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Reuse of Oyster Shell Waste as Antimicrobial Water Treatment Agent by Silver Ion Exchange

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A water treatment agent with antimicrobial activity(Ag-Os) was created by exchanging silver ion(Ag^+) on calcined oyster shell powder. The desorption of the exchanged silver ion was negligible, thereby indicating a stable antimicrobial water treatment agent. The sterilization effect of Ag-Os on underwater microorganisms was then investigated. An MIC (Minimum Inhibitory Concentration) test result indicated that Ag-Os had an excellent sterilization effect on G-germs, such as *Escherichia coli* and *Pseudomonas aeruginosa*. Most germs were annihilated with an Ag-Os concentration of 200 ppm and contact time of 60 minutes. The sterilization effect was mainly dependent on the contact time. The zeta potential of the Ag-Os powder adsorbed on sand was measured relative to the concentration of exchanged silver ion. As the concentration of the exchanged silver ion increased, the surface charge density of the anions on the surface of the Ag-Os powder adsorbed on sand also increased. Accordingly, this result indicated that a higher silver ion than ion exchange capacity was present on the particle surface due to adsorption. Consequently, this increased concentration of exchanged silver ion would appear to significantly enhance the sterilization power.

Key words : oyster shell powder, silver ion, ion-exchange, sterilization, antimicrobial activity, zeta potential

1. Introduction

The amount of oyster shell produced is 9 times more than that of *oysters*[1]. Currently, only about 30% of oyster shell is reused and the rest is abandoned as waste. As a result oyster shell waste causes many problems such as oceanic pollution and a bad smell due to the organic substance attached to the shell. In addition, the treatment of oyster shell waste is expensive. Consequently, the reuse of oyster shell waste is needed in terms of preventing environmental pollution and reducing treatment *cost*[2].

Accordingly, in this study, the reusability of oyster shell as a water treatment agent with antimicrobial activity was examined. Oyster shell was crushed and calcined to create activity on the surface, thereby increasing ion exchange capacity. Thereafter, silver ion was exchanged on the processed oyster shell powder to produce antimicrobial activity. Among metal ions with antimicrobial activity, silver ion was selected for the ion exchange because of its high bond energy with oxygen in a molecular frame to form a stable bond and high selectivity of the metal ion on the powder *surface*[2~3]. In addition, because the desorption concentration of exchanged silver ion is within the regulation range, this eliminates the risk of water pollution if it is used as a water treatment *agent*[2].

The sterilization effect of Ag-Os on underwater microorganisms was investigated by the MIC (Minimum Inhibitory Concentration) test and by measuring the number of viable cells relative to the contact time. To determine the sterilization mechanism, the sterilization effect was examined relative to the agitation speed.

In order to highlight any correlation between the sterilization effect and the concentration of the exchanged silver ion, the zeta potential of the Ag-Os adsorbed on sand was measured using a specially

designed streaming potentiometer.

2. Materials and Methods

2.1. Manufacture of Activated Powder from Oyster Shell

The oyster shell was washed by tap water to remove any salt and then dried for 3 days in the atmosphere. The dried oyster shell was crushed by a jaw crusher and calcined at 900℃ for 3 hours. The calcined oyster shell was then reduced to a powder by ball milling and finally dried at 110℃ for 24 hours.

2.2. Manufacture of Water Treatment Agent with Antimicrobial Activity(Ag-Os)

Based on previous analysis, oyster shell powder has a basic structure of SiO_2 and Al_2O_3 which can also be seen in zeolite, a typical inorganic carrier for ion exchange. Furthermore, due to the existence of Na, which is a highly selective ion in an ion exchange reaction, oyster shell powder is expected to be exchangeable with an antimicrobial metal *ion*[4].

