Study of Modified Polypropylene Nonwoven Cloth. II. Antibacterial Activity of Modified Polypropylene Nonwoven Cloths

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ABSTRACT: Removal of *E. coli* from water by modified polypropylene (PP) nonwoven cloths which were prepared through radiation-induced grafting of 4-vinyl pyridine (4-VP) onto PP nonwoven cloths and followed by quaterization was carried out by filtration. The results showed that the content and structure of the pyridinium group on PP nonwoven cloths were important factors to affect their antibacterial activity. The antibacterial activity increased with the number of piled sheets of the used nonwoven cloths and decreased with increase of the viable bacterial cell concentrations in the influent and with filtration rates. The activity detection results found that modified PP nonwoven cloths possessed the ability to capture the bacterial cell alive, and no morphological changes of adhered bacterial cells were observed using SEM; thus, the surfaces of the modified PP nonwoven cloths may not be bactericidal, but bacteriostatic.
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Key words: polypropylene nonwoven cloth; pyridinium group; *E. coli,* antibacterial activity; extent of removal

INTRODUCTION

In general, antibacterial agents of low molecular weight are used for sterilizing water, but they have a problem of residual toxicity of the agents, even when suitable amounts of the agents are used.[1] Recently, to prevent such a residual toxicity of the agents, polymeric agents having antibacterial activity have been developed.[2–4] As compared with conventional antibacterial agents of low molecular weight, polymeric antibacterial agents have the advantages that they are nonvolatizable, chemically stable, and hard to permeate through the skin of a man or animal. So, they can significantly reduce losses associated with volatilization, photolytic decomposition, and transportation. Moreover, increased efficiency, selectivity, and handling safety are additional benefits. Polymeric antibacterial agents offer great promise for enhancing the efficacy of some existing antibacterial agents as well as reducing the environmental problems associated with others.[5]

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But some polymeric antibacterial agents such as pyridinium resin are not suitable for some applied requirements, because of their low surface area, high cost, poor workability, and bad mechanical properties. To overcome these drawbacks, some researchers have already attempted to bear antibacterial agents onto polymeric matrixes. Nonaka et al.[6] studied the antibacterial activity of resin-containing triethylenetetramine side chains and/or thiol group–metal complexes. The results showed that the resins, which contain both triethylenetetramine side chains and thiol groups, bearing silver ions exhibited high antibacterial activity against bacteria, especially *Escherichia coli (E. coli),* without the residual silver ions in water after contacting with bacteria. Bucheńska[7] examined graft polymerization of acrylic acid (AA) onto PA6 yarn. The resultant fibers, containing a carboxylic group in their structure, were additionally modified with penicillin, neomycin, or gentamycin to obtain antibacterial fibers in relation to Gram-positive and Gram-negative microorganisms (*S. aureus, E. coli, P. aeruginosa*). The release of antibiotics into solution proceeded for quite a long time, after which there was still enough antibiotic on the fibers to provide them with antibacterial properties. Park et al.[8] used a preirradiation grafting method to graft AA onto polypropylene (PP) fabric. Antibacterial activity on metallic complexes of AA-grafted PP fabric were evaluated by viable cell counting. An Ag-complexed fabric had strong biocidal effect for all bacteria. Kawabata et al.[9] coated nonwoven cloth with a small amount of the polymer by soaking in a dilute organic solution of the pyridinium-type polymer following by drying. The treated cloth was found to be effective in removing microorganisms from water. Since the nonwoven cloth does not show marked resistance to the flow of water, filtration through the nonwoven cloth may have practical application to the treatment of water. Because there is only an affinity between the polymeric matrix and the antibacterial agent, the antibacterial agent is easy to release from the polymeric matrix during handling, so it does not possess endurance and safety antibacterial properties.

In this study, we made a systematic investigation into the antibacterial activity of modified PP nonwoven cloth against *E. coli* suspended in sterilized distilled water and discussed the factors influencing antibacterial activity of this material.

