope(SEM). The effects of the percentage of grafting of the grafted cellulose fibers on bactericidal activity were also studied. The spread plate method was used to characterize the bactericidal activity. The disinfection process was further investigated by directly observing the morphology of the bacterial cells adsorbed on the antibacterial fibers with SEM and measuring extracelluar total protein concentration in suspension. The poly(DMAE-BC)-grafted cellulose ?ber was found to exhibit particularly high activity against *E.coli*.

Keywords: Surface grafting, QAS, antibacterial, cellulose fiber, thiocarbonate-H_2O_2 redox system

1 Introduction

Surface contact disinfecting technique is a newly developed method for water sterilization, which is performed in a packed bed of solid media on which bactericide is immobilized (1). Compared to conventional sterilization processes using small bactericides dissolved in water, the surface contact method appears to have practical advantages in terms of being free of secondary contamination by bactericides and a high speed, as a result of high concentration of disinfectant at solid surface (2–4). Because the grafting layer is bonded with matrix in chemical bond, the antibacterial surface is quickly connected with matrix, so it possesses endurance and safety antibacterial properties.

Cellulose is one of the most abundant natural polymers on earth. It is biodegradable, renewable and easily available at low costs. Modification of cellulose by graft copolymerization techniques allows one to chemically change the cellulose chain by introducing functional polymeric chains, which leads to new cellulose products with new properties (5). The graft copolymerization of monomers onto cellulosic fiber has been carried out by different techniques, such as reversible addition-fragmentation chain transfer (RAFT) polymerization, (6) atom transfer radical polymerization(ATRP), (7, 8) thiocarbonate-H2O2 chemical initiation method, (9) and ceric(IV) ion chemical initiation method (10,11). The ceric (IV) ion initiated grafting offers great advantages of forming radicals at cellulose backbone through a single electron-transfer process to promote grafting of monomer onto cellulose, and was used extensively (11).

Low-molecular-weight or polymeric quaternary ammonium salts, quaternary phosphonium salts, and pyridinium salts show antimicrobial activities and are most commonly used. Some works involving preparation and performance of antibacterial cellulose grafted with quaternary ammonium salts, quaternary phosphonium salts, and pyridinium salts have been reported. Lee (8) has grown an antimicrobial polymer directly on the surfaces of paper using atom transfer radical polymerization (ATRP).

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The tertiary amine 2-(dimethylamino)ethyl methacrylate was polymerized directly onto Whatman #1 filter paper. Following the polymerization, the tertiary aminogroups were quaternized using an alkyl halide to produce a large concentration of quaternary ammonium groups on the polymer-modified surfaces. Nonaka (11) studied the graft copolymerization of methacryloyloxyethyl trimethyl ammonium chloride (METAC) and tributyl-4-vinylbenzyl phosphonium chloride (TRVB) on loofah fiber using ammonium cerium nitrate, the most extensively used initiator.

In the previous article, we also discussed the modification of cellulose fiber by grafting QAS with ceric ions as the initiator (12,13). But as we know, ceric ion, a type of heavy metal ions, poses potential harm to human health in water treatment, and from an economical point of view, the price of ceric ammonium nitrate is higher than that of ferrous ammonium sulfate and H2O2 (14, 15). Because of its low cost and high activity, hydrogen peroxide, in the form of Fenton's reagent ($FeSO_4$-H_2O_2) as a redox initiator, has been used for grafting vinyl monomers onto starch, cellulose, wool, gelation, sodium alginate, natural rubber, and synthetic polymers such as poly(ethylene terephthalate) (9,16). However, none of these studies have been focused on directly grafting QAS monomers onto cellulose fiber.

The purpose of this work was using the $FeSO_4$-H_2O_2 initiation system to directly prepare cationic cellulose-P(DMAE-BC) grafted copolymer containing quaternary ammonium groups. Using *E. Coli* as the model bacteria, the spread plate method was used to characterize the bactericidal activity. The disinfection process of the bacteria adsorbed on the antibacterial fibers was further investigated by observing the cell morphology with scanning electron microscope. The extracelluar total protein concentration of *E. Coli* suspension contacted with grafted fiber was also determined.

