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Removal of micro-organisms by filtration through unwoven cloth coated with a pyridinium-type polymer

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SUMMARY

Unwoven cloth coated with 32 mg/g of a copolymer of *N*-benzyl-4-vinylpyridinium chloride and styrene was found to be effective in removing micro-organisms from water. In experiments demonstrating removal of *Escherichia coli* by filtration through ten sheets of the unwoven cloth, the rate of removal was 99·99% at a filtration rate of 2·6 cm/h, and remained at 99% even at a high filtration rate of 300 cm/h and a low influent concentration of the bacterial cells such as 10^3 cells/ml. The rate of removal tended to increase with a decrease in the influent bacterial concentration. Seven other bacteria and two yeasts were effectively removed by filtration through the unwoven cloth. Filtration through the unwoven cloth was also effective in removing spores of fungi from water but was not very effective in removing bacteriophage T4 from aqueous solution.

INTRODUCTION

Water disinfection processes involve the removal and destruction of micro-organisms by both physical and chemical means. The most common process involves treatment with chlorine and other related chemicals, but the formation of trihalomethanes and other toxic organohalogen compounds is a serious defect in this procedure [1–6], since these compounds are suspected of being carcinogenic [7]. These compounds generally do not easily undergo biological degradation, and can therefore become concentrated in the food chain in the environment. Methods suggested for removing trihalomethanes from water include adsorption on powdered or granulated carbon [6], aeration, and coagulation of precursors prior to chlorination [8–10]. However, the rate of removal of the organohalogen compounds by adsorption is not always satisfactory, and the presence of the remaining coagulants such as polyethylenimine in the treated drinking water is not desirable, because they may be toxic.

During the course of studies to develop an alternative method of disinfection using insoluble polymeric materials, cross-linked poly(vinylpyridinium halide) was found to have a novel and remarkable ability to remove bacteria from water [11]. When suspensions of bacteria (10^5–10^8 cells/ml) were passed through a column packed with the insoluble pyridinium-type resin at a flow rate of 0·8–1·4

bed volumes per hour, 97–100% of the viable cells were eliminated from the suspensions during the treatment. Mechanistic studies revealed that the insoluble pyridinium-type resin irreversibly captured bacteria on the surface. The resin strongly removed bacteriophage T4 [12] as well as pathogenic human viruses [13] from aqueous solution. The insoluble pyridinium-type resin developed in our laboratory is expected to be useful for the removal of micro-organisms from water, avoiding the production of organic pollutants such as organohalogen compounds.

However, regeneration of the resin that has been used for removing micro-organisms from water is extremely difficult, because the capturing interaction between the resin surface and microbial cells is very strong. Thus, the resin is obviously economically unsuitable, at least at the present stage, for practical water treatment. In the present study therefore we attempted to use unwoven cloth coated with a small amount of pyridinium-type polymer instead of the insoluble pyridinium-type resin. A co-polymer of *N*-benzyl-4-vinylpyridinium chloride and styrene (designated as soluble BVPS in this report) was used as a soluble pyridinium-type polymer. Soluble BVPS is a linear polymer, not a cross-linked polymer, and is soluble in organic solvents. However, the polymer contains 50 mol% of styrene as a hydrophobic component, and is insoluble in water; the solubility of soluble BVPS in water is therefore negligible under our experimental conditions. Unwoven cloth was coated with a small amount of soluble BVPS by soaking in a dilute organic solution of the polymer, and then dried. This cloth was found to be effective in removing micro-organisms from water. Since the unwoven cloth does not show marked resistance to the flow of water, filtration through the unwoven cloth may have practical application to the treatment of water.

MATERIALS AND METHODS

Unwoven cloth coated with soluble pyridinium-type polymer

4-Vinylpyridine and styrene were purified as reported previously [14]. The co-polymer of 4-vinylpyridine with styrene was prepared by co-polymerization using azobisisobutyronitrile (AIBN) as an initiator. The resulting co-polymer was converted to soluble BVPS by reaction with an equimolar amount of benzyl chloride.