To give antimicrobial activity to the oyster shell powder, an antimicrobial metal ion was exchanged with metal ions(P^{+1}) with an oxidation number of +1 which were positioned to maintain electrical neutrality. These metal ions, such as Na and K, do not participate in the formation of the structure itself, yet exist to maintain a charge balance, therefore, such an ion exchange with other ions is not expected to cause any change in the molecular structure.

The sterilization power of antimicrobial metal ions is known to be in the order of $Hg > Cu > Zn > Fe > Pb$[3]. In this study, silver ion was selected to give antimicrobial activity to the oyster shell powder because of its inherent advantages over the other antimicrobial metal ions, including a high selectivity for the P^{+1} ion in the oyster shell powder since Ag^{+1} ion has a relatively small atomic radius, the same oxidation number as the P^{+1} ion, and a high bonding energy with oxygen in the molecular structure of the oyster shell powder, thereby producing a stable *bond*[2~3]. In addition, because its regulation in water pollution ranges from 50 to 100 ppb, the probable physical desorption Ag^{+1} ion will preclude the chance of water pollution while being used as a water treatment agent.

About 10-40g of activated oyster shell powder was put in 100-250ml of deionized water and the solution agitated at 60℃ for 90 minutes. The pH of the solution was maintained at 6.5 using acetic acid. For the ion exchange, 0.339-1.335g of $AgNO_3$ was dissolved in the solution and then agitated for 24 hours. Thereafter, the ion-exchanged powder was separated from the solution using vacuum filtration, washed with deionized water, and dried at 105℃ for 60 minutes. Finally, it was crushed and dried again to produce an antimicrobal water treatment agent(Ag-Os).

From the experiments, it was found that 99.9% of the silver ion was exchanged on the activated shell powder, whereas the desorption of the ion-exchanged silver ion was less than 0.03%. Consequently, the successfully ion-exchanged Ag-Os was expected to be stable while being used as a water treatment agent.

2.3. Analysis of Activated Oyster Shell Powder and Ag-Os

The calcined oyster shell powder was quantitatively analyzed by XRF(X-ray Fluorescence) and the results are shown in Table 1. About 99% of the powder was composed of CaO and the other components were SiO^2 and Al_2O_3, which can be seen in zeolite, a typical inorganic carrier for ion exchange.

Table 1. Quantitative analysis of calcined oyster shell powder by XRF

Component	wt%
SiO_2	1.07
CaO	98.87
Al_2O_3	0.05

The thermal behavior of oyster shell was examined by TG/DTA(Termal Gravimetric/Differential Thermal Analysis) and, the phase change relative to heating was analyzed by XRD(X-ray Diffraction). The pH change relative to the calcination temperature was measured using a pH meter. The size and shape of the calcined shell

powder and Ag-Os was analyzed by SEM(Scanning Electron Microscopy).

The density and pore characteristics of the calcined oyster shell powder and Ag-Os were measured using a Porosimeter and the results are summarized in Table 2. From the BET(Brunauer-Emmett-Teller) measurement results, the specific surface area of the calcined oyster shell powder and Ag-Os was found to be $3m^2/g$ and $2m^2/g$, respectively.

The element composition of the calcined oyster shell powder and Ag-Os was analyzed using an ICP(Inductively Coupled Emission Spectrophotometer) and the results are summarized in Table 3. For both samples, Ca and P were the main elements. The Ag content of Ag-Os was about 0.088%, which was about 50 times higher than that of the calcined oyster shell powder.

2.4. Investigation of Sterilizing Characteristics of Ag-Os

To investigate the sterilizing effect of the antimicrobial water treatment agent(Ag-Os), an MIC

Table 2. Density and pore characteristics of Ag-Os measured by porosimeter

	Average Pore Diameter (Å)	Pore Area (m^2/g)	Bulk Density (g/mL)	Skeletal Density (g/mL)	Porosity (%)
Calcined Powder	12844	4.020	0.3865	0.7713	49.89
Ag-Os	15930	3.013	0.7114	2.0301	64.96

