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The Mode of Action of Sodium Hypochlorite in the Cleaning Process

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The action of sodium hypochlorite (NaOCl) solution in the cleaning of alumina (Al_2O_3) particles fouled with bovine serum albumin (BSA) or pectin was studied as a function of pH. The efficacy of NaOCl in the cleaning process was evaluated in the pH region where OH^- alone exerted no significant action on BSA or pectin removal. The efficiency and the rate of the removal of BSA or pectin increased with increasing available chlorine concentrations and pH. The pH dependence of the efficacy of NaOCl could be explained on the basis of the equilibrium between undissociated hypochlorous acid (HOCl) and dissociated hypochlorite ion (OCl^-). The efficacy of NaOCl in the removal of BSA or pectin depended on the concentration of OCl^-, but not on HOCl. Size-exclusion chromatographic analysis showed that BSA molecules were partially decomposed by the action of NaOCl, whereas no significant variation in the molecular weight of pectin molecules took place. The difference between the actions of OCl^- in the removal of BSA and pectin could probably be ascribed to the reactivity of OCl^- with their molecules. It could be concluded that the concentration of OCl^- was a major factor determining the actual NaOCl concentration required for an efficient cleaning treatment.

Key words : Sodium hypochlorite/Cleaning/Dissociated hypochlorite ion/Hydroxide ion/Removal of organic matter.

INTRODUCTION

In the food industry, to produce a safe and wholesome product, the most important factors are the cleanliness and sterility of the food-processing equipment. Cleaning and sterilization are complementary processes and they are performed as separate and combined operations. In general, cleaning should precede the sterilizing operation since it is far easier to sterilize clean surfaces than soiled surfaces.

Chlorine has been widely used as a reliable disinfectant or bleaching agent in food industry settings and hospital, health-care and potable water treatment facilities (Clegg, 1962; Rutala and Weber, 1997). Sodium hypochlorite (NaOCl) is a more convenient material to use because it is an aqueous product and relatively stable in its concentrated and diluted forms. In aqueous solution, NaOCl exhibits a dynamic balance as shown by the following:

$$NaOCl + H_2O \rightleftarrows HOCl + NaOH \rightleftarrows OCl^- + H^+ + OH^- + Na^+ \quad (1)$$

When NaOCl solution is used for the purpose of sterilization, the active species is believed to be undissociated hypochlorous acid (HOCl), but not dissociated hypochlorite ion (OCl^-) (Wojtowicz, 1979). HOCl is a weak acid with a dissociation constant of pK_a=7.5 (30℃) in aqueous solution, and the dissociation of HOCl to H^+ and OCl^- depends on pH (Morris, 1966). The optimal pH of the formation of HOCl is in the weakly acidic region. The germicidal activity of HOCl is due to its penetration through the cell membrane (lipid bilayer) because of its electrical

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neutrality. HOCl and OCl^- have been reported to react with a wide variety of biological molecules such as proteins (Hawkins and Davies, 1998; Hazell et al., 1993 and 1994), amino acids (Nightingale et al., 2000), peptides (Heinecke et al., 1993), lipids (Spickett et al., 2000), and DNA (Prutz, 1998) at physiological pH conditions. Although the mechanism of the germicidal activity of HOCl is still poorly understood, it is postulated that HOCl acts directly or indirectly as hydroxyl radicals (Dukan et al., 1999; Rosen and Klebanoff, 1982) on damaging the membrane and DNA, disrupting enzyme activity, and perhaps on the loss of ion regulation.

A concentrated NaOCl solution is a strong base solution (pH > 12) due to the presence of NaOH, and therefore most of the chlorine exist as a less germicidal form, i.e., OCl^-. As the pH of NaOCl solution decreases, more HOCl is formed and the germicidal activity increases. On the other hand, NaOH in NaOCl solution is considered to stabilize free available chlorine in the form of OCl^- and to reduce the corrosive effect of OCl^- on metal. In addition, high concentrations of OH^- can dissolve a wide range of organic materials, i.e., proteins, polysaccharides, and fats. Therefore, a concentrated NaOCl solution appears to be suitable for use in the cleaning operation. The question then arises which of the forms, i.e., HOCl and OCl^-, in a dilute NaOCl solution has the stronger cleaning power. However, the abilities of HOCl and OCl^- to remove the organic soils from solid surfaces have not been fully understood.