EXPERIMENTAL

Materials

4-Vinyl pyridine (4-VP) was first grafted onto PP nonwoven cloth as described before,[10] and then the grafted samples were quaternized with halohydrocarbon (quaterization agent). Quaterization was achieved by immersing a known weight of the grafted samples in ethanol containing twice the calculated weight (based on the amount of 4-VP in grafted samples) of halohydrocarbon (*RX*). The samples were refluxed at 70–80°C for 6 h, then removed and thoroughly washed with ethanol and water. Finally, the samples were dried and the increase in weight (W_1) due to the quaterization reaction was noted. The pyridinium content, which represents the amount of pyridinium (mmol) per gram of the sample, were calculated according to the increase in weight (W_1). The quaterization extent of the samples could also be calculated as follows:

$$\text{Quaterization extent } (\%) = \frac{W_1}{W_2} \times 100\ (\%) \quad (1)$$

where W_2 is the theoretical weight of the quaterization agent needed for complete quaterization of grafted 4-VP.

The obtained grafted PP nonwoven cloths and the corresponding modified PP nonwoven cloths containing the pyridinium group were designated as PP-*g*-4VP and Q-PP-*g*-4VP, respectively. The halohydrocarbons used in our experiments were benzyl bromide (BzBr), benzyl chloride (BzCl), ethyl bromide (EtBr), butyl bromide (BuBr), and hexadecyl bromide (HdBr). The halohydrocarbon usually used was BzBr unless otherwise mentioned. The results of the composition analysis of the Q-PP-*g*-4VP samples are given in Table I.

Bacteria

E. coli MIG 1.45 was used as the test bacteria for the experiments. The strain was provided from the Microorganism Institute of Guangdong. *E. coli* were incubated at 37°C for 24 h on a nutrient agar plate. Then, the bacterial cell suspensions were prepared as described before,[5] with sterile distilled water used as the suspending medium.

Removal of Bacteria from Water by Filtration Using the Samples of Modified PP Nonwoven Cloths (Q-PP-*g*-4VP)

Removal of bacteria by simple filtration using Q-PP-*g*-4VP samples was carried out using a

Table I Composition Analysis of Modified PP Nonwoven Cloths with Pyridinium Group

Samples (Q-PP-*g*-4VP)	Pg (%) (PP-*g*-4VP)	Quaterization Agents	Quaterization Extent (%)	Pyridinium Content (mmol/g)
A_1	2.3	BzBr	92.8	0.19
A_2	8.6	BzBr	95.7	0.64
A_3	18.8	BzBr	97.6	1.17
A_4	39.2	BzBr	98.1	1.81
A_5	53.7	BzBr	98.8	2.10
A_6	2.3	BzCl	91.7	0.19
A_7	2.3	EtBr	91.3	0.19
A_8	2.3	BuBr	90.2	0.19
A_9	2.3	HdBr	89.0	0.19

glass instrument similar to a condenser. On the bottom of the inner glass column of the instrument is a simple stage, which acts as a supporting nonwoven cloth sample and has no effect on the filtrating process. The diameter of the inner glass column is 34 mm and the length is between 150 and 200 mm. The Q-PP-*g*-4VP sample was placed on the sample stage and the prepared bacterial suspension was added from the inlet of the inner glass column at a given rate so as to make the bacterial suspension pass through the Q-PP-*g*-4VP sample, thus being filtrated. The effluent suspension was collected with the aseptic container at the outlet located under the sample stage. The filtration test was usually carried out at room temperature. The extent of removal was calculated according to the following equation:

$$\text{Removal } (\%) = \frac{N_{(\text{inf.})} - N_{(\text{eff.})}}{N_{(\text{inf.})}} \times 100\ (\%) \quad (2)$$

where $N_{(\text{inf.})}$ and $N_{(\text{eff.})}$ are the viable bacterial cell concentrations in the influent and effluent suspensions, respectively, in the unit of cells/mL.

Scanning Electron Microscopy

The Q-PP-*g*-4VP sample used to remove the *E. coli* cell suspension was collected and repeatedly rinsed with sterilized physiological saline. Subsequently, the sample was fixed chemically with a glutaraldehyde and osmium oxide solution, and then the sample was dehydrated with a graded ethanol series. After dehydration, the sample was immediately soaked in absolute isoamylacetate, dried in a critical point dryer (Model DX-1, EIKO Co), mounted on a sample stand, and coated with gold in an ion fine coat (Model JFC-1100, JEOL). Observation of the sample was done under a scanning electron microscope (Model JSM-T300, JEOL).[5]