Table 3. Element analysis for calcined oyster shell powder and Ag-Os by ICP

Sample	Element	Wave(nm)	Con(ppm)	R.S.D.	Con.(%)
Calcined Oyster Shell Powder	Ca	393.336	109	0.85	43.04
	P	214.914	135	0.51	17.03
	Ag	328.068	0.172	0.56	0.0017
	Mg	279.806	18.5	1.5	0.19
	Na	558.995	13.5	0.73	0.14
	Fe	258.588	1.02	1.9	0.010
	Mn	259.373	0.646	0.96	0.0065
	Zn	213.856	0.191	1.3	0.0019
	Sr	338.071	4.61	1.2	0.045
	K	766.490	0.380	1.8	0.0037
	Al	396.152	8.58	0.44	0.083
Ag-Os	Ca	393.366	94.8	0.64	38.46
	P	214.914	153	0.12	19.89
	Ag	328.068	8.75	1.2	0.088
	Mg	279.806	8.95	1.5	0.090
	Na	588.995	14.1	0.93	0.14
	Fe	258.588	0.956	0.25	0.0097
	Mn	259.373	0.060	1.1	0.0061
	Zn	213.856	0.269	1.5	0.0027
	Sr	338.071	3.61	1.2	0.036
	K	766.490	0.571	3.9	0.0057
	Al	396.152	20.4	0.50	0.20

(Minimum Inhibitory Concentration) test was performed and the number of viable cells was measured relative to the contact time using *Escherichia coli, Pseudomonias aeruginosa,* and *Klebsiella* pneumoniae as G^- germs and *Staphylococcus aureus, P.vulgaris,* and *Bacillus subtlis* as G^+ germs.

For this experiment, a 200 ppm concentration of Ag-Os was prepared in a nutrient broth and contacted with $10^6/ml$ of the germs in a shaking incubator at 35℃. Thereafter, samples were collected every 10 minutes from 0 to 60 minutes and cultivated in a nutrient agar for 24 hours at 35℃. The composition of the nutrient agar was 3g of beef extract, 5g of peptone, 15g of agar, and 1000ml of distilled water. The number of viable cells was counted using a colony counter. For the MIC test, the optical density was 660nm.

The effect of the agitator speed on the sterilization was examined. For *E.coli, P.vulgaris,* and *S.aureus* with 10 ppm of Ag-Os, the number of viable cells was measured after contact times of 5, 10, 15, 20, and 30 minutes and various agitator speeds ranging from 50 to 150 rpm.

2.5. Zeta Potential of Ag-Os Adsorbed on Sand

A streaming potentiometer was designed to measure the zeta potential of the silver ion exchanged zeolite powder adsorbed on sand. The cell for the streaming potentiometer, shown schematically in Fig. 1, consisted of a thick-walled acryl tube with tapped ends to receive two snuggly fitting plugs. O-rings were used at both ends of the tube to prevent any solution leakage. This design allowed for the easier packing of the particle. The platinum leads to the electrodes consisted of a platinum mesh for an increased electrode surface area. These platinum electrodes were then connected to a voltameter. Platinum was used for the electrode material to *avoidpolarization*[5].

The solution reservoir was a 4-liter polyethylene container with a mechanical on-off valve. The height of the outlet was varied to produce different hydrostatic pressures across the cell. This design also facilitated a measuring streaming potential (E) for the driving pressure (ΔP) ranging from 8 to 17 cmHg. Plus a vertically equipped plug was used to prevent channeling,

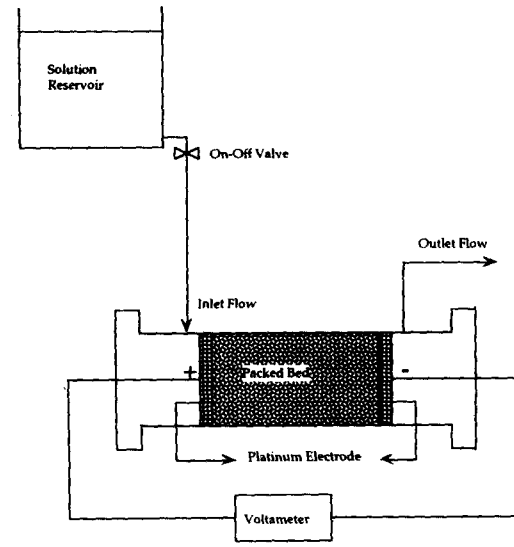

Fig. 1. Schematic diagram of streaming potentiometer.