Polymerizations were carried out in a 5 l, round-bottomed, three-necked flask equipped with a mechanical stirrer, a reflux condenser and a gas inlet. A mixture of 4-vinylpyridine (315 g, 3·0 mol), styrene (312 g, 3·0 mol) and AIBN (4·4 g, 26·8 mmol) was added to 1·5 l of ethanol under a nitrogen atmosphere and with stirring at 80 °C. After 6 h the reaction mixture was allowed to cool to room temperature. Benzyl chloride (342 g, 2·7 mol) was added to the mixture and allowed to react at 80 °C for 5 h under a nitrogen atmosphere. Soluble BVPS thus prepared was isolated by pouring the contents of the flask into ethyl acetate, and the precipitate was dried *in vacuo* to constant weight. Soluble BVPS thus obtained contained 2·94 mmol/g of the pyridinium group, measured according to a conventional procedure used in our previous work [15]. The intrinsic viscosity of soluble BVPS was 0·23 dl/g, as determined in ethanol containing 10 g/l of $MgCl_2 \cdot 6H_2O$ at 30 °C.

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Unwoven cloth WE-8890, made of pure 1·5-denier rayon and provided by the Japan Vilene Co. Ltd, Tokyo, Japan, was used in these experiments. The cloth was 0·5 mm thick and the surface area was 2020 cm^2/g as reported by the supplier. This unwoven cloth was soaked in a 0·5 wt% solution of soluble BVPS in 60/40 (v/v) methanol-acetone mixture at room temperature overnight, and was dried at room temperature in a hood. The amount of soluble BVPS coated on the surface of the unwoven cloth was 32 mg/g, based on the increase of weight during the coating procedure. The coated polymer layer was estimated to be about 0·16 μm thick.

As mentioned, bacteriophage T4 was not satisfactorily removed from water by filtration through the unwoven cloth, especially at a high filtration rate. In this case, therefore, the amount of soluble BVPS coated on the surface of unwoven cloth was increased up to 222 mg/g by repeating the soaking procedure several times.

Bacteria and yeasts

Escherichia coli B ICR B00120 (Institute for Chemical Research, Kyoto University, Collection, Kyoto, Japan), *Pseudomonas aeruginosa* IFO 3080, *Bacillus subtilis* IFO 3037, *Staphylococcus aureus* IFO 3060, *Serratia marcescens* IFO 3046, *Alcaligenes faecalis* IAM B141-1 (Institute for Applied Microbiology, University of Tokyo, Collection, Tokyo, Japan), *Achromobacter polymorph* ICR B00880, and *Arthrobacter simplex* ATCC 6946 were used as test bacteria. *Saccharomyces cerevisiae* IFO 1662 and *Candida utilis* IFO 0619 were used as test yeasts. *E. coli*, *P. aeruginosa*, *B. subtilis*, and *Staph. aureus* were incubated for 24 h at 37 °C. *Serr. marcescens* and *Sac. cerevisiae* were incubated for 24 h at 30 °C. *Alc. faecalis*, *Ach. polymorph* and *Arth. simplex* were incubated for 48 h at 30 °C. *C. utilis* was incubated for 24 h at 25 °C. The nutrient broth used for the culture of bacteria was prepared by dissolving 3·0 g of meat extract and 5·0 g of peptone into 1000 ml of water at pH 7·2. The medium used for the culture of yeasts was prepared by dissolving 20·0 g of glucose and 2·0 g of yeast extract into 1000 ml of water at pH 6·0. Cells were harvested by centrifugation at 3000 rev./min for 10 min at room temperature, and washed repeatedly with sterile physiological saline solution. Physiological saline was used to avoid autolysis of micro-organisms during the treatment. The salinity does not affect the adhesion process [11].

Fungi

Mucor racemosus f. *racemosus* IFO 4581 was used as a test fungus. *M. racemosus* was cultured by incubation at 22 °C for 10 days in a 300 ml Erlenmeyer flask containing 50 ml of growth medium. The medium was prepared by dissolving 6·25 g of maltose, 6·25 g of malt extract, 1·25 g of KH_2PO_4, 1·0 g of yeast extract, 0·625 g of $MgSO_4 \cdot 7H_2O$, 0·625 g of peptone and 20·0 g of agar in 1000 ml of water at pH 6·0. After incubation, 80 ml of sterilized physiological saline solution was added to the Erlenmeyer flask and shaken for 3 h. The mycelium was eliminated from the supernatant suspension by filtration using four sheets of gauze. Spores were harvested by centrifugation of the filtrate at 3000 rev./min at room temperature for 5 min, and were washed with sterilized physiological saline solution.