2 Experimental

2.1 Materials and Characterization

All reagents were used without further purification. Cellulose fibers were kindly donated by Procter & Gamble Co. Carbon disulphide, sodium hydroxide, ferrous sulphate, and hydrogen peroxide, were used as received from Shanghai Chemical Reagents Co. ltd., China. Methacryloxylethyl benzyl dimethyl ammonium chloride (DMAE-BC) was synthesized in the lab (17). The *E. Coli* was kindly provided by the Department of Chemical Engineering, School of Biochemical Engineering, Tsinghua University, China. Fourier transform infrared (FTIR) spectra (wavenumber range 400–4000cm^{-1}) were recorded using a Nicolet MAGNA-IR 560 FTIR Spectrometer. The morphology of cellulose fibers and morphological changes of the adsorbed cells adsorbed on antibacterial fibers were explored by SEM (JSM35CF, JEOL).

2.2 Thiocarbonation of Cellulose Fiber

The cellulose was placed in a glass vessel containing the given thiocarbonation solution (NaOH:CS_2 1:1 w/v). The material to liquor ratio was 1:25, and the temperature was kept at 30°C. The contents in the vessel were continuously stirred throughout the thiocarbonation reaction. After 2 h, the solution was drained, and the fiber was thoroughly washed with distilled water until the washing liquor acquired pH 7. The cellulose fiber treated in this method will be referred to as cellulose thiocarbonate (9).

2.3 Synthesis of QAS Grafted Cellulose Fiber by the Chemical Method

The cellulose thiocarbonate was placed in a sealed glass vessel containing the $FeSO_4$ aqueous solution at pH 2. Then a specific amount of monomer (methacryloxylethyl benzyl dimethyl ammonium) was added and nitrogen was introduced for 10 min, followed by the addition of a desired amount of initiator (H_2O_2). The grafting process was carried out at a proper temperature. The grafted fibers obtained were purified by extraction of homopolymer and the unreacted monomer with boiled water and acetone.

The samples were dried and the QAS group content on grafted cellulose fiber was measured by weight. The percentage of grafting (*Pg*) was calculated as follows:

$$Pg(\%) = \frac{Wg - W0}{W0} \times 100\%$$

Where W_0 and W_g are the weights of the initial and grafted fibers, respectively.

2.4 Removal of Bacteria Cell from Water with Grafted Cellulose

A E.coli JM105 cell was inoculated at 36°C for 18 h on a nutrient agar plate before use. Then the bacteria cell suspensions were mixed with axenic water by 1:4. 100 ml dilute bacteria solution and grafted cellulose fibers were mixed at 37°C in a test tube shaker at 150 rpm. At a specified time, the surviving bacteria were counted by the spread plate method to assess their bactericidal activities (18).

3 Results and Discussion

The mechanism of cellulose reacting with carbon disulphide to yield cellulose thiocarbonate and the cellulose thiocarbonate grafting initiated by Fe^{2+}-H_2O_2 system was suggested by Hebeish (9). The factors, such as reaction time, temperature, monomer concentration, initiator concentration, coinitiator concentration, which influence the grafting yield in the grafting process, were studied.