RESULTS AND DISCUSSION

Effect of the Content and Structure of Pyridinium Group of Q-PP-*g*-4VP Samples

Removal of *E. coli* from water by simple filtration using Q-PP-*g*-4VP samples in this work was carried out by passing bacteria suspensions through the filtration apparatus, and the filtration was easily accomplished. Figure 1 shows the effect of pyridinium content on the extent of removal when one sheet of the Q-PP-*g*-4VP sample was used. The extent of removal increased with increase in the pyridinium content, but it leveled off when the pyridinium content was higher than 1.81 mmol/g. When the pyridinium content was 0.19 mmol/g, the extent of removal was 95.27%. On the other hand, in the corresponding control experiments using PP nonwoven cloth, the extent of removal was only 72.63%, while in another control experiment using the unquaternized sample of PP-*g*-4VP [percent of grafting (Pg), 2.3%], the extent of removal was 84.42%. The difference in the extent of removal indicates that the strong ability of the Q-PP-*g*-4VP sample to remove bacterial cells from water is attributed to the interaction between the bacterial cells and the pyridinium groups on the PP nonwoven cloth acting as functional groups. Because the pyridinium group is a group of quaternary ammonium salts possessing positive charges and because the bacterial cells usually have negative charges,[11] there is a strong electrostatic interaction between pyri-

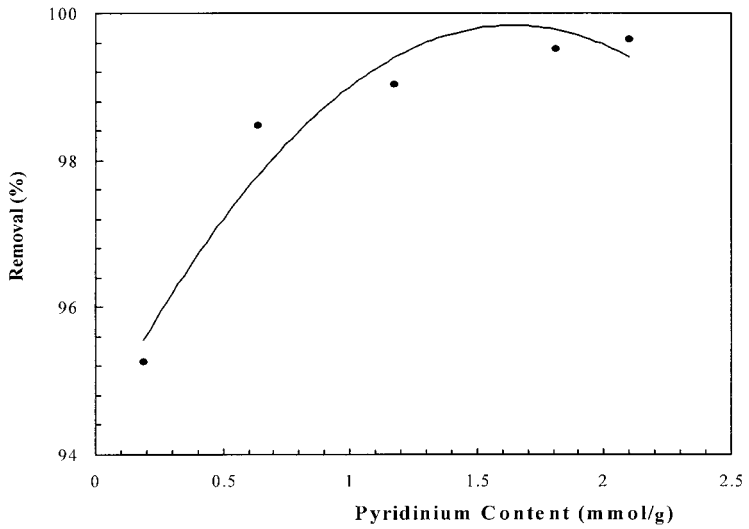

Figure 1 Effect of the pyridinium content on the extent of removal for *E. coli:* $N_{(inf.)}$, 7.55×10^6 cells/mL; filtration rate, 7.5 mL/min; collected effluent bacteria volume, 20 mL; one sheet of sample.

dinium groups and bacterial cells. Obviously, the higher the pyridinium group content in the Q-PP-*g*-4VP sample, the stronger the electrostatic interaction that can occur between the bacterial cells and the sample; thus, the sample can remove bacteria more efficiently.

The antibacterial activity was also different with the change of the pyridinium-group structure. The influence of different halohydrocarbons (*RX*) with the same Pg on the extent of removal is shown in Table II. When *R* was Bz with different *X*, it was found that the $N_{(eff.)}$ of A_1(BzBr) was lower than that of A_6(BzCl), or the extent of removal of A_1(BzBr) was higher than that of A_6(BzCl). There were hydrated layers in the surface of the Q-PP-*g*-4VP samples due to the solvent water action. Because the hydrated capacity of Cl^- was higher than that of Br^-, the hydrated layer thickness of Cl^- was greater.[12] The thinner the hydrated layer thickness, the nearer the gap of the Q-PP-*g*-4VP samples and the bacterial cells was; thus, the electrostatic interaction between them was stronger. However, when the used quaterization agent *RX* has the same *X* and a different *R* group, the extent of removal decreased in the following order: A_1(BzBr) > A_9(HdBr) > A_8(BuBr) > A_7(EtBr). A_1(BzBr) possesses the strongest antibacterial activity, which might attribute to the peculiar structure. As *R* was a linear alkyl, the longer the carbochain, the better antibacterial activity it shows. It has been reported that the target site of antibacterial agents is the cytoplasmic membrane of the bacteria. The phospholipids in the cytoplasmic membranes of bacteria are phosphoglycerides that have both a hydrophilic end (phosphate, often with other po-