Bacteriophage

Bacteriophage T4 IFO 20004 was used as a test virus for this work. The phage was propagated in *E. coli* strain B in a medium prepared by dissolving 10·0 g of peptone, 3·0 g of meat extract, 5·0 g of yeast extract, 2·5 g of NaCl, and 8·0 g of KH_2PO_4 into 1000 ml of water at pH 7·2. The phage was inoculated into *E. coli* culture at the logarithmic growth phase, and the culture maintained at 37 °C overnight. After multiplication of the phage, the suspension was centrifuged at 3000 rev./min at room temperature for 10 min. The supernatant was used as the T4 phage suspension, after filtration through a membrane made of cellulose acetate having a pore size of 0·45 μm. In a previous report from this laboratory [12], bacteriophage T4 in such supernatant was shown to be effectively removed by adsorption on the resin surface.

Removal of micro-organisms by filtration through unwoven cloth coated with soluble BVPS

All procedures were performed under aseptic conditions. The filtration experiments were carried out using a glass column, 25 by 30 mm, with two silicone-rubber stoppers connected by a glass inlet to a suspension of organisms. Sheets of the unwoven cloth coated with soluble BVPS were placed in layers in the column unless otherwise stated; 10, 20 and 40 for the filtration of bacteria and yeasts, spores of fungus, and bacteriophage T4, respectively. Prior to the filtration experiments, the unwoven cloth was washed with sterilized water at a flow rate of 2·6 cm/h for 2 h, in order to remove residual organic materials. The amount of total organic carbon contained in the effluent solution became negligible after the 2 h washing. After washing, suspensions of micro-organisms were passed through the filtration apparatus at room temperature using a peristaltic pump. Aliquots of the effluent suspension were tested for the concentration of variable micro-organisms. The rate of removal of viable micro-organisms was based on the difference between viable cell concentrations of influent and effluent suspensions.

Concentration of viable micro-organisms

For bacteria and yeasts, 0·5 ml portions of the influent or effluent suspensions were quickly mixed with 4·5 ml of sterile physiological saline, and decimal serial dilutions prepared from this in saline. Surviving bacteria or yeasts were counted on nutrient media by the spread-plate method. The medium used for the culture of *E. coli* was deoxycholate hydrogen sulphide lactose (DHL) agar (Nissui). The medium used for the culture of other bacteria was prepared by dissolving 3·0 g of yeast extract, 10·0 g of peptone, 10·0 g of NaCl, and 15·0 g of agar into 1000 ml of water at pH 7·0. The medium used for the culture of yeasts was prepared by dissolving 10·0 g of yeast extract, 10·0 g of malt extract, and 15·0 g of agar into 1000 ml of water at pH 6·0. After inoculation, plates with bacteria were incubated at 37 °C and those with yeasts at 30 °C. Colonies of yeasts and *E. coli* were counted after 42 h and colonies of other bacteria after 24 h. The counting was done in quintuplicate every time.

Concentrations of spores of *M. racemosus* were evaluated as follows. A 0·5 ml portion of influent or effluent suspensions was mixed with 4·5 ml of sterilized physiological saline solution, and then decimal serial dilutions prepared in

physiological saline solution. A 1·0 ml portion of these dilutions was mixed with 10·0 ml of a culture medium. The medium was prepared by dissolving 6·25 g of maltose, 6·25 g of malt extract, 1·25 g of KH_2PO_4, 1·0 g of yeast extract, 0·625 g of $MgSO_4 \cdot 7H_2O$, 0·625 g of peptone and 10·0 g of agar into 1000 ml of water at pH 6·0, and was kept at 45–50 °C. The mixture was solidified by cooling to room temperature, and incubated at 22 °C for 42 h. The colonies were counted in quintuplicate.

Bacteriophage T4 was assayed using plates of peptone soft agar. This assay procedure was done in quintuplicate every time.

RESULTS

Removal of Escherichia coli *from water*

Fig. 1 shows the influence of the filtration rate on the rate of removal of *E. coli*. The rate of removal decreased with an increase in filtration rate, but remained level at 99% even at the high filtration rate of 300 cm/h. On the other hand, in the corresponding control experiments using ten sheets of untreated cloth, the rate of removal of *E. coli* was 82% even at the slow filtration rate of 2·6 cm/h. At the high filtration rate of 300 cm/h the rate of removal was only 29%. This difference in the rate of removal between filtration through the coated unwoven cloth and that through the uncoated unwoven cloth can be attributed to the ability of soluble BVPS to capture bacterial cells.