The objective of this study was to investigate the mode of action of NaOCl in the cleaning process. We used protein-fouled or acidic polysaccharide-fouled alumina (Al_2O_3) particles as a model cleaning system. The effect of NaOCl on the removal of protein and polysaccharide was studied as a function of pH. This paper describes that the efficacy of NaOCl in the cleaning process depends on the concentrations of OCl^- in conjunction with OH^-.

MATERIALS AND METHODS

Chemicals and materials

Bovine serum albumin (BSA; Fraction V, Lot M7G9556) and citrus pectin (Lot LKE1944) were obtained from Nacalai Tesque Inc. (Kyoto) and Wako Pure Chemical Industries, Ltd. (Osaka), respectively. α-Al_2O_3 particles (Sumicorundum AA-5; Lot YM6601) were obtained from Sumitomo Chemical Co., Ltd. (Tokyo). The specific surface area and mean diameter of Al_2O_3 particles were 0.5 m^2/g and 4.6 μm, respectively. Before use, Al_2O_3 particles were washed several times with deionized water to remove ionic impurities by centrifugation (2,000 × g for 5 min) and then were dried at 150℃ for 4h. NaOCl (Lot DWF2416) was purchased from Wako Pure Chemical Ind. Ltd. (Osaka). All other chemicals were of analytical grade and were purchased from commercial sources.

Preparation of the NaOCl solution as the cleaning agent

The concentration of NaOCl was determined as available chlorine (AC) by the iodometric method (American Public Health Association, 1989). The reagent NaOCl was diluted with a deionized water to approximately 200 to 1,100 mg AC/l. The pH values (1.5 to 12.7) of diluted NaOCl solutions were adjusted by drop-wise addition of HCl or NaOH (0.1 M solutions) under conditions of constant agitation. The concentration of dissociated OCl^- was calculated using an acid dissociation constant (pK_a) of 7.53 (Morris, 1966).

Fouling experiments

The BSA-fouled or pectin-fouled Al_2O_3 particles were prepared at 40℃ by introducing 25 ml of BSA solution (3g/l, 10^{-3} M KNO_3, pH 5.2) or pectin solution (3g/l, 10^{-3} M KNO_3, pH 3.0) and 5 g of Al_2O_3 particles into a 50-ml polypropylene tube, which was then laid on its side in a water bath and reciprocally shaken (140 rpm) for 2h (Takehara et al., 2000). The amounts of BSA and pectin adsorbed on Al_2O_3 particles were 4.2 and 1.5 mg/m^2, respectively.

Cleaning experiments

Batchwise cleaning was conducted at 40℃ by introducing a 1-g portion of BSA-fouled or pectin-fouled Al_2O_3 particles and 5 ml of NaOCl solution into a 25-ml glass vial, which was then laid on its side in a water bath and reciprocally shaken (140 rpm) for 2h as described previously (Takehara et al., 2001). The efficiency of cleaning was determined as the percentage of the amount of BSA or pectin removed out of the initial amount of BSA or pectin adsorbed on Al_2O_3 particles.

Continuous cleaning was conducted at 40℃ in a stainless steel column (4 mmϕ × 50 mm) by feeding the cleaning solution from the bottom of the column at a flow rate of 0.25 ml/min (space time=1.5min) as described previously (Urano and Fukuzaki, 2002). After continuous cleaning with and without NaOCl, the particles were rinsed with the KNO_3 solution (pH 5.6) and withdrawn from the column to determine the amount of protein remaining on the Al_2O_3 particles. The amount of BSA or pectin remaining on the Al_2O_3 particles at any time t (Γ) was calculated by the

following:

$$\Gamma = \Gamma_f + \sum_{i=1}^{N} C_i V_i \quad (2)$$

where Γ_f is the amount of BSA or pectin remaining on Al_2O_3 particles after cleaning; i is the fraction number; C_i and V_i are the BSA or pectin concentration and volume of the i-th fraction, respectively; and N is the total number of fractions. The natural logarithm of the amount of BSA or pectin remaining on Al_2O_3 particles ($\ln \Gamma$) was plotted against t. A graph of $\ln \Gamma$ versus t was analyzed as a function of t (see below).