In this study, 400 micron sand adsorbed by 0.01 and 0.05M silver ion-exchanged oyster shell powder was used as the packing material. The length of the packing bed was 5cm. 10^{-3} and 10^{-5} M NaCl solutions were used as the conductivity solutions. If the solution is forced through a porous plug of solid particles bearing an electric double layer, the movement of ions causes a convection which creates a potential difference(streaming potential) across the ends of the plug[6,7]. Then, since the zeta potential is proportional to $E/\Delta P$ (streaming potential versus driving pressure), its value can be obtained from the slope of $E/\Delta P$.

The Smoluchowski equation was used to calculate the zeta potential as *follows*[5]:

$$\zeta = \frac{4\pi\eta\lambda E}{\varepsilon P}$$

where,

ζ = zeta potential(mV)
η = solution viscosity
λ = specific conductivity
ε = dielectric constant
E = streaming potential
P = pressure drop across the bed

The pressure drop across the bed was obtained using the Ergun *equation*[8].

$$\Delta P = K_2 Q/k_1 \psi_s^2) + 1.75Q^2/(k_1 \psi_s)$$

where,

$$k_1=\frac{\varepsilon^3 D_p \pi^2 r^4}{(1-\varepsilon)L\rho}$$

$$k_2=\frac{150(1-\varepsilon)\pi r^2 \mu}{\rho}$$

ΔP=pressure drop across the bed
ε =Porosity
ρ =solution density
ψ_S=sphericity
Q=volumetric flowrate
μ =solution viscosity
L=bed length
D_p=particle diameter
r=bed radius

3. Results and Discussion

3.1. Physical Properties of Calcined Oyster Shell Powder and Ag-Os

From the TG/DTA analysis shown in Fig. 2, it was found that at 680℃, the powder weight started to decrease and an exothermic reaction started at 710℃. An exothermic peak appeared at 880℃ along with a 36.3% decrease in the powder weight.

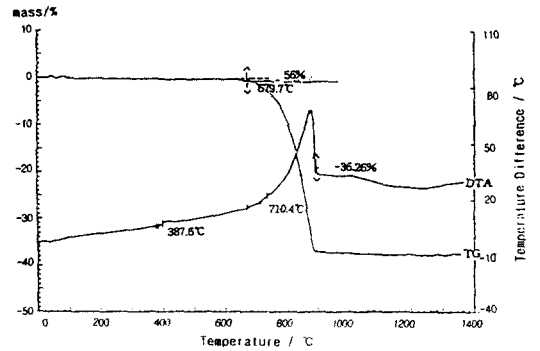

Fig. 2. TG and DTA curves for activated oyster shell powder.

The phase change of the powder relative to the calcination temperature was analyzed by XRD. As shown in Fig. 3, the XRD analysis showed that only $CaCO_3$ existed at 650℃, whereas CaO and $CaCO_3$ coexisted at 750℃. With an increase in temperature above 750℃, the diffraction intensity of $CaCO_3$ decreased, yet that of CaO increased. Above 950℃, the diffraction of $CaCO_3$ vanished.