Fig. 2 shows the influence of the influent concentration of *E. coli* on the rate of removal and on the effluent concentration. The rate of removal remained higher than 99% even at a low influent concentration of bacteria such as 10^3 cells/ml, and tended to increase with a decrease in the influent bacterial concentration. Thus, filtration through the unwoven cloth coated with soluble BVPS seems to be effective at low bacterial concentrations.

Fig. 3 shows the effect of the number of piled sheets of unwoven cloth on the rate of removal. The rate of removal increased with the number of piled sheets. The removal ratio was 94% even when 5 sheets of the unwoven cloth were used, and reached 99·93% when 15 sheets were used. More effective removal of micro-organisms from drinking water supply appears to be easily accomplished by increasing the number of piled sheets of the unwoven cloth. In the control experiments that involved untreated cloth, increasing the number of piled sheets was not very effective for the removal of *E. coli*, and the rate of removal was 66% even when 15 sheets of the unwoven cloth were used.

In order to evaluate the capacity of the unwoven cloth for removing microbial cells on the surface, the *E. coli* suspension was filtered through one sheet of the unwoven cloth coated with soluble BVPS. The breakthrough curve is shown in Fig. 4. The total capacity to capture *E. coli* cells was approximately evaluated as $2{\cdot}3 \times 10^5$ cells/cm^2 for the unwoven cloth coated with soluble BVPS.

In practical application, however, the breakthrough capacity (i.e. the total number of cells removed before microbial cells become detectable in the filtrate) would be more important than the total removing capacity shown in Fig. 4. Therefore, another filtration experiment was performed using ten sheets of treated cloth and a suspension containing $2{\cdot}03 \times 10^3$ cells/ml of *E. coli*, and the breakthrough capacity measured. Fig. 5 shows the breakthrough curve. The

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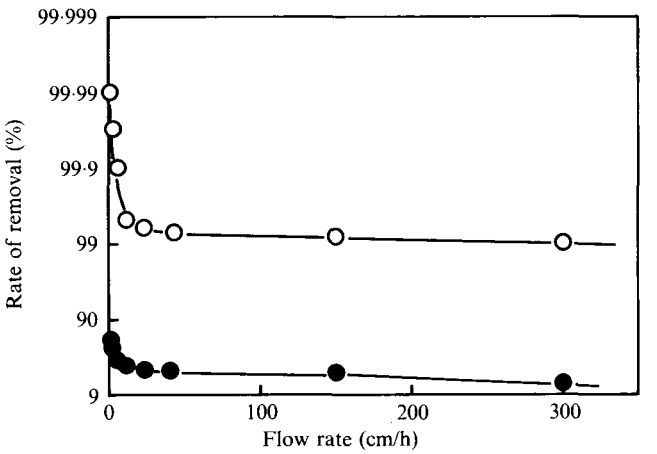

Fig. 1. Influence of the filtration rate on the rate of removal for *E. coli*. ○, Filtration through 10 sheets of unwoven cloth coated with 32 mg/g of soluble BVPS; ●, control experiment carried out using 10 sheets of unwoven cloth that was not coated with the pyridinium-type polymer; influent concentration, $8{\cdot}34 \times 10^6$ cells/ml.

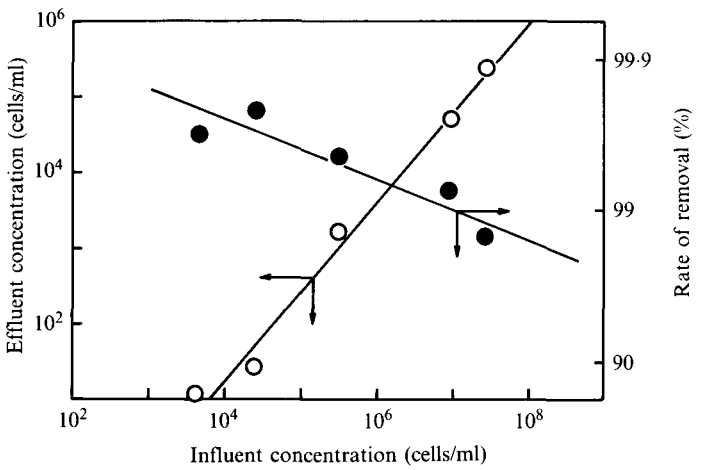

Fig. 2. Influence of the influent concentration of *E. coli* on the rate of removal (●), and on the effluent concentration (○), in the filtration through 10 sheets of unwoven cloth coated with 32 mg/g of soluble BVPS at a filtration rate of 34·2 cm/h.

breakthrough capacity was estimated to be $5{\cdot}4 \times 10^2$ cells/cm^2 for the treated cloth.