Kinetic analysis

To analyze the nonlinear curve characterizing the removal of BSA or pectin, the concept that two first-order reactions occur independently and simultaneously during the cleaning process was used (Urano and Fukuzaki, 2002). In this study, we assumed that one first-order reaction proceeds by the action of OH^- (Eq. 3a) and the other by the action of $HOCl/OCl^-$ (Eq. 3b):

$$\ln \Gamma^{OH} = \ln \Gamma_0^{OH} - k^{OH} t \quad (3a)$$

$$\ln \Gamma^{OCl} = \ln \Gamma_0^{OCl} - k^{OCl} t \quad (3b)$$

where k^{OH} and k^{OCl} are the removal rate constants of BSA or pectin based on the actions of OH^- (BSA^{OH} or $pectin^{OH}$) and $HOCl/OCl^-$ (BSA^{OCl} or $pectin^{OCl}$), respectively; Γ^{OH} and Γ^{OCl} are the amounts of BSA^{OH} or $pectin^{OH}$ and of BSA^{OCl} or $pectin^{OCl}$ remaining on Al_2O_3 particles, respectively, at any given time t; Γ_0^{OH} and Γ_0^{OCl} are the amounts of BSA^{OH} or $pectin^{OH}$ and of BSA^{OCl} or $pectin^{OCl}$ on Al_2O_3 particles at zero time, respectively. Here,

$$\Gamma = \Gamma^{OH} + \Gamma^{OCl} \quad (4)$$

Substituting Eqs. 3a and 3b into Eq. 4 and expressing Eq. 4 throughout as a natural logarithm give the following integrated model:

$$\ln \Gamma = \ln\{\exp(\ln \Gamma_0^{OH} - k^{OH} t) + \exp(\ln \Gamma_0^{OCl} - k^{OCl} t)\} \quad (5)$$

The kinetic parameters of Eq. 5 were calculated by a data-fitting procedure based on the nonlinear least-squares regression method.

Analyses

BSA and pectin dissolved in solution or remaining on Al_2O_3 particles were determined using a total organic carbon analyzer described previously (Takahashi et al. 2003). Variation in the molecular weights of BSA and pectin after cleaning was measured by size-exclusion high-performance liquid chromatography as described previously (Urano and Fukuzaki, 2001).

RESULTS

Batch cleaning system

The efficiency of BSA removal from Al_2O_3 particles was measured in the presence and absence of NaOCl at different pH values during batch cleaning. In the cleaning without NaOCl, i.e., with dilute HCl or NaOH solutions alone, the efficiency of BSA removal was less than 10% in the pH range of 1.5 to 10.8, whereas it increased markedly at pHs above 11 (Fig. 1A). No significant amount of BSA was removed in the pH range of 1.5 to 6.5 even in the presence of sufficient NaOCl. At pHs above 6.5, the efficacy of NaOCl became greater with increasing pH and it depended on the AC concentration. This suggests that a certain concentration of OH^- is required for the appearance of the cleaning action of NaOCl. To clarify the action of NaOCl, we put aside the data obtained