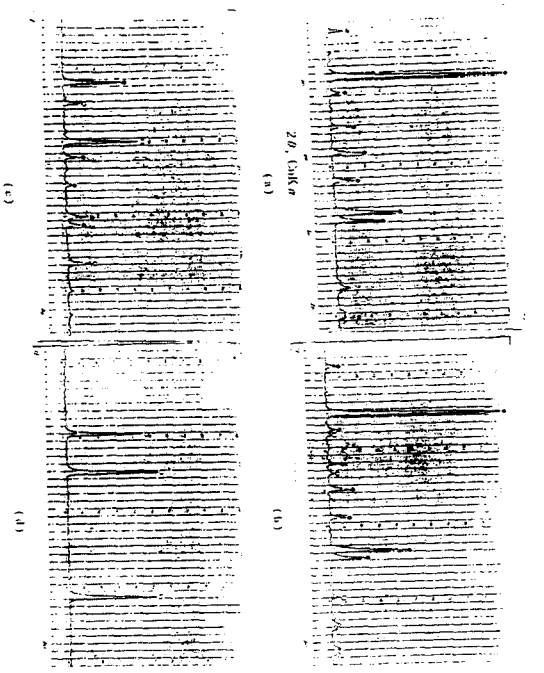

Fig. 3. XRD patterns for oyster shells calcined at (a)650, (b)750, (c)850 and (d)950℃.

Relative to the calcination temperature, the pH of the calcined oyster shell powder in a 0.1 wt% aqueous solution at 21℃ was measured using a pH meter. The pH measurement and XRD analysis results, as summarized in Table 4, show that the pH of the solution increased and the phase changed from $CaCO_3$ to CaO as the calcination temperature increased. Generally, the CaO formed on the calcined oyster shell powder was not reduced to $CaCO_3$, which is characteristic of a strong base. $CaCO_3$ did decompose to CaO and CO_2 depending on the changes in temperature and pH, and this erupted CO_2 gas can inhibit the ion exchange.

Based on the above analysis, it was determined that the exothermic peak resulted from the reaction $CaCO_3$-->$CaO+CO_2$. Consequently, calcined oyster shell powder prepared at above 950℃ would appear to be the most suitable as an inorganic carrier for an ion exchange.

The surface properties of the calcined oyster shell powder and Ag-Os were analyzed by SEM. As seen in Fig. 4, the diameter of the particles ranged between 0.5-15 microns and the size was not uniform.

Table 4. Effect of Ag-Os on the growth of microorganism

Microorganism	Concentration of Ag-Os (ppm)				
	0	25	75	125	175
Encherichia coli(G^-)	0.414	0.404	0.291	0.221	0.120
Pseudomonas aeruginosa(G^-)	0.672	0.549	0.288	0.090	0.050
Klebsiella pneumoniae(G^-)	0.432	0.441	0.436	0.398	0.112
Staphylococcus aureus(G^-)	0.481	0.483	0.393	0.302	0.181
Bacillus subtlis(G^+)	0.458	0.476	0.322	0.189	0.061

*increased O.D. after 24 hours incubation at 35℃

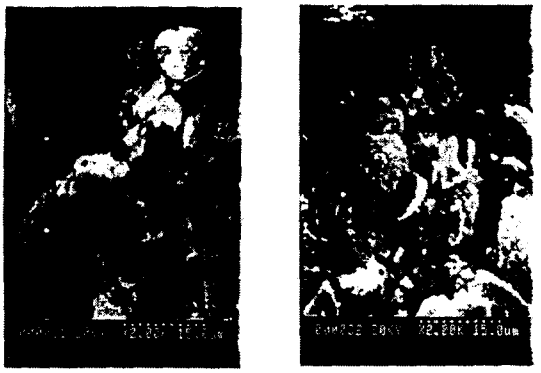

Fig. 4. Surface of Ag-Os and the calcined oyster shell powder photographed by SEM(X2000)

3.2. Sterilizing Characteristics of Ag-Os for Underwater Microorganisms

An MIC test was conducted to characterize the sterilization effect of Ag-Os on underwater microorganisms and the results are shown in Table. 5. As seen in Table 5, Ag-Os showed a strong sterilization power with G^- germs. However, *K. pneumoniae* was insensitive to Ag-Os because it has a narrow *membrane*[9]. Consequently, it would appear that Ag-Os was only effective with G-germs without a narrow membrane. This result was consistent with previous results reported by Yamamoto et *al*[3].

