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Disinfection of *Escherichia coli* by Using H^+ and OH^- Form Ion-exchange Resins, Part 2

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Abstract The effectiveness of various porous ion-exchange resins in H^+ and OH^-forms on the disinfection of *Escherichia coli* (*Ecoli K-12*) has been probed. Water suspensions of up to 10^8 *E.coli cells*／cm^3 were passed through a glass column packed with various ion-exchange resins (0.5～2.0g). The killing effectiveness of the column was determined by using plate counts. Quanternary ammonium strong based anion-exchange resin in OH^- form (IRA-938), which has enough macropores to encapsulate *E.coli* cells, was effective to the devitalization of *E.coli*. The mixed bed of OH^- resins and a sulfonic acid H^+ form cation-exchange resin (TAS3-3), which was noneffective by itself, was found to have remarkable germicidal efficiency by a synergistic effect of OH^- and H^+ ions on the resins. The mixed bed of the two resins has the feasibility of anew and handy "disinfectant" for water treatment.

Key word : Disinfection, Bacteria, H^+ and OH^- form Ion-exchange Resins

Introduction

It is well known that chlorine is a strong disinfectant to microorganisms such as *Escherichia coli* and viruses, and has long been used for production of drinking water. Recently, however, it has become known that the chlorine in water reacts with a variety of organic impurities and converts them into carcinogens.[1,2]. Thus, it is urgent that effective non-chlorine based disinfectants be developed. It appears that the triiodide from3-5) of a quaternary ammonium anion-exchange resin is one of such disinfectants. So far, however, there has been an anxiety[6,7] that it is slightly dissolved in water, especially when some organic compounds are contained in water.

Meanwhile, it has been found that counter ions such as I^{3-}, H^+ and OH^- on the surface of ion-exchange resins exist in concentrated states of about 1～6 M[8,9] Therefore, if the concentrated indissoluble acid H^+ or basic OH^- ions are fully utilized, it seems that they can devitalize *Escherichia coli*. Thus, the purpose of this work is to examine the feasibility of employing these H^+ and OH^- form ion-exchange resins as a new disinfectant for the treatment of water.

Material and method

Ion-exchange resins and Bacterial suspension: Macroreticular basic quanternary ammonium anion-exchange resins and sulfonic acid cation-exchange resin (Organo Co. Japan) were used. The resin particles, sieved to 28～32mesh, were pretreated in a column with aqueous solution of hydrochloric acid (1 mol・dm^{-3}) and Sodium hydroxide (1 mol・dm^{-3}), and then with ethanol and water.

After this treatment, the resins were converted to H^+, Na^+, OH^- and Cl^- forms with excess solutions of hydrochloric acid (1 mol・dm^{-3}), Sodium hydroxide (1 mol・dm^{-3}) and Sodium hydrochloride (1 mol・dm^{-3}), respectively.

Repeated washings with distilled water were carried out before drying at room temperature. The dried resins were stored in a desiccator with a relative humidity of 20% prepared with sulfuric acid and water 20℃. *Escherichia coli K-12* W3110 was grown in nutrient broth for 20 h. at 37℃ and cells were diluted in distilled water to the desired concentrations.

Proceduer: These resins (0.5, 1.0, 2.0g) weight into a glass column (length:200 mm, diameter:10 mm) and aqueous suspensions of *E.coli* with 10^8～10^4 cells・cm^{-3} were passed through the column at a flow late of