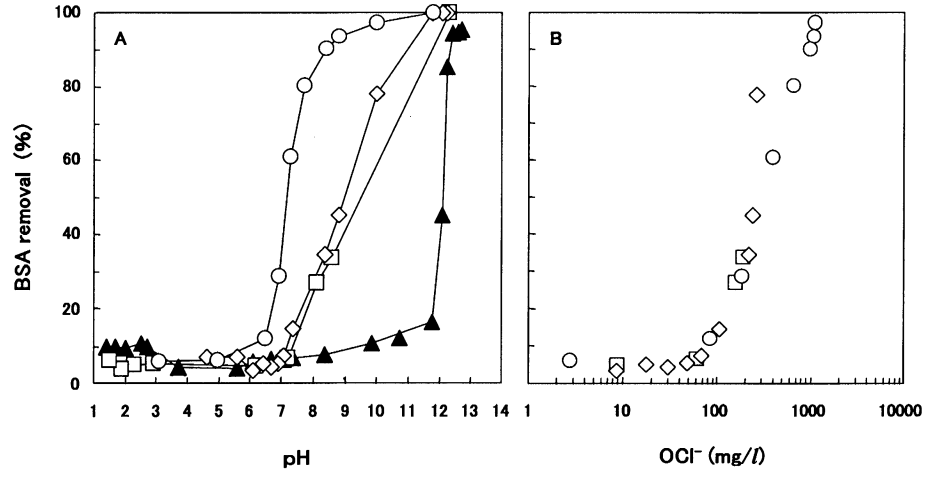

FIG. 1. Effects of pH and AC concentration on the efficiency of BSA removal from Al_2O_3 particles during batch cleaning. (A) Plotted as a function of pH; (B) plotted as a function of the OCl^- concentration. Cleaning was conducted in a 25-ml glass vial for 2 h at 40℃ with reciprocal shaking (140 oscillations per min). Symbols: ▲, Dilute HCl or NaOH solution alone; □, 200 mg AC/l of NaOCl solution; ◇, 250 mg AC/l of NaOCl solution; ○, 1,100 mg AC/l of NaOCl solution.

at pHs above 11, where the cleaning action of NaOH alone was pronounced, and plotted the efficiencies of BSA removal against the concentrations of dissociated OCl^- (Fig. 1B). It was noted that the removal of BSA occurred in an OCl^--dependent manner. The acceleratory threshold concentration of OCl^-, beyond which the effect of NaOCl appeared, was 60 mg/*l*.

Figure 2 shows the effects of pH and the AC concentration on the efficiency of pectin removal from Al_2O_3 particles during batch cleaning. Similar to the results of BSA removal, in the cleaning without NaOCl, the efficiency of pectin removal was considerably low (<10%) in the pH range of 2.5 to 10.2, but above which it increased considerably with increasing pH (Fig. 2A). In the cleaning with NaOCl, large fractions of pectin were removed even when using a low-pH NaOCl solution. The efficiency of pectin removal depended on pH and the AC concentration. The relationship between the efficiency of pectin removal and OCl^- concentration is shown in Fig. 2B, except the data obtained at pHs above 10.2. It is clear that the efficacy of NaOCl on pectin removal also depended largely on OCl^- concentration.

Continuous cleaning system

Figure 3 shows the time course of BSA removal

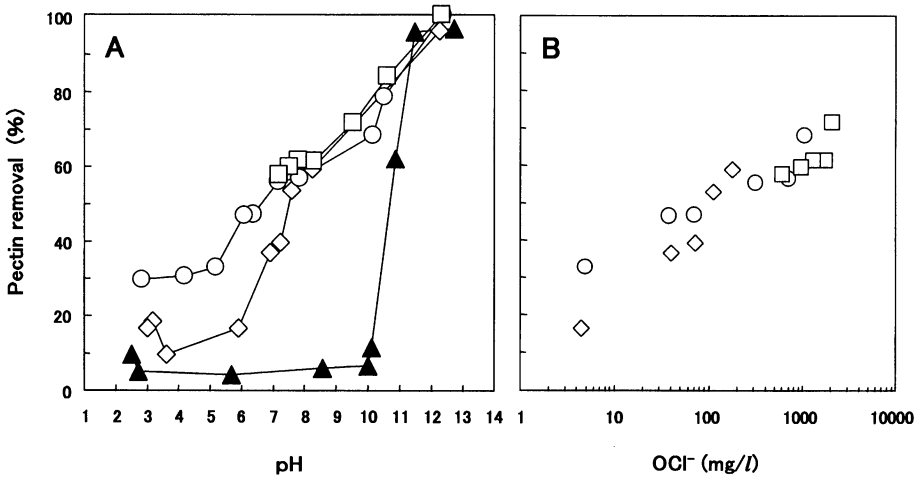

FIG. 2. Effects of pH and AC concentration on the efficiency of pectin removal from Al_2O_3 particles during batch cleaning. (A) Plotted as a function of pH; (B) plotted as a function of the OCl^- concentration. Cleaning conditions are given in the legend to Fig. 1. Symbols: ▲, Dilute HCl or NaOH solutions alone; ◇, 250 mg AC/*l* of NaOCl solution; ○, 1,100 mg AC/*l* of NaOCl solution; □, 2,200 mg AC/*l* of NaOCl solution.