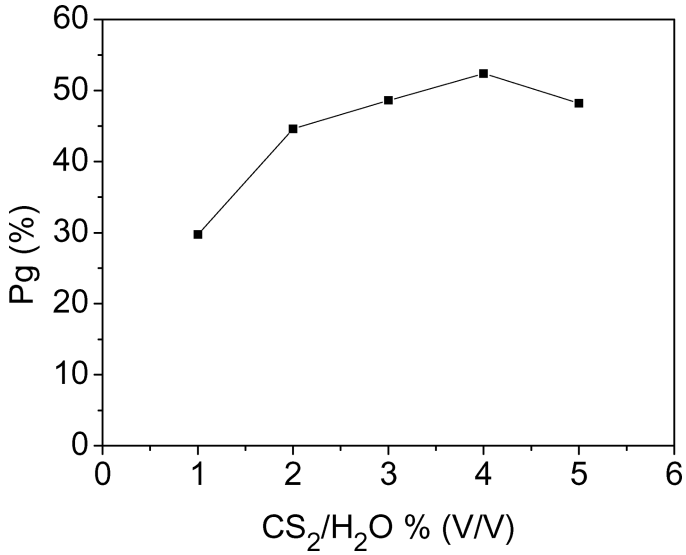


Fig. 1. The relationship between Pg and concentration of thiocarbonation solution (T = 30°C M = 0.8 mol/L t = 4 h).

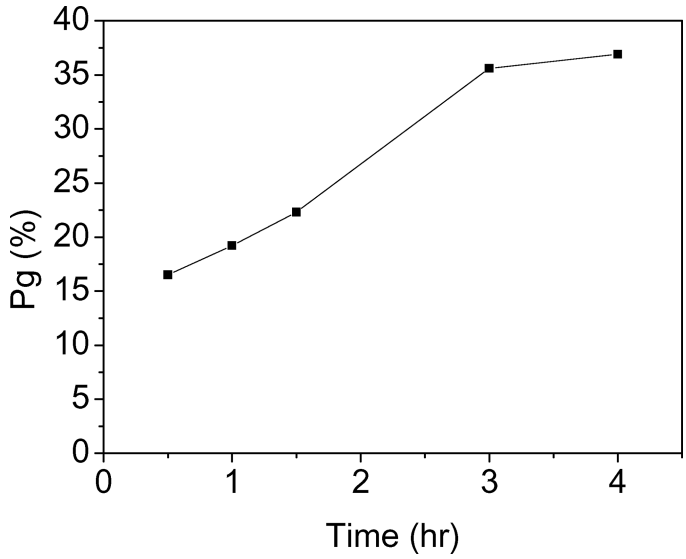


Fig. 2. The relationship between Pg and grafting reaction time (T = 30°C M = 0.8 mol/L H_2O_2 = 0.3%).

3.1 Concentration of Thiocarbonation Solution

The effect of concentration of thiocarbonation solution on the percent of grafting is shown in Figure 1. It is clear that the Pg obviously increases with the increase of concentration of thiocarbonation solution below 4%, and then decreases. By increasing the thiocarbonation solution concentration, even more thiocarbonate groups were introduced in the cellulose main chain. Above a particular concentration, there will be an abundance of cellulose macroradical, which contributes largely in the termination of growing chains; thus, the Pg decreases.

3.2 Reaction Time

Figure 2 shows the effect of reaction time on the percent of grafting polymer add-on. The percentage of grafting increased with increasing reaction time. With increasing reaction time, the amounts of the hydroxyl radical and macroradical, as well as the conversion of the monomer increased. Then, branches increased, that is, the extent of grafting increased; 4 h is a must for preparing high Pg grafted fiber.

3.3 Concentration of Monomer

Figure 3 indicates the effect of concentration of DMAE-BC on the percent of grafting polymer add-on. When the amount of the monomer was increased, the grafting reaction was speeded up and the Pg increased. The obvious increment in Pg by increasing monomer concentration could be attributed to the greater availability of monomer molecules in the vicinity of the cellulose at a higher monomer concentration.

3.4 Reaction Temperature

Figure 4 shows the effect of reaction temperature on the percent of grafting polymer add-on. The grafting degree increased with elevating the reaction temperature below 40°C, but slowly decreased above 40°C. When the reaction temperature is elevated, the reactive activity of DMAE-BC increased and the reaction rate of Fe^{2+} and H_2O_2 increased to give more hydroxyl radicals. The grafting macromolecular motion can also be strengthened with high temperature. Therefore, the grafting degree increased with increasing temperature. When the temperature is high, the homopolymer chain motion was strengthened and some of the