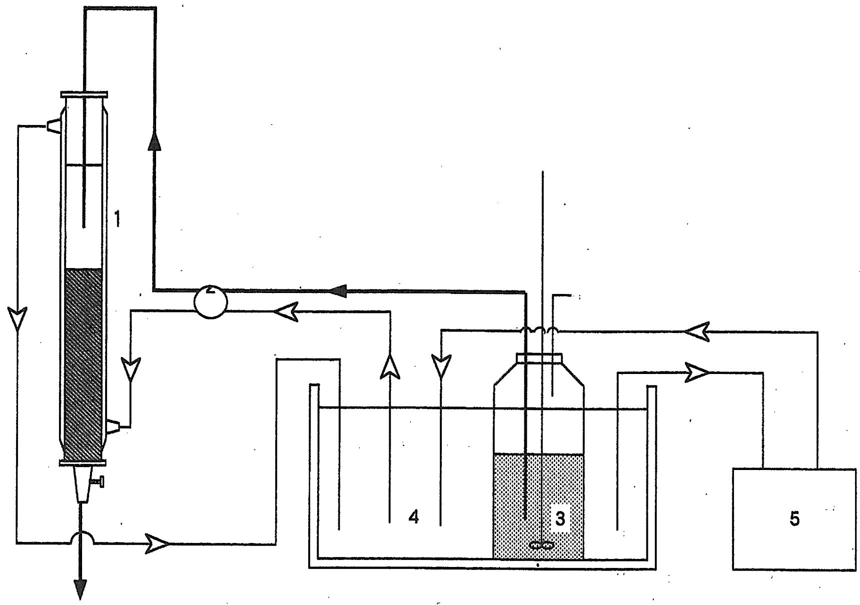

Fig.1 Schematic diagram of disinfection sysytem
1 : Ion-exchange column, 2 : Quantitative pump 3 : Water suspension of E.coli,
4,5 : Water Bath at 15℃

Table1. Disinfection effect of HCl、NaOH and NaCl aqueous solutions

$$\text{Viable ratio} : \frac{\text{Viable count in solution (cells / cm}^3\text{)}}{7.9\times10^7 \text{ (cells / cm}^3\text{)}} \times 100$$

Measured Value / Solvent system	Contact time (min)	Viable count (cells/cm³)	Viable ratio (%)
Water	1	7.9×10^7	100
	30	5.1×10^7	65
	60	4.8×10^7	61
HCl (0.01M)	1	1.9×10^6	2.4
	30	1.7×10^5	2.2×10^{-1}
	60	1.3×10^5	1.6×10^{-1}
HCl (1M)	1	0 (<10)	0
	30	0	0
	60	0	0
NaOH (0.01M)	1	6.6×10^4	8.4×10^{-2}
	30	4.0×10^4	5.1×10^{-2}
	60	2.4×10^4	3.0×10^{-2}
NaOH (1M)	1	0	0
	30	0	0
	60	0	0
NaCl (1M)	1	7.9×10^7	100
	30	7.0×10^7	89
	60	7.3×10^7	92

Table 1. Disinfection effect of HCl, NaOH and NaCl aqueous solutions

$$\text{Viable ratio} : \frac{\text{viable count in solution (cells/cm}^3\text{)}}{7.9\times10^7 \text{ (cells/cm}^3\text{)}} \times 100$$

2cm^3 • min^{-1} at 15° C. The column effluents were taken every 10cm^3 and they were used in making plate counts. The sample plates were incubated for 24h at 37℃ and the number of the viable *E.coli* cells were then counted to determine viable cell concentrations. A schematic diagram of the measurement system is represented in Fig.1.

Result and discussion

Firstly, germicidal effect of H^+ and OH^- ions was investigated and shown in Table.1. From the table, both H^+ and OH^- ions were found to be effective even in the concentration of 0.01M and the *E.coli* with a high cell concentration of 10^8 cells • cm^{-3} was completely devitalized within contact time of only 1min in 1M hydrochloric acid and 1M sodium hydroxide aqueous solutions. 1M sodium chloride solution and pure

Table 2. Germicidal capabilities of various porous ion-exchange resins

$$\text{Viable ratio} : \frac{\text{viable count in effluent (cells/cm}^3)}{\text{viable count in influent (cells/cm}^3)} \times 100$$

Ion-exchange resins	Ion form	Amount of resin (g)	Original cell concentration (cells/cm^3)	Effluent Volume (cm^3)	Viable ratio (%)
Amberlyst 15	H^+	2.0	10^6	20	30
XT-1009	H^+	2.0	10^6	20	30
XT-1009	Na^+	2.0	10^6	20	30
TAS 3-3	H^+	2.0	10^6	20	20
TAS 3-3	Na^+	2.0	10^6	20	30
Amberlyst A-26	OH^-	2.0	10^6	20	15
IRA-938	OH^-	0.5	10^6	50	2
IRA-938	Cl^-	0.5	10^6	50	7