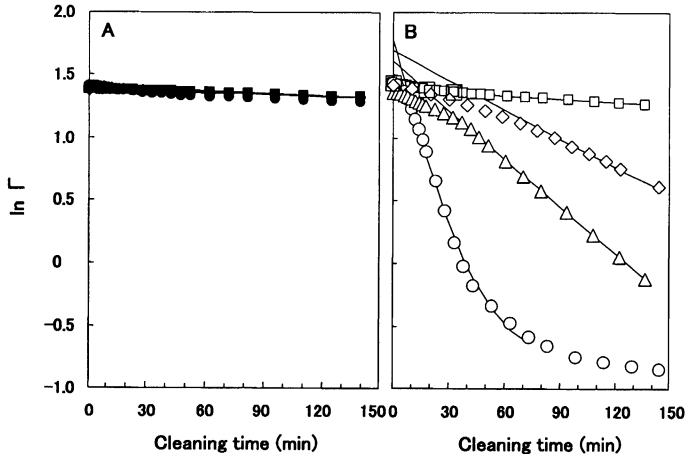

FIG. 3. Curves characterizing BSA removal from Al_2O_3 surfaces during continuous cleaning with dilute HCl or NaOH solutions (A) and with dilute NaOCl solutions (B). Cleaning was conducted in a stainless steel column at 40°C for 150 min by continuously feeding the dilute HCl or NaOH solutions, and NaOCl solutions at various pH from the bottom of the column at a space time of 1.5 min. Shaded symbols (HCl or NaOH solution): ■, pH 5.2; ◆, pH 7.2; ●, pH 10.3. Unshaded symbols (NaOCl solution):□, 1,030 mg AC/*l* at pH 5.2; ◇, 640 mg AC/*l* at pH 7.2; △, 890 mg AC/*l* at pH 7.5; ○, 1,020 mg AC/*l* at pH 10.3.

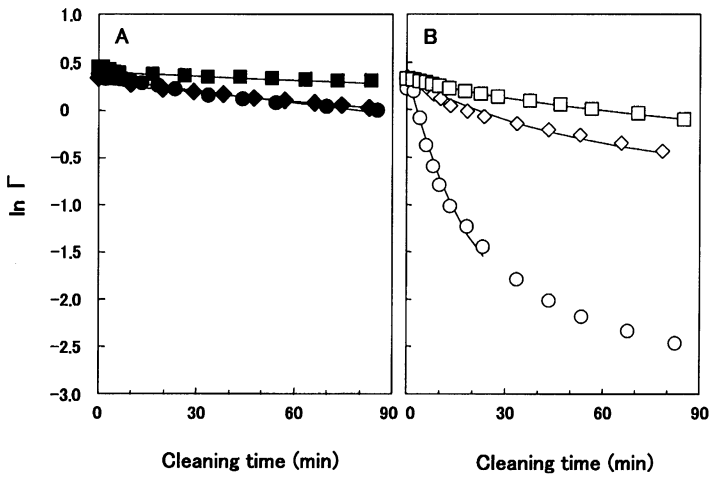

FIG. 4. Curves characterizing pectin removal from Al_2O_3 surfaces during continuous cleaning with dilute HCl or NaOH solutions (A) and with dilute NaOCl solutions (B). Cleaning conditions are given in the legend to Fig. 3. Shaded symbols (HCl or NaOH solution): ■, pH 5.2; ◆, pH 7.2; ●, pH 10.3. Unshaded symbols (NaOCl solution): □, 1,030 mg AC/*l* at pH 5.2; ◇, 640 mg AC/*l* at pH 7.2; ○, 1,020 mg AC/*l* at pH 10.3.