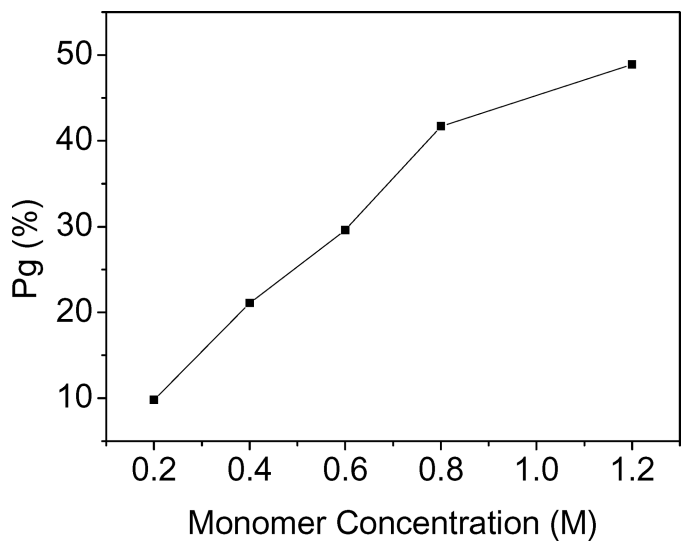


Fig. 3. The relationship between Pg and concentration of monomer (T = 30°C t = 4 h H_2O_2 =0.3%).

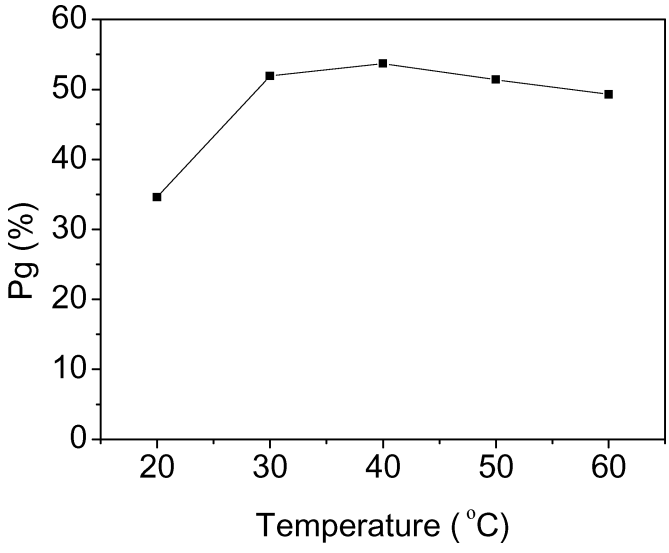


Fig. 4. The relationships between Pg and grafting reaction temperature (t = 4 h M = 0.8 mol/L H_2O_2 = 0.3%).

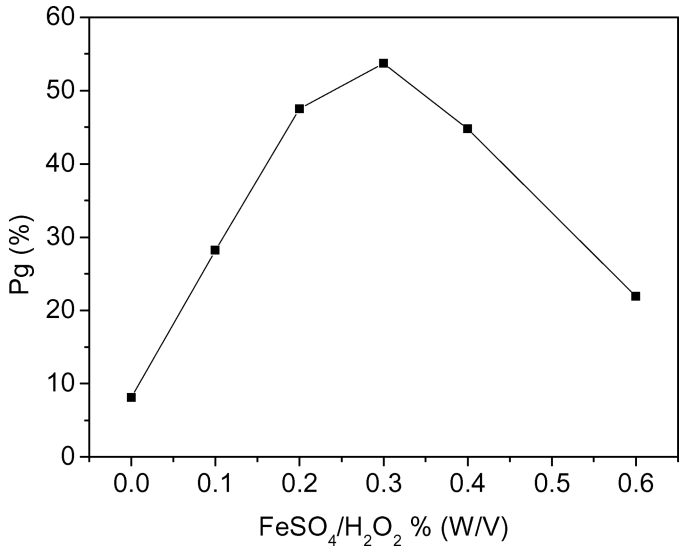


Fig. 6. The relationship between Pg and concentration of initiator (T = 30°C M = 0.8 mol/L H_2O_2 = 0.3%).