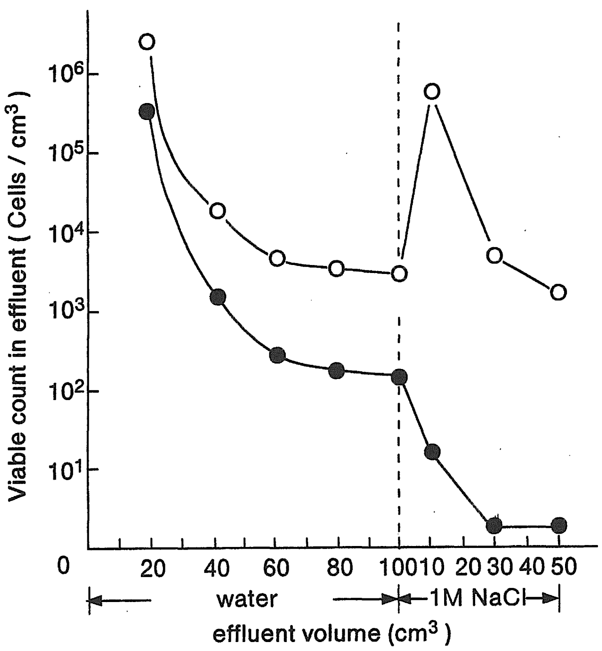

Fig.2 Comparison of germicidal capabilities between OH^- and Cl^- forms of IRA-938
-○- : Cl^- form (0.5g) -●- : OH^- form (0.5g)

water were found to be almost noneffective. From the result of Table 1, H^+ and OH^- forms of ion-exchange resins are considered to have germicidal capabilities, because H^+ and OH^- ions on the surface of ion-exchange resins have been found to exist in concentrated states of at least 1M.[9)]

Disinfection capabilities of several porous type ion-exchange resins packed in the column are compared in Table 2. From the table, OH^- and Cl^- forms of an anion-exchange resin named as IRA-938 were found to be effective, whereas H^+, Na^+ and OH^- forms of other cation- and anion-exchange resins were almost noneffective.

This IRA-938 is a super macroreticular basic quaternary ammonium anion-exchange resin, that is, it has large pores (diameter: 2.5～23 μm) enough to encapsulate *E.coli* whose diameter id about 1 μm, while other ion-exchange resins are usual porous resins and have smaller pores into which *E.coli* can not enter. These result suggested that for effective devitalization, it is necessary for *E.coli* to be captured in pores and brought in full contact with the concentration ions on the surface of the pores. The difference between OH^- and Cl^- forms of IRA-938, however, should be investigated, because Cl^- ions are considered to be investigated from the result of Table 1. After packed OH^- or Cl^- form IRA-938 (0.5g) was saturated with *E.coli* cells by passing 100 cm^3 of water suspensions of *E.coli* with a high concentration of 10^8 cells/cm^3, 100cm^3 of germ-free water was passed through each of the resins and then 50 cm^3 of 1M NaCl aqueouss olution was followed. Viable counts of *E.coli* cell in water and NaCl effluents were determined and shown in Fig.2. From the figure, it was found that in general OH^- forms resin is more effective than Cl^- form resin but difference between viable counts in water effluents passed through the two form resins are smaller than that between viable counts in 1M NaCl effluents. Notice that the ratio of the former is 10 times at the water effluents volume of 100 cm^3, whereas that of the latter reaches 10^4 times at the NaCl effluents volume of 10 cm^3 and the difference is almost infinity at effluents volume of 30 cm^3. Moreover, it was found that viable count at the NaCl effluent volume of 10 cm^3 passed through Cl^- resin is 5×10^5 cells/cm^3 and the value is larger than that of 3×10^3 cells/cm^3 at the water effluent volume of 100 cm^3 passed through the same Cl^- resin.