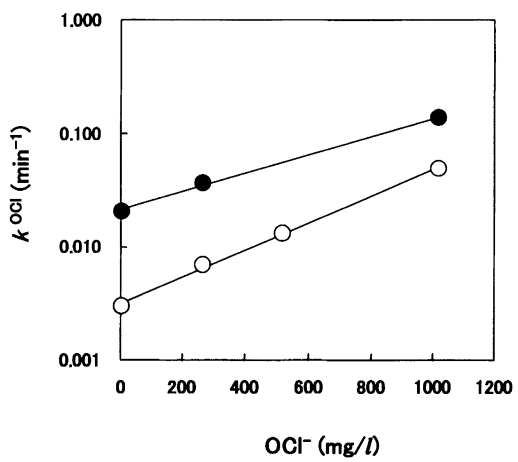

FIG. 5. The k^{OCl} values estimated for the removal of BSA or pectin as a function of the OCl^- concentration. Symbols: ○, BSA removal; ●, pectin removal.

from Al_2O_3 particles during continuous cleaning with and without NaOCl. In the cleaning without NaOCl (Fig. 3A), BSA removal proceeded at very low rate and removal values at each pH during 150 min of cleaning approximately formed a steady linear pattern. These suggest that BSA removal apparently follows first-order kinetics under these cleaning conditions (Eq. 3a). From the slopes of the graphs, the k^{OH} values at pH 5.2, 7.2, 10.3 were estimated to be 0.00043, 0.00039, and 0.00047 min^{-1}, respectively. On the other hand, the rate of BSA removal was accelerated markedly by the presence of NaOCl, especially at a higher pH (i.e., higher OH^- concentrations) (Fig. 3B). In addition, the lag period observed in the initial stage of cleaning became shorter with increasing pH. The lag phase appears to be the time that is needed for the diffusion of OCl^- into the dried BSA layer on Al_2O_3 particles and for reaching the steady state of the reaction of OCl^- with BSA. After a lag period, a fast desorption phase occurred under each given condition. To estimate the k^{OCl} from curves of BSA removal, the k^{OH} values estimated above were substituted into eq. 5, which was then fitted to the experimental data, except for the data in lag phase, based on a nonlinear regression method. For analysis of the cleaning with the NaOCl solution of pH 7.5, the average value (0.00043 min^{-1}) was substituted into Eq. 5. Solid lines in Fig. 3B were derived from the computer analysis (multiple correlation coefficient, R, = 0.996 to 0.999). The estimated k^{OCl} values at pH 5.2, 7.2, 7.5, and 10.3 were 0.0030, 0.0069, 0.0131, and 0.0493 min^{-1}, respectively.

Figure 4 shows curves characterizing pectin removal from Al_2O_3 particles during continuous cleaning with and without NaOCl. In the cleaning without NaOCl (Fig. 4A), the rate of pectin removal tended to increase slightly with increasing pH. In an each removal curve, small amounts of pectin (ca. 0.15 mg/m^2) were removed rapidly at the beginning of cleaning (~8 min), and afterwards removal values formed a linear pattern. From the slopes of the linear portions, the k^{OH} values at pH 5.2, 7.2, 10.3 were estimated to be 0.00126, 0.00307, and 0.00675 min^{-1}, respectively. NaOCl accelerated the pectin removal markedly, depending on pH (Fig. 4B). To estimate the k^{OCl} from curves of pectin removal, the k^{OH} values estimated above were substituted into Eq. 5, which was then fitted to the experimental data as described above, resulting in solid lines in Fig. 4B (R = 0.993 to 0.999). The estimated k^{OCl} values at pH 5.2, 7.2, 10.3

were 0.0207, 0.0363, and 0.137 min^{-1}, respectively.