Fig. 5 shows the number of viable cells relative to the contact time. Ag-Os showed a strong sterilization effect on *E. coli*, whereas *K. pneumoniae* was the most resistant to sterilization. This result was also consistent with the MIC test result. However, most of the germs were sterilized with a 200 ppm concentration of Ag-Os after 30 minutes of contact time.

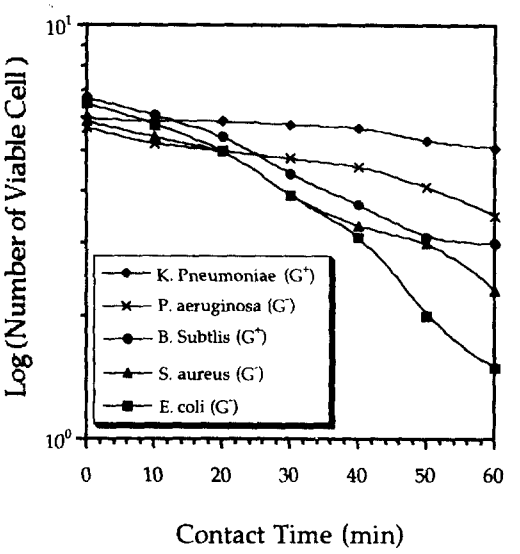

Fig. 5. Viable cell number treated with 200ppm Ag-Os vs contact time.

With a 10 ppm concentration of Ag-Os, the number of viable cells for *E.coli, P.vulgaris,* and *S.aureus* was measured at an agitator speed of 50 and 150 rpm and the results are shown in Fig. 6. As shown in this figure, the number of viable cells decreased as the agitator speed increased. This result indicates that an increase in both the contact time and frequency between the antimicrobial water treatment agent and the microorganism cells increased the sterilization effect.

As mentioned above, the desorbed concentration of exchanged silver ion was almost negligible and thus, it would seem that almost no physical adsorption of silver ion onto the calcined oyster shell powder surface occurred during the ion exchange reaction.

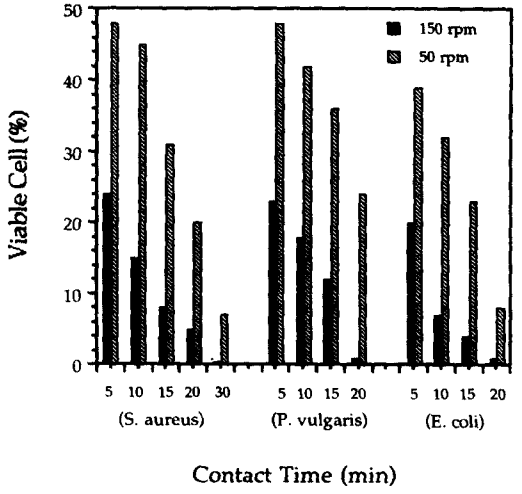

Fig. 6. Effect of agitator speed on number of viable cell.

3.3. Measurement of Zeta Potential for Ag-Os using Streaming Potentiometer

The antimicrobial water treatment agent was prepared by exchanging silver ion with metal ion on the calcined oyster shell powder. During the ion exchange process, an additional amount of silver ion can be adsorbed onto the powder surface. In this experiment, the probable adsorption of more silver ion than the ion exchange capacity was examined by measuring the surface charge density of the particles using a specially designed streaming potentiometer.