Removal of other bacteria and yeasts from water

Experiments on the removal of several other bacteria and yeasts by filtration through ten sheets of unwoven cloth coated with 32 mg/g of soluble BVPS were performed in a similar manner at a filtration rate of 2·6 cm/h unless otherwise stated. The results are summarized in Table 1. All of the viable cells were effectively removed and the rate of removal was more than 99·4%. In the corresponding control experiments using untreated cloth, the rates of removal of

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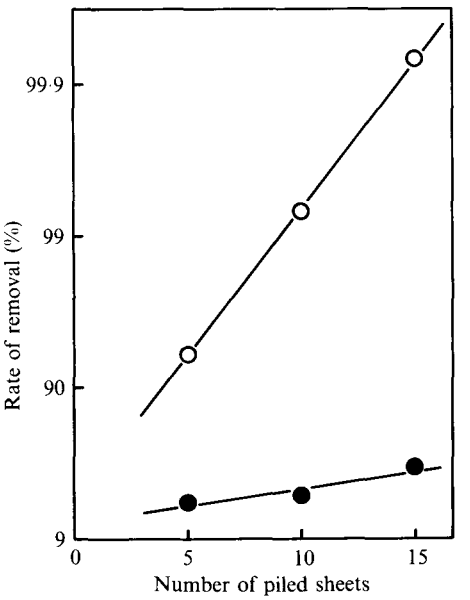

Fig. 3. Effect of the number of piled sheets of unwoven cloth on the rate of removal for *E. coli*. ○, Filtration through piled sheets of unwoven cloth coated with 32 mg/g of soluble BVPS; ●, control experiment carried out using piled sheets of unwoven cloth that was not coated with the pyridinium-type polymer; influent concentration, $6{\cdot}07 \times 10^6$ cells/ml; filtration rate, 34·2 cm/h.

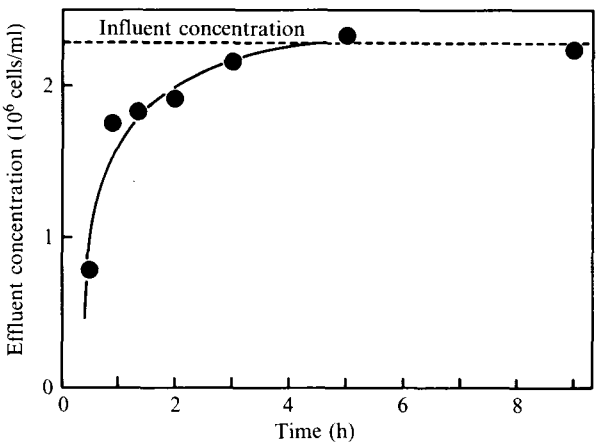

Fig. 4. A breakthrough curve for the removal of *E. coli* from water by filtration through 1 sheet of the unwoven cloth coated with 32 mg/g of soluble BVPS. Influent concentration, $2{\cdot}28 \times 10^6$ cells/ml; filtration rate, 2·6 cm/h.

these micro-organisms from water were considerably lower than those of the corresponding filtrations through the BVPS-coated cloth. Ease of removal largely depended on the type of micro-organism. *Arth. simplex* and *Staph. aureus* were most effectively removed from water, whereas *P. aeruginosa* was removed to a much less extent. The ease of removal was related to the order of affinity of bacterial cells with insoluble pyridinium-type resin [16].