Table II Effect of Different Quaterization Agents on Extent of Removal for *E. coli*

Samples	Quaterization Agents	Viable Cell Concentrations (cells/mL)		
		Influent	Effluent	Removal (%)
A_1	BzBr	7.55×10^6	3.57×10^5	95.27
A_6	BzCl	7.55×10^6	4.82×10^5	93.62
A_7	EtBr	6.93×10^6	7.53×10^5	89.13
A_8	BuBr	6.93×10^6	6.33×10^5	90.86
A_9	HdBr	6.93×10^6	5.86×10^5	91.54

[a] Filtration rate, 7.5 mL/min; one sheet of Q-PP-*g*-4VP sample; collected effluent bacteria volume, 20 mL.

Table III Effect of the No. Plied Sheets of QPP-*g*-4VP Samples on the Viable Bacterial Cell Concentration in the Effluent [$N_{(eff.)}$]

	$N_{(eff.)}$ (cells/mL), at the Following No. Piled Sheets				
Samples	1	2	5	7	10
PP nonwoven cloth	1.52×10^6	1.37×10^6	1.04×10^6	9.96×10^5	9.05×10^5
A_1	2.57×10^5	5.05×10^4	2.81×10^3	4.23×10^2	28
A_5	2.02×10^4	5.03×10^3	6	0	0

[a] $N_{(inf.)}$, 5.61×10^6 cells/mL; filtration rate, 7.5 mL/min; collected effluent bacterial volume, 20 mL.

lar residues attached to it) and a hydrophobic end (two long-chain fatty acid tails possessing a carbon number of 12–20). The antibacterial agents possessing a long alkyl chain are assumed to strongly interact with the cytoplasmic membranes owing to an affinity of the molecular structure, leading to the higher activity.[13] Therefore, the hydrophobic end of antibacterial agents must possess a suitable carbochain length to make the molecular attack a lipid phase.

Effect of the Number of Piled Sheets

The antibacterial activity increased with the number of piled sheets of the used Q-PP-*g*-4VP samples, as shown in Table III. When one sheet of the sample was used under the same conditions, as for the A_1 sample with a low pyridinium content, the $N_{(eff.)}$ was 2.57×10^5 cells/mL, and for the A_5 sample with a high pyridinium content, the $N_{(eff.)}$ was 2.02×10^4 cells/mL. But the $N_{(eff.)}$ were only 2.81×10^3 cells/mL and 6 cells/mL when five sheets of the A_1 and A_5 samples were used and decreased to 28 and 0 cells/mL using 10 sheets of the samples. Effective removal of bacteria from water appeared to be easily accomplished by increasing the number of piled sheets of the Q-PP-*g*-4VP samples. In the control experiments that used PP nonwoven cloth to remove bacteria from the water, an increase in the number of piled sheets was not very effective for the removal of bacteria, and the $N_{(eff.)}$ was 9.05×10^5 cells/mL even when 10 sheets of samples were used.

Effect of the Viable Bacterial Cell Concentrations in the Influent Suspension [$N_{(inf.)}$]

Figure 2 describes the extent of removal versus time curves for filtrating bacteria at various $N_{(inf.)}$ when one sheet of A_1 was used. The extent of removal increased with decrease in the $N_{(inf.)}$ at the same filtration time. As to the given $N_{(inf.)}$, the extent of removal decreased with the duration of filtration time. Because the bacterial cells' adsorption on or adhesion to the surface of A_1 increase more and more with duration of filtration time, the ability to adsorb or adhere bacterial cells becomes worse and worse, resulting in losing efficacy in the end. At the same filtration time, the higher the $N_{(inf.)}$, the more contact chances between A_1 and the bacterial cells, and the more bacterial cells can be adsorbed on or adhered to the surface of A_1. Therefore, the effective surface of A_1 for adsorbing or adhering bacterial cells becomes less as the filtration proceeds. The higher the $N_{(inf.)}$, the more quickly the effective surface decreases, so the extent of removal is lower.

Effect of Filtration Rate

Figure 3 shows the effect of the filtration rate on the extent of removal when one sheet of the sample was used. The extent of removal for A_1 decreased with increase in the filtration rate, but it remained level at 89.56% even at a high filtration rate of 33.3 mL/min, while the extent of removal for PP nonwoven cloth was only 75.16% even at a slow filtration rate of 2.1 mL/min. At a high filtration rate of 33.3 mL/min, the extent of removal was only 36.55%.