Fe^{2+} ions can be oxided into Fe^{3+} ions. These may be why the grafting degree decreased when the temperature was elevated above 40°.

3.5 Concentration of Initiator (H_2O_2)

Figure 5 indicates that the extent of grafting increased with increasing the concentration of initiator (H_2O_2) to a small extent. The grafting copolymerization took place in the heterogeneous medium. When the amount of H_2O_2 is increased, the amount of the hydroxyl radical formed in the medium increased both the grafting copolymerization and homopolymerization can easily occur. Therefore, increasing the concentration of H_2O_2 below 0.4% resulted in the grafting degree increasing (5).

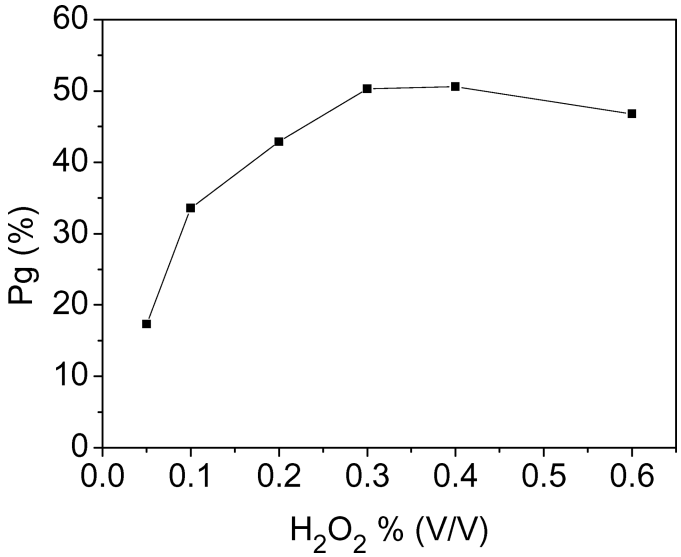


Fig. 5. The relationship between Pg and concentration of initiator (T = 30°C M = 0.8 mol/L t = 4 h.

3.6 Concentration of Coinitiator ($FeSO_4$)

Figure 6 shows the effect of Mohr's salt on the Pg. The addition of Mohr's salt to the grafting system led to a great increase in the Pg, except for the concentration of Mohr's salt of more than 0.2% ($FeSO_4$/ H_2O_2) (W/V).

3.7 FTIR Analysis

The grafting copolymerization was confirmed by the infrared (IR) spectrum. The FTIR spectra of cellulose fiber, grafted cellulose fiber (Pg 29.7%) are given in Figure 7(a), (b). Compared with the FTIR spectrum of original cellulose (Fig. 7(a)), the FTIR spectrum of grafted cellulose (Fig. 7(b)) showed a new peak at 1740 cm^{-1} due to ester group stretching peak of DMAE-BC.

3.8 Bacteria Removal Performance of Grafted Cellulose Fiber

Figure 8 shows a plot of log (survivors) vs. contact time for grafted cellulose fibers. It was seen that the removal takes place rapidly at first, then slows down and levels off. All fibers make the viable cell number in the bacterial suspension decease greatly in several minutes. The rate and the extent of removal increase with an increase in the QAS group content. For the grafted cellulose fiber with a low QAS group content (Pg = 28%), about 10^9 cells ml/L of bacteria contacted with 0.8 g cellulose fiber declined to about 10^7 cells ml/L in 5 min. But in 5 min, about 10^9 cells