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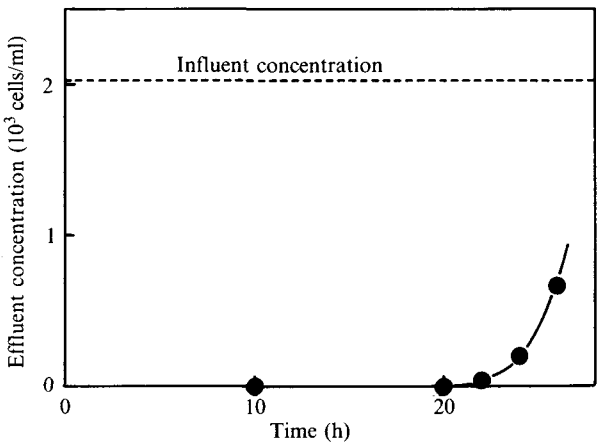

Fig. 5. A breakthrough curve for the removal of *E. coli* by filtration through 10 sheets of the unwoven cloth coated with 32 mg/g of soluble BVPS. Influent concentration, $2{\cdot}03 \times 10^3$ cells/ml; filtration rate, 2·6 cm/h.

Table 1. *Removal of various bacteria and yeasts from water by filtration through unwoven cloth coated with soluble BVPS**

Microorganism	Concentration (cells/ml) Influent	Effluent	Removal (%)	
Bacteria				
E. coli	$8{\cdot}34 \times 10^6$	$8{\cdot}97 \times 10^2$ ($1{\cdot}50 \times 10^6$)	99·99	(82)
E. coli†	$8{\cdot}34 \times 10^6$	$5{\cdot}84 \times 10^4$ ($4{\cdot}26 \times 10^6$)	99·3	(49)
P. aeruginosa	$1{\cdot}06 \times 10^7$	$4{\cdot}84 \times 10^4$ ($1{\cdot}92 \times 10^5$)	99·5	(98)
B. subtilis	$2{\cdot}20 \times 10^6$	$1{\cdot}94 \times 10^2$ ($2{\cdot}61 \times 10^4$)	99·991	(99)
Staph. aureus	$8{\cdot}60 \times 10^7$	$2{\cdot}40 \times 10^1$ ($2{\cdot}23 \times 10^6$)	99·99997	(97)
Staph. aureus†	$8{\cdot}60 \times 10^7$	$6{\cdot}13 \times 10^3$ ($1{\cdot}38 \times 10^7$)	99·993	(84)
Serr. marcescens	$1{\cdot}11 \times 10^7$	$6{\cdot}80 \times 10^4$ ($7{\cdot}16 \times 10^5$)	99·94	(94)
Alc. faecalis	$1{\cdot}76 \times 10^7$	$9{\cdot}22 \times 10^3$ ($5{\cdot}88 \times 10^6$)	99·95	(67)
Ach. polymorph	$7{\cdot}82 \times 10^7$	$1{\cdot}04 \times 10^3$ ($1{\cdot}69 \times 10^7$)	99·999	(78)
Arth. simplex	$1{\cdot}14 \times 10^7$	0 ($1{\cdot}21 \times 10^6$)	100	(89)
Arth. simplex†	$1{\cdot}14 \times 10^7$	$5{\cdot}20 \times 10^1$ ($4{\cdot}12 \times 10^6$)	99·9995	(64)
Yeasts				
Sac. cerevisiae	$1{\cdot}86 \times 10^6$	$6{\cdot}92 \times 10^3$ ($8{\cdot}04 \times 10^4$)	99·6	(96)
C. utilis	$3{\cdot}87 \times 10^7$	$5{\cdot}93 \times 10^3$ ($3{\cdot}04 \times 10^5$)	99·98	(92)

* Filtrations were carried out using 10 sheets of unwoven cloth coated with 32 mg/g of soluble BVPS at room temperature and at a filtration rate of 2·6 cm/h. Control experiments were performed for each run using unwoven cloth that was not coated with the pyridinium-type polymer, and the results are given in parentheses.

† The filtration rate was 34·2 cm/h.