The estimated k^{OCl} values for the removal of BSA or pectin were plotted against OCl^- concentrations (Fig. 5). The k^{OCl} values increased exponentially with the OCl^- concentration. Semi-log graphs of k^{OCl} versus OCl^- concentration for BSA and pectin gave the following relationship (R^2: correlation coefficient):

$$\text{BSA} : k^{OCl} = 0.0031 \times \exp(0.0027 \times [OCl^-]) \quad (R^2 = 0.998) \quad (6)$$

$$\text{Pectin} : k^{OCl} = 0.0213 \times \exp(0.0018 \times [OCl^-]) \quad (R^2 = 0.998) \quad (7)$$

Fragmentation after NaOCl cleaning

Variation in the molecular weights of BSA and pectin removed by NaOCl cleaning was examined by size-exclusion high-performance liquid chromatography (Fig. 6). In the cleaning with the NaOH solution of pH 12.5, the peaks assigned to removed BSA and removed pectin emerged at the same retention times (t_R) as those corresponding to native BSA and native pectin, respectively (dotted lines in Figs. 6A and 6B). The chromatogram of the BSA removed by the cleaning with the NaOCl solution (1,130 mg/l) at pH 10.3 (Fig. 6Ab) showed that the large single peak in Fig. 6Aa disappeared, whereas two peaks appeared at later t_R (arrow in Fig. 6Ab), after subtracting peaks assigned to the NaOCl solution (Fig. 6Ac). The above two peaks were fractionated in a lump, and this fraction was found to show a positive reaction to Folin-Ciocalteu reagent, a polypeptide-assay reagent (Lowry et al., 1951). This result suggested that the appeared peaks in Fig. 6Ab were assigned to low-molecular fragments (peptides) derived from the decomposition of BSA by the oxidative action of NaOCl. Similar peaks presumably assigned to the decomposed fragments were observed when BSA-fouled Al_2O_3 particles were once cleaned with the NaOCl solution (1,130 mg/l) at pH 5.3 followed by with the NaOH solution of pH 12.5 (data not shown). On the other hand, the chromatogram of the pectin removed by the cleaning with the NaOCl solution (1,130 mg/l, pH 10.3) exhibited one peak at the same t_R as that corresponding to native pectin (Fig. 6Bb). Any peaks assigned to fragments derived from pectin decomposition could not be detected due to the interference by the peaks assigned to refractive index of the NaOCl solution itself (Fig. 6Bb and 6Bc). In all fractions obtained at later t_R than that corresponding to native pectin in Fig. 6Bb, the pectin fragments could not be detected distinctly because of their very small amounts.