Since *E.coli* has negatively charged groups owing to various phspholipids and proteins contained on the surface of it, it is considered that the negative groups were partially anion-exchanged for Cl^- or OH^- ions of IRA-938 and the *E.coli* cells were strongly held on the surface of the resin. Thus, the strongly held *E.coli* cells were eluted with 1M NaCl solutions by anion-exchange reactions between the negative groups of *E.coli* on the surface of the pores and Cl^- ions in solution, while *E.coli* cells merely encapsulated in the pores were eluted with water.

Further, the reason of the large difference of viable counts between the NaCl effluents passed through OH^- and Cl^- forms of IRA-938 is considered to be caused by the fact that the strongly held *E.coli* cells on the surface of the OH^- form resin were devitalized by disinfection effect of the concentrated basic OH^-i ons on the surface of it, while the *E.coli* cells on the surface of the Cl^- form resin were alive owing to the noneffective Cl^- ions. This interesting fact was als supported by the preliminary result that some substances, having absorption peaks near 260 nm were discovered in the NaCl effluents passed through the OH^- resin. They are considered to be wreckage of the devitalized *E.coli* cells, becouse they were not discoveredin the effluents through the Cl^- resin. In this manner, OH^- form of IRA-938 was found to have germicidal capability.

Mixed effect of OH^- form anion-exchange resin(IRA-938) and a H^+ form cation-exchange resin (TAS 3-3) is shown in Table 3. From the table, the mixed bedof the OH^- resin (0.5g) and the H^+ resin (0.5g) was found to be have remarkable germicidal capability, that is, its capability is about 10 times that of the OH^- resin (cf. Second and fourth columns).

This result is interesting because the single H^+ form resin itself, which is a porous sulfonic acid cation-exchange resin, is almost noneffective (cf. third column). Thus, this remarkable effect of the mixed bed is considered to be a synergistic effect of both

Table 3. Mixed effect of OH^- form anion - exchange resin (IRA938) and H^+ form cation - exchange resin (TAS 3 - 3)

$$\text{Viable ratio}: \frac{\text{Viable count in effluent (cells/cm}^3\text{)}}{\text{Viable count in inffluent (cells/cm}^3\text{)}} \times 100$$

Ion - exchange capacities of the OH^- resin and the H^+resin are 3.1 and 3.0mg eq /g, respectively.

Ion form (amount) / Measured Value	IRA-938 OH^- (1g)	TAS 3 − 3 H^+ (1g)	IRA-938 OH^- (0.5g) + TAS 3 − 3 H^+ (0.5g)
Influent cell concentration (cells/cm³)	4×10⁷	5×10⁷	4×10⁷
Effluent Volume (cm³)	100	100	100
Viable ratio (%)	10	90	0.9

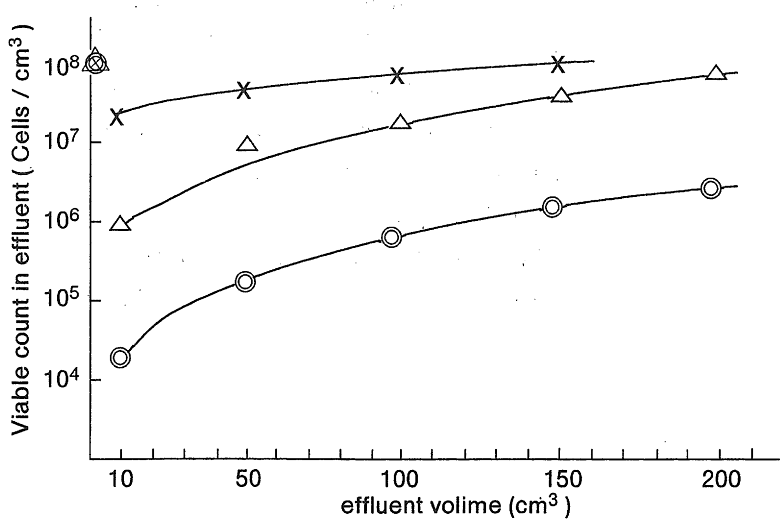

Fig.3 Comparison of the germicidal effect of the mixed bed with those of the OH^- resin and the H^+resin
-×- : 1g of the H^+ resin
-△- : 1g of the OH^- resin
-◎- : 0.5g of the H^+ resin and 0.5g of the OH^- resin