When the bacterial suspensions pass through the samples, the bacteria may be cutoff due to surface adsorption or adhesion of the samples. There are more bacterial cells to adsorb on or adhere to their surface due to a longer contact time at a lower filtration rate, so the extent of removal is higher. Along with increasing the filtration rate, it is unfavorable for samples to adsorb or adhere bacterial cells at a shorter contact time; therefore, the extent of removal is lower. Because there is a strong adherence between A_1

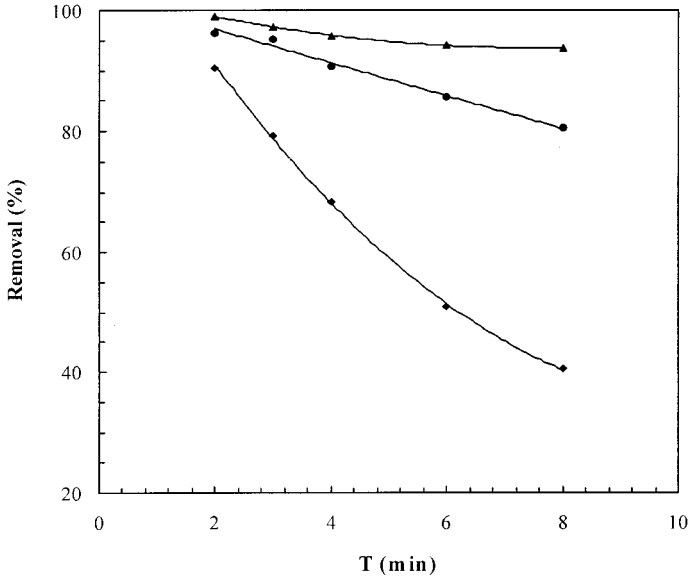

Figure 2 Effect of viable bacterial cell concentrations in the influent suspension [$N_{(inf.)}$] on the extent of removal by sample A_1. $N_{(inf.)}$: (◆) 6.32×10^7 cells/mL; (●) 5.93×10^6 cells/mL; (▲) 3.78×10^5 cells/mL; filtration rate, 7.5 mL/min; one sheet of A_1.

and the bacterial cells attributed to electrostatic interaction, the extent of removal is much higher than that of PP nonwoven cloth.

Activity Detection of the Adhered Bacterial Cells on Q-PP-*g*-4VP Samples

Actually, the antibacterial activity of the Q-PP-*g*-4VP samples can also be called surface antibacterial activity.[14] To examine whether the surface antibacterial activity of the samples is bactericidal activity or bacteriostatic activity, the experiment of activity detection was carried out.

The sample, which had been used in the filtration test, was washed repeatedly with sterilized physiological saline. Then, the moisture on the sample surface was sucked up with sterilized filter paper. Finally, the thus-treated sample was

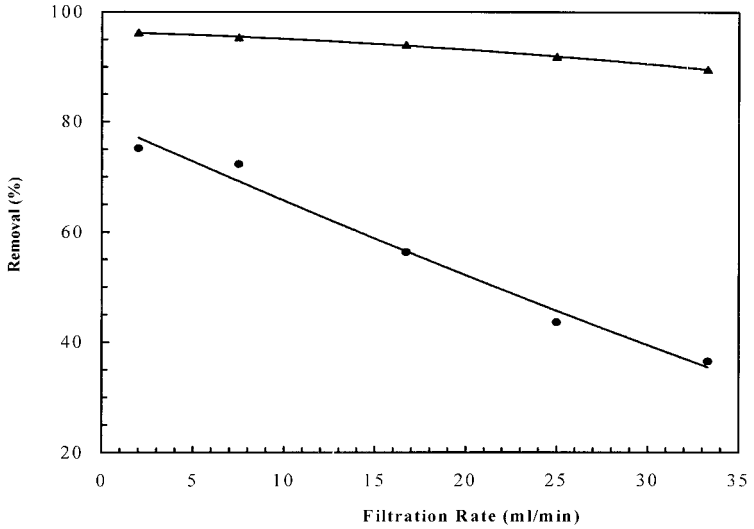

Figure 3 Effect of filtration rate on extent of removal for *E. coli:* (●) A_1; (▲) PP nonwoven cloth; $N_{(inf.)}$, 8.15×10^6 cells/mL; collected effluent bacteria volume, 20 mL; one sheet of sample.