The volumetric flow-rate(Q, ml/sec) of the conductivity solution was plotted as a function of the pressure drop(ΔP, cmHg) across the packed bed. The volumetric flow-rate and pressure drop ranged from 21.7 to 39.0ml/sec and from 7.9 to 17.3 cmHg, respectively. Under these experimental conditions, the streaming potential (E) was then measured with ΔP and the results are shown in Figs. 7, 8, and 9. These plots were used to obtain the slope, E/ΔP, and the Zeta potential was finally calculated based on E/ΔP using Smoluchowski's equation, as summarized in Table 6.

As seen in Table 6, the negative surface potential increased as the concentration of silver ion increased during the ion exchange. This would

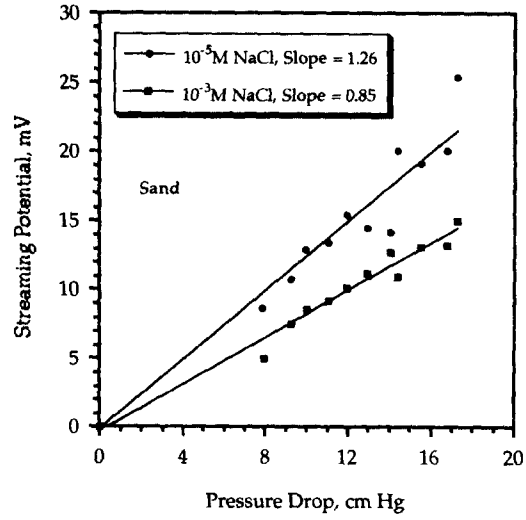

Fig. 7. Streaming potential vs pressure drop for sand.

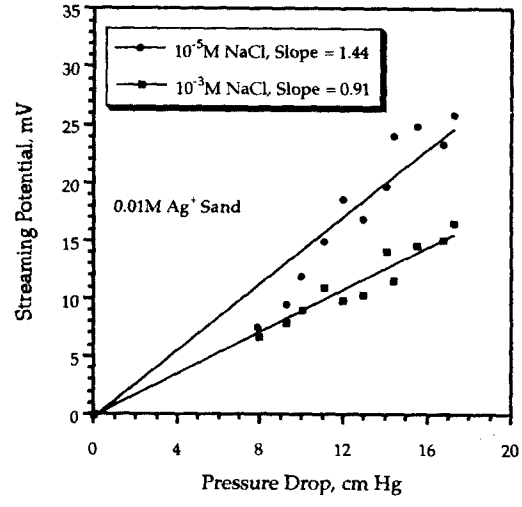

Fig.8. Streaming potential vs pressure drop for 0.01 M Ag^+ sand.

Table 5. Zeta potential of particle with concentration of NaCl

	Sand	0.01M Ag^+ Sand	0.05M Ag^+ Sand
10^{-3}M NaCl	-191	-218	-245
10^{-5}M NaCl	-133	-138	-159

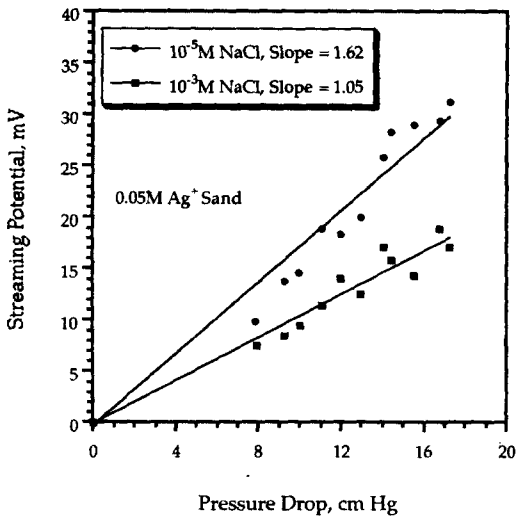

Fig. 9. Streaming potential vs pressure drop for 0.05 M Ag^+ sand.

appear to be due to the fact that the increased positive charge on the particle surface can attract more anions in the solution by an electrostatic force, thereby resulting in an additional adsorption of silver ion than ion exchange capacity. Consequently, this increased concentration of silver ion during the ion exchange process would seem to significantly enhance the sterilization effect due to the increased antimicrobial silver ion on the particle surface.