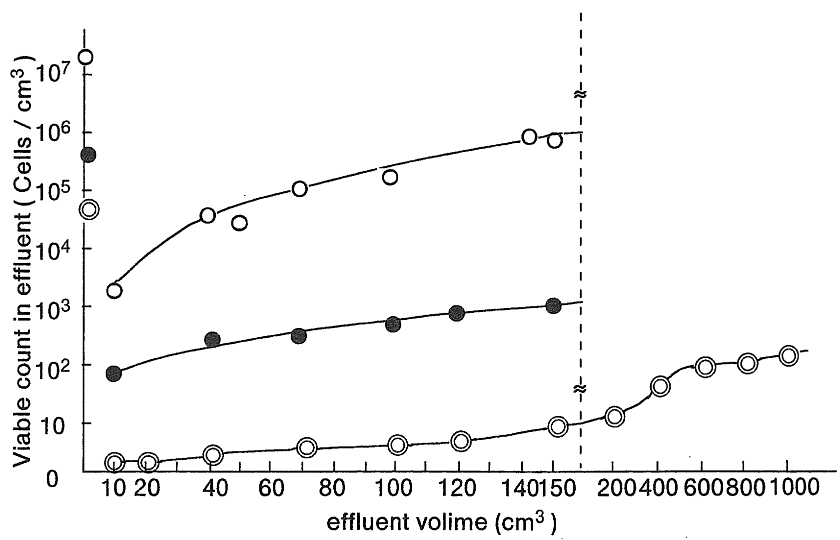

Fig 4 Disinfection capabilities of the mixed bed column (0.5g of the H^+ resin and 0.5g of the OH^- resin)
-○- : Influent cell concentration is 3×10^7 (cells/cm³)
-●- : Influent cell concentration is 3×10^5 (cells/cm³)
-◎- : Influent cell concentration is 3×10^4 (cells/cm³)

OH^- and H^+ ions which are found to be essentially effective from the result of Table 1. The remarkable synergistic effect is clearly shown in Fig.3 where effluent behavior of the suspension (10^8 cells/cm^3) through the mixed bed is compared with those through the OH^- resin and the H^+ resin. In Fig.4, disinfection capabilities of the mixed bed (0.5g of the OH^- resin and 0.5g of the H^+ resin) are represented.

Notice that viable count is about 10^2 cells/cm^3, even after the treatment of 1000 cm^3 of *E.coli* suspension with a cell concentration of 10^5 cells/cm^3. The viable count of 10^2 cells/cm^3 smaller tha n the value of 3×10^3 cells/cm^3 which is the allowable limit of waste water in Japan.

Taking the results obtained into account, we can conclude that the mixed bed has the feasibility of a new and handy disinfectant employed for water treatment.

Acknowledgements

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1) Y.Baba,. Kagaku To Kogyo(Chemistry and Chemical Industry) **31**, 492-493(1978).

2) T.Suzuki, and L.T.Fan, J. Fermen . Technol. **57**, 578-581(1979)

3) S.L.Taylor, L.R.Fina and J.L.Lambert, Appl. Microbiol.**20**, 720(1970)

4) L.R.Fina, and L.Lambert, Proc 2nd.World congr. Int. Water Resources assoc., New Delhi, India Vol.2 **53**(1975).

5) T.Tawaratani, H.Tsuji and I.Shibasaki, J. Ferment. Technol. **54**, 158(1976).

6) G.L.Hatch, L.Lambert, and L.R.Fina, Ind. Eng. Chem. Prod. Res. Dev. **19**, 259-263(1980)

7) T.Suzuki, Y.Hayakawa, and Y.Matsumura, J. Chem. Soc., Faraday Trans. I **77**, 2901-2905(1981).

8) T.Suzuki, and Y.Hayakawa, J.Phys. Chem. **83**, 1178-1180(1979).

9) N.Ayuzawa, T.Suzuki, and Y.Hayakawa, Denki Kagaku(J. Electrochem. Japan)**49**, 227-230(1981)