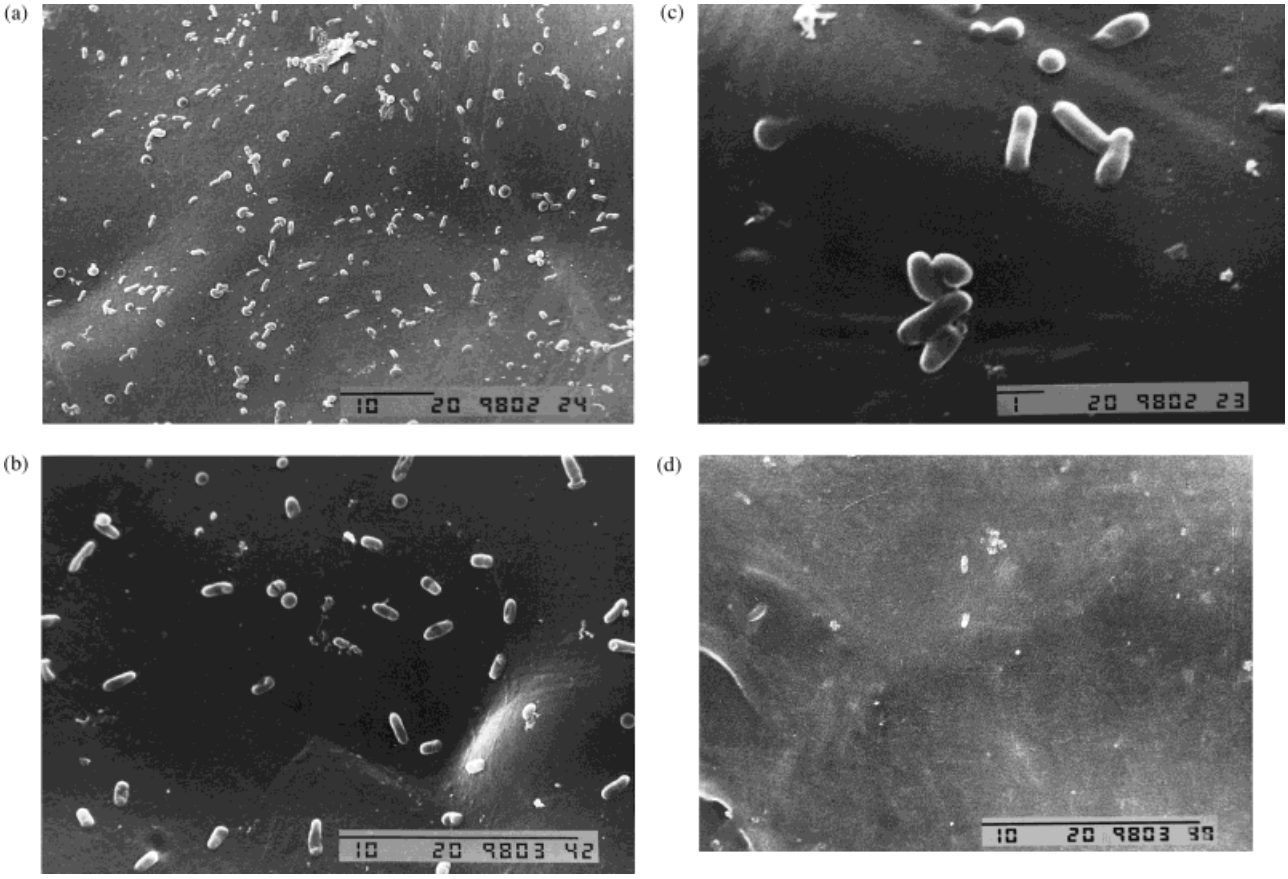

Figure 4 SEM photographs of the surface of samples adhered *E. coli* cells: (a) A_1 (×2000); (b) A_1 (×5000); (c) A_1 (×10,000); (d) PP nonwoven cloth (×5000).

placed into a nutrient broth. The turbidity of the nutrient broth was observed on being cultured at 37°C for 18 h. If it becomes turbid, the multiplication of *E. coli* is proved to occur in it. For the sake of prudence, the cultured broth was inoculated on nutrient agar plates and the inoculated plates were cultured at 37°C for 12–18 h. As a result, *E. coli* colonies appeared on the incubated plates, indicating that the Q-PP-*g*-4VP samples have the ability to capture bacterial cells alive; thus, the surface of the samples may not be bactericidal, but bacteriostatic. Because the free pyridinium polymers can pass through cell walls to cytoplasmic membranes of bacteria, they show bactericidal activity. However, when the pyridinium polymers are covalently attached to the surfaces with short spacers, it is unlikely that they penetrate through the cell walls of bacterial cells. Therefore, their surface antibacterial activity was bacteriostatic.