Based on the above results, it was also found that the surface charge density decreased as the concentration of the conductive solution increased. This may be attributed to the fact that the thickness of the electric double layer formed around each particle was reversibly proportional to the concentration of the conductive solution. Therefore, the ionic potential on the particle surface decreased because of a shrinkage in the electric double layer.

4. Conclusions

Oyster shell powder calcined at above 950℃ was mainly comprised of CaO and the other components were SiO_2 and Al_2O_3. This structure was confirmed to be suitable for silver ion exchange to produce antimicrobial activity.

About 99.9% of the antimicrobial silver ion was exchanged on the calcined oyster shell powder and the desorption of the ion-exchanged silver ion was less than 0.03%. Consequently, successfully ion-exchanged calcined oyster shell powder(Ag-Os) would be expected to be stable while being used as a water treatment agent.

For the sterilization effect, an MIC test result indicated that Ag-Os was effective against G-germs except for those with a narrow membrane, such as *K.Pneumoniae.* Based on the measurement of the viable cell number relative to the contact time, it was found that *E.coli* was the most sensitive whereas *K.pneumoniae* was the most resistant to sterilization. This result was also consistent with the MIC test result. However, most of the germs were sterilized with a 200 ppm concentration of Ag-Os after 60 minutes of contact time.

With a 10 ppm concentration of Ag-Os, the number of viable cells for *E.coli, P.vulgaris,* and *S.aureus* decreased as the agitator speed increased. Consequently, it would appear that the sterilization effect of Ag-Os for underwater microorganisms is mainly dependent upon the contact time rather than the concentration of Ag-Os.

The negative surface potential increased as the concentration of silver ion for ion exchange increased. This would seem to be due to the fact that an increased positive charge on the particle surface can attract more anions in the solution by an electrostatic force, thereby resulting in an additional adsorption of silver ion than ion exchange capacity. Consequently, the increased concentration of silver ion during the ion exchange process would appear to significantly enhance the sterilization effectiveness because of the increased antimicrobial silver ion on the particle surface.

References

[1] Kim, M. P. and J. D. Han, 1997, Adsorption properties of oyster shell powder as landfill cover, J. of Korean Society of Environmental Engineers., 19, 97~110

[2] Shin, C. H., B. I. Noh., and M. C. Jo., 1997, Development of water treatment agent with adsorption and antimicrobial activity using oyster shell", Final Project Report for Department of Agriculture and Fishery., Pusan, Korea.

[3] Yamamoto, T., S. Uchida, and Y. Kurihara,

1991, Disinfection of zeolite containing metal ion, J. Antibact. Antifungi Agents., 19, 425~431.

[4] Chen, N. Y. and F. Thomas, 1994, Molecular transport and reaction in zeolites, New York, UCH Publishers Inc.

[5] Jo, M. C., 1989, Effect of aggregate pretreatment with ASA on the asphalt-aggregate bond, Auburn, AL., Department of Chemical Engineering, Auburn University, U.S.A., M.S. Thesis.

[6] Horn, J.M. and G.Y. Onoda Jr., 1997, Streaming potential and noncreeping flow in porous beds", J. of Colloid and Interface Science., 2, 61.

[7] Robinson, M., J. A. Pa나, and D. W. Fuerstenau, 1960, Surface charge of alumina and magnesia in aqueous media, J. of American Ceramic Society, 47, 516~521.

[8] McCabe, W. L., J. C. Smith, and P. Harriot, 1993, Unit Operations of Chemical Engineering, McGraw-Hill, 5th ed. 151-154.

[9] Ressel, A. D. and W. B. Hugo, 1994, Antimicrobial activity and action of silver, Prog. Med. Chem., 31, 351~390.