Removal of spores of fungi from water

The results are summarized in Table 2. When 10 sheets of the unwoven cloth were used, the rate of removal was 98% at a filtration rate of 2·6 cm/h, whereas the rate of removal was 87% in the corresponding control experiment using 10 sheets of untreated cloth. On the other hand, when 20 sheets of the unwoven cloth were used, the rate of removal reached at 99·994%; the rate of removal was 99%

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Table 2. *Removal of spores of* Mucor racemosus *from water by filtration through unwoven cloth coated with soluble BVPS**

No. of piled sheets	Concentration (spores/ml) Influent	Effluent	Removal (%)
10	$1{\cdot}15 \times 10^3$	$2{\cdot}85 \times 10^1$ ($1{\cdot}50 \times 10^2$)	98 (87)
20	$1{\cdot}80 \times 10^4$	$1{\cdot}00 \times 10^0$ ($2{\cdot}40 \times 10^2$)	99·994 (99)

* Filtrations were carried out using unwoven cloth coated with 32 mg/g of soluble BVPS at room temperature and at a filtration rate of 2·6 cm/h. Control experiments were performed for each run using unwoven cloth that was not coated with the pyridinium-type polymer, and the results are given in parentheses.

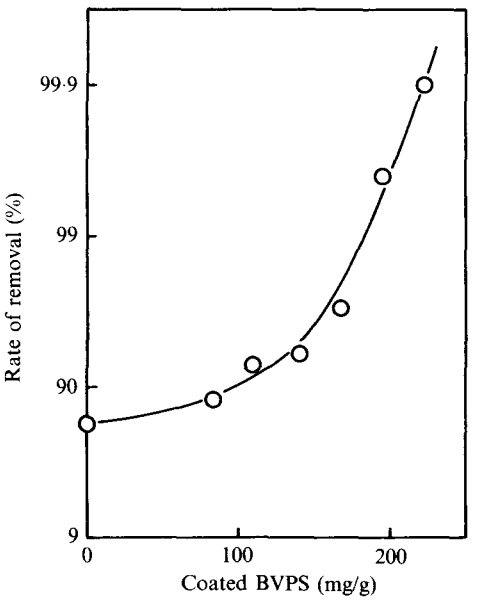

Fig. 6. Effect of the amount of soluble BVPS coated on the surface of unwoven cloth on the rate of removal for bacteriophage T4. Influent concentration, $2{\cdot}5 \times 10^8$ p.f.u./ml; the number of piled sheets of the unwoven cloth, 40; filtration rate, 2 cm/h.

in the corresponding control experiment. Thus, filtration through the unwoven cloth coated with soluble BVPS is an effective method for removing spores of fungi.

Removal of bacteriophage T4 from water

The results are summarized in Figs. 6–8. Filtration was not very effective for removing the virus from water, when compared with bacteria and yeasts. Therefore we used 40 sheets of unwoven cloth coated with 222 mg/g of soluble BVPS to remove bacteriophage T4 from water.

The efficiency of removing bacteriophage T4 from water increased with the amount of soluble BVPS coated on the surface of unwoven cloth (Fig. 6). Coating of more than 200 mg/g of soluble BVPS appeared to be necessary to remove more than 99% of the virus at the filtration rate of 2 cm/h. The rate of removal rapidly

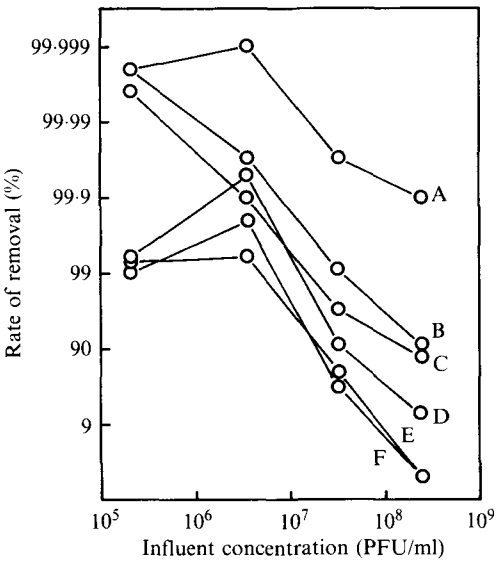

Fig. 7. Effect of influent concentration on the rate of removal for bacteriophage T4. Filtration rate (cm/h): A, 2; B, 4; C, 6; D, 10; E, 14; F, 20. The number of piled sheets, 40.