Morphologies of Bacterial Cells Adhered onto the Samples

E. coli cells adhered onto A_1 or PP nonwoven cloth were observed using SEM. Figure 4(a–c) shows the SEM of *E. coli* cells adhered onto A_1 with the amplification of 2000, 5000, and 10,000, respectively. It can be seen in Figure 4(a) that there were many *E. coli* cells adhered to the surface of A_1. With the amplification increasing, the morphologies of adhered *E. coli* cells that display a shaft shape could be clearly seen, as shown in Figure 4(b,c). It was also found that the surface antibacterial activity of A_1 may be bacteriostatic, as evidenced by no morphological changes of the bacterial cells adhered on the surface of A_1. Figure 4(d) shows the SEM of *E. coli* cells adhered to the surface of PP nonwoven cloth with an amplification of 5000. It was observed that there were only a few *E. coli* cells adhered to its surface.

CONCLUSIONS

Removal of *E. coli* from distilled water using modified PP nonwoven cloth containing the pyridinium group (Q-PP-*g*-4VP) was carried out by filtration, and the following conclusions have been made from the results obtained in this work:

1. The content and structure of the pyridinium group in Q-PP-*g*-4VP were important factors to affect their antibacterial activity. The extent of removal increased with increasing pyridinium group content. With the same grafted sample, when the used halohydrocarbon or quaterization agent (*RX*) was different, the extent of removal was also different. It was found that the extent of removal of the sample using the quaterization agent BzBr was the highest.
2. The antibacterial activity increased with increasing the number of piled sheets of the used samples. Effective removal of bacteria from water appears to be easily accomplished by increasing the number of piled sheets of the Q-PP-*g*-4VP samples. In the control experiments that used PP nonwoven cloth to remove bacteria from the water, an increase in the number of piled sheets was not very effective.
3. The extent of removal for bacteria decreased with increase of the viable bacterial cell concentrations in the influent suspension [$N_{(\text{inf.})}$] and with filtration rate.
4. The activity detection result found that the modified PP nonwoven cloths possessed the ability to capture bacterial cells alive, and no morphological changes of the adhered bacterial cells were observed by SEM. Thus, the surfaces of the modified PP nonwoven cloths may not be bactericidal, but bacteriostatic.

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REFERENCES

1. Kawabata, N. Prog Polym Sci 1992, 17, 1.
2. Kawabata, N.; Takagishi, K.; Mishiguchi, M. React Polym 1989, 10, 269.
3. Tashiro, T. J Appl Polym Sci 1991, 43, 1369.
4. Kawabata, N.; Teramoto, K.; Ueda, T. J Microbiol Methods 1992, 15, 101.
5. Li, G.; Shen, J.; Zhu, Y. J Appl Polym Sci 1998, 67, 1761.
6. Nonaka, T.; Uemura, Y.; Enishi, K.; Kurihara, S. J Appl Polym Sci 1996, 62, 1651.
7. Bucheńska, J. J Appl Polym Sci 1996, 61, 567.
8. Park, J. S.; Kim, J. H.; Nho, Y. C.; Kwon, O. H. J Appl Polym Sci 1998, 69, 2213.
9. Kawabata, N.; Inoue, T.; Tomita, H. Epidemiol Infect 1992, 108, 123.
10. Tan, S. Doctoral Thesis, South China University of Technology, China, 1998; p 20.
11. Kawabata, N.; Ueno, Y.; Torii, K.; Matsumoto, T. Agric Biol Chem 1987, 51, 1085.
12. Li, G. Doctoral Thesis, South China University of Technology, China, 1995; p 101.
13. Kanazawa, A.; Ikeda, T.; Endo, T. J Appl Polym Sci 1994, 53, 1237.
14. Kanazawa, A.; Ikeda, T.; Endo, T. J Appl Polym Sci 1994, 54, 17.