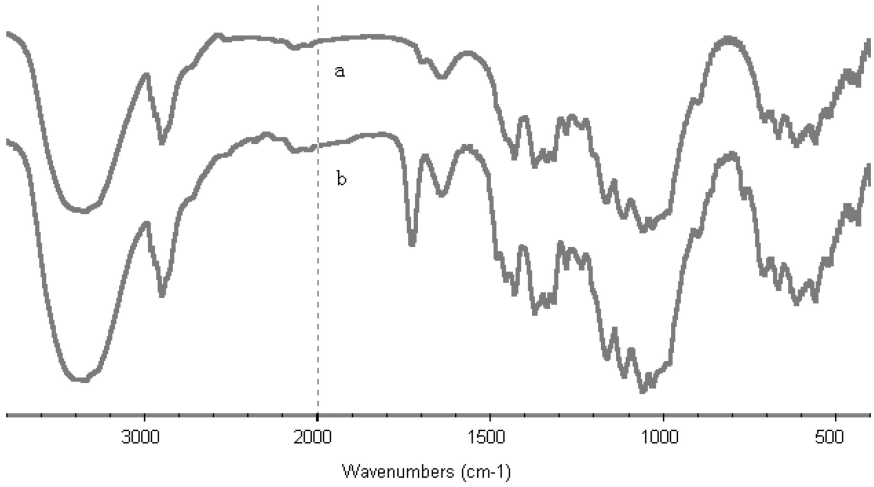


Fig. 7. FTIR spectra of grafted cellulose fiber (a, cellulose fiber; b, Pg = 29.7%)

ml/L of bacteria contacted with the same amount high QAS group content (Pg = 51%) fiber declined to about 10^3 cells ml/L. From the differences in the extent and rate of bacteria removal, it can be concluded that the antibacterial activity of the grafted fiber initiated by Fe^{2+}-H_2O_2 is attributed to the interaction between the bacterial cells and the QAS groups on the cellulose fiber. The removal ability and trend is similar to the grafted fibers initiated by ceric ion.

3.9 Bacteria Removal Performance of Grafted Cellulose Fiber

It is very difficult for intracellular protein, a kind of macromolecular in the cell, to penetrate through the cell membrane. So, the extracellular protein concentration of cell suspension in which bacteria were naturally incubated is about 0.03~0.0 4mg/l, a rather low concentration. At the action of some surfactants, such as QAS, the permeability of bacterial membrane will increase (19), then induce the increase of extracellular protein concentration in suspension.

The plot of extra celluar total protein concentration versus time is shown as Figure 9. It is clear that the protein concentration obviously and instantly increases with adding the grafted fibers. For the grafted cellulose fiber with a low QAS group content (Pg = 21%), the protein concentration instantly rise to about 0.1 mg/ml in 5 min, and remains steady at this concentration. This means the permeability of cells increases obviously. For the grafted