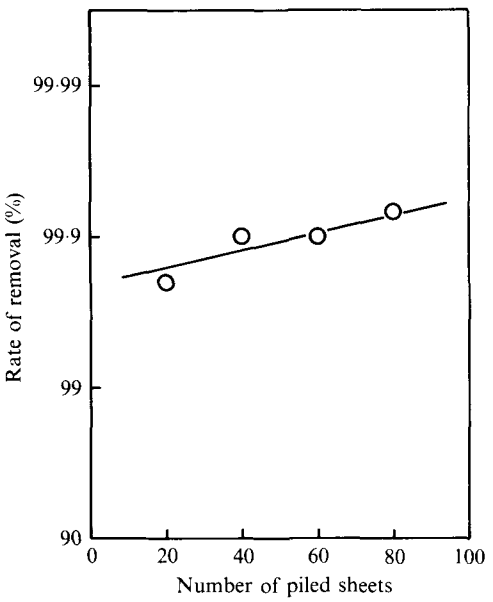

Fig. 8. Effect of the number of piled sheets of unwoven cloth on the rate of removal for bacteriophage T4. Influent concentration, $2{\cdot}5 \times 10^8$ p.f.u./ml; the amount of soluble BVPS coated on the surface of unwoven cloth, 222 mg/g.

decreased with an increase in filtration rate (Fig. 7). Although an increase in the number of piled sheets of the unwoven cloth showed some improvement (Fig. 8), a slow filtration rate was necessary for significant removal of the virus from water. These observations indicate a low efficiency for removing the virus by filtration

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through the unwoven cloth coated with soluble BVPS, when compared with bacteria and yeasts.

DISCUSSION

This study shows that unwoven cloth coated with a pyridinium-type polymer has an excellent ability to remove bacteria and yeasts from water. In experiments removing *E. coli* by filtration through 10 sheets of unwoven cloth coated with 32 mg/g of soluble BVPS, the rate of removal was 99·99% at a filtration rate of 2·6 cm/h. Although the rate of removal decreased with an increase in the filtration rate, the level remained at 99%, even at the high filtration rate of 300 cm/h. The filtration rate used in practical water supplies is in the order of 30–120 m/day, i.e. 125–500 cm/h. Therefore Fig. 1 indicates satisfactory removal of micro-organisms at a practical filtration rate. The remove efficiency increased with the number of sheets of cloth, suggesting that high removal efficiency can easily be accomplished. Removal by filtration was reliable even at a low influent concentration of bacterial cells, and the rate of removal showed a tendency to increase with a decrease in the influent bacterial concentration. When ten sheets of unwoven cloth coated with 32 mg/g of soluble BVPS were used, the unwoven cloth captured $1{\cdot}1 \times 10^6$ cells/g of *E. coli* until the bacteria became detectable in the effluent suspension during the filtration. Although ease of removal largely depended on the type of micro-organism, and *Arth. simplex* and *Staph. aureus* were removed the most efficiently and *P. aeruginosa* the least efficiently, all of the test bacteria and yeasts were effectively eliminated from water by filtration. There was no marked resistance against the flow of water through the unwoven cloth, and may have practical applicability in treating drinking water. When the cloth is properly prepared, there would be no possibility of the pyridinium-type polymer leaking into the treated water. Thus, the new method of filtration is expected to be useful in effectively removing micro-organisms from water as an alternative method to conventional chlorination.

As was pointed out in a previous report from this laboratory [11], insoluble pyridinium-type resin captures micro-organisms in a living state. Therefore in the case where the water contains nutrient, the micro-organisms can grow on the surface of the resin as well as on the treated cloth. Thus the filtration method would only be suitable for purification of water which does not contain nutrients for micro-organisms.

Spores of fungi are well known for showing a strong resistance against thermal sterilization. Conventional autoclaving is not always reliable in this case. In recent years, automats for warm coffee with cream and other drinks containing nutrient have come into wide use, especially in Japan. It is feared that fungi or endospore-forming bacteria may multiply in these drinks during storage at high temperature. However, filtration through the unwoven cloth coated with 32 mg/g of soluble BVPS may be effective in removing spores of fungi from water.

The new filtration method was not very effective in removing virus from water. This can be attributed to the fact that viruses are much smaller than bacteria and yeasts. The probability of capturing pollutants on the surface of unwoven cloth coated with soluble BVPS may depend on the opportunity of contact of pollutants with the surface of unwoven cloth, and may decrease with decrease of the size of

the pollutants. Virus appears to be too small, compared with the pore size of the unwoven cloth. It seems necessary to use membranes and other materials with pores smaller than those of unwoven cloth. Further research on effectively removing viruses from water, as well as the removal of animal viruses and protozoa organisms from water by the filtration method, are required.

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