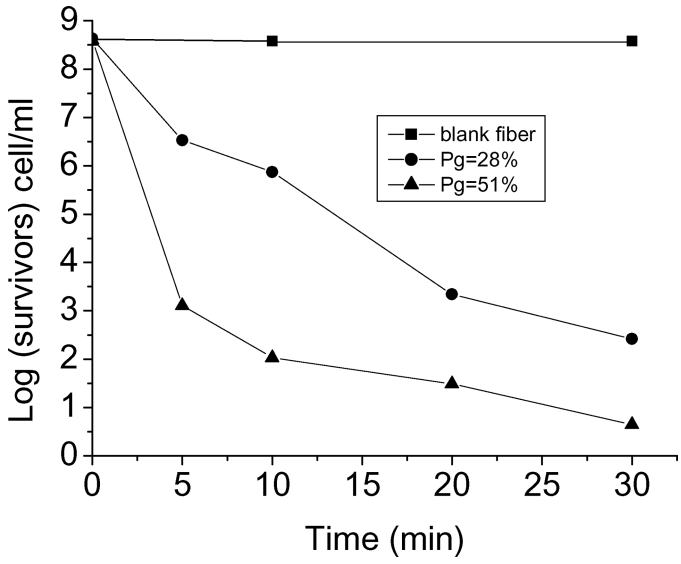


Fig. 8. Adsorption of bacteria as function of the grafted ratio of fiber

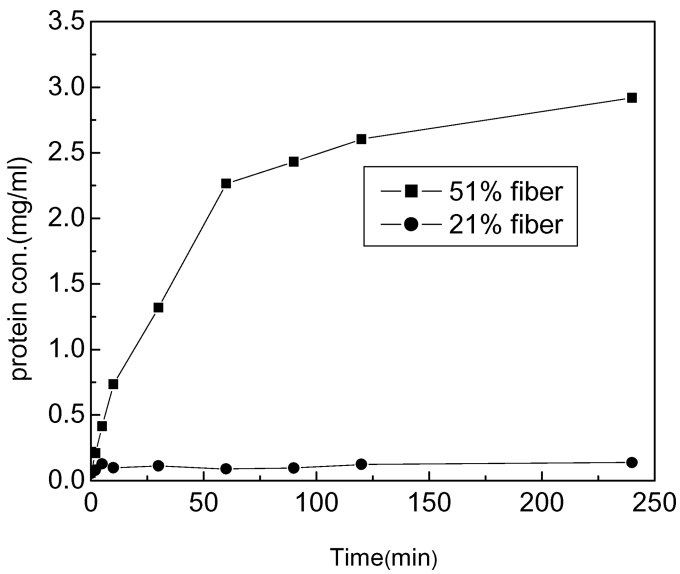
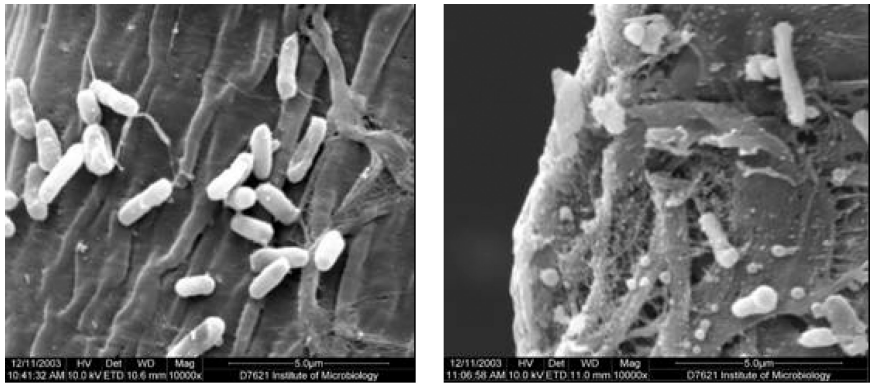


Fig. 9. Change of concentration of extra celluar protein



A ×10000 B ×10000

Fig. 10. SEM photographs of the surface of samples adhered *E. coli* cells

cellulose fiber with a high QAS group content ($Pg = 51\%$), the protein concentration steadily increases to 2.5 mg/ml in about 60 min. This cannot be just explained by a change of cell permeability.

Figure 10 shows the morphological changes of bacterial cells in contact with grafted cellulose fiber (Pg = 51%). Picture (A) and (B) shows the surfaces of the grafted cellulose fiber on which the bacteria were adsorbed for 3 h. The membrane of many cells is disintegrated, and fibrous, and the cellular material accumulates as a result of the leakage and cytolysis of the cells. Some bacteria conglutinate together and some break into fragments. The results obtained by SEM suggest that in addition to the bacterial removal function, the grafted fibers also have the bactericidal function.

4 Conclusions

In this article, quaternary ammonium salt (QAS) monomer, methacryloxylethyl benzyl dimethyl ammonium, was grafted onto the surface of cellulose fiber with $FeSO_4$-H_2O_2 redox system as the initiator. FTIR proved the existence of Cellulose-*g*-QAS. The percent of grafting was influenced by several parameters such as reaction time, temperature, monomer concentration, initiator concentration, and co-initiator concentration. The proposed procedures for the preparation of DMAE-BC grafted on cellulose fiber are simple and efficient. The QAS grafted cellulose fibers prepared by $FeSO_4$-H_2O_2 redox chemical initiation method can rapidly remove *E. coli* cell from water. By directly observing the morphology of the bacterial cells adsorbed on the antibacterial fibers and measuring extracelluar total protein concentration, it was also proved that the antibacterial fibers have bactericidal activity against *E. coli*.

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