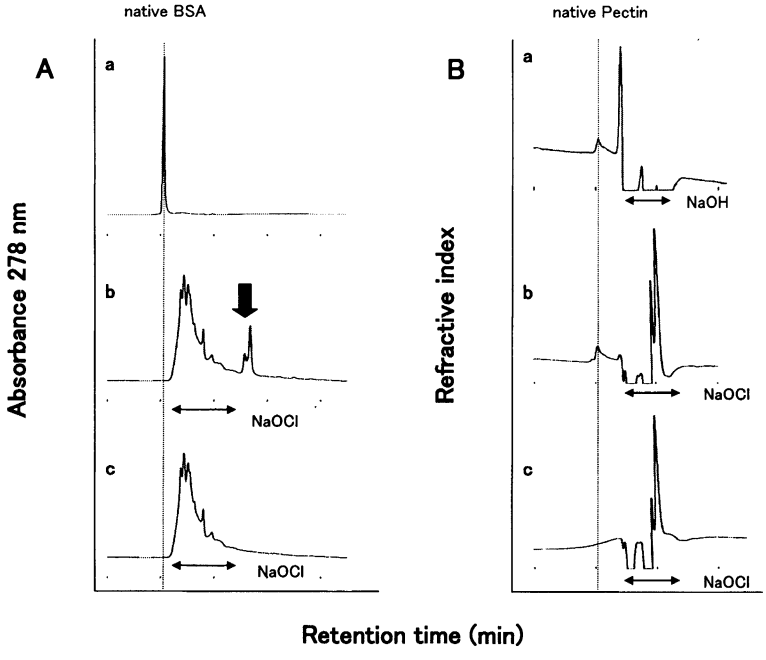

FIG. 6. Size-exclusion chromatograms of the removed BSA (A) and pectin (B) during batch cleaning. Curves Aa and Ba: molecules removed by NaOH solution at pH 12.6; Curves Ab and Bb: molecules removed by NaOCl solution (1,130 mg AC/l) at pH 10.3; Curves Ac and Bc: peaks assigned to the NaOCl solution (1,130 mg AC/l) at pH 10.3. Arrow in Fig. 6Ab denotes the peaks belonging to low-molecular-weight fragments (peptides). Chromatography was performed on Shodex SB-802.5HQ column at 278 nm (A) or by refractive index (B) with 50 mM phosphate buffer (pH 6.8) containing 0.3 M NaCl at a flow rate of 0.75 ml/min.

DISCUSSION

In this study, the action of NaOCl in the cleaning process was evaluated in the pH region where no significant amount of BSA or pectin was removed by the action of OH^- alone. The effect of NaOCl on the removal of BSA or pectin was enhanced by an increase in pH, i.e., OH^- concentration, and AC concentration. The pH dependence of the efficacy of NaOCl can be explained on the basis of the $HOCl-OCl^-$ equilibrium. It was shown that the efficiency (Figs. 1 and 2) and the rate (Figs. 3 to 5) of the removal of BSA or pectin depended on the OCl^- concentration. These findings imply that the presence of OCl^- plays an important role in the appearance of the action of NaOCl in the cleaning process.

It was clearly indicated that OCl^- was the active species in the removal of BSA (Fig. 1B). Contrary to the expectation, undissociated HOCl exerted little action in the removal of BSA in spite of its high concentrations. The primary function of OCl^- in BSA removal is to decompose the BSA molecules into low-molecular-weight fragments by its oxidative action (Fig. 6A). The Cl atom in HOCl and OCl^- behaves as Cl^+, a strong electrophile, and combines with a pair of electrons in parts of the substrate that have high electron densities (Wojtowicz, 1979). Among the functional groups of amino acid residues on the protein molecule, the C=C double bond, amino groups, and thiol groups are susceptible to the electrophilic attack of Cl^+ (Nightingale et al., 2000). The peptide bond (amide bond) becomes also a target of the Cl^+ attack since it has a partial double-bond character. Cysteine and methionine residues react rapidly with HOCl/OCl^- to give oxoacids and cystine (from Cys) (Winterbourn and Brennan, 1997) and sulphoxides (from Met) (Pereira et al. 1973). BSA is reported to contain 17 disulfide bonds between Cys residues (Foster, 1977). These disulfide bonds would be broken by the oxidative action of Cl^+ derived from $HOCl/OCl^-$. The chlorination reactions of proteins with $HOCl/OCl^-$ occur mainly on the free amino groups of amino acid residues, e.g., lysine and tyrosine, to give chloramines under physiological conditions (Clark et al., 1986; Domigan et al., 1995). Hawkins and Davies (1998 and 1999) have reported that free amino groups of lysine residues, which are present in many proteins at a much higher level than other reactive residues, are converted into unstable chloramines via the reaction with $HOCl/OCl^-$, which were in turn broken down into nitrogen-centered radicals via the homolysis of N–Cl bonds:

$$\begin{array}{l} R-CH_2-NH_3^+ \xrightarrow{HOCl/OCl^-} R-CH_2-NH_2^+-Cl \xrightarrow{\text{homolysis of N-Cl bond}} \\ R-CH_2-\dot{N}H_2^+ \rightleftarrows R-CH_2-\dot{N}H \end{array} \quad (8)$$

They concluded that the chloramine formation and nitrogen-centered radicals are key species involved in the $HOCl/OCl^-$-induced backbone fragmentation of proteins. In this study, the marked increase in the efficiency of BSA removal was also observed in the pH range of 6 to 10 (Fig. 1A). It is possible that some targets, e.g., amino groups, on proteins become more sensitive to OCl^- than to HOCl with varying concentrations of OH^-. Most of the side-chain amino groups on proteins are thought to be protonated at pHs below 10 because of their high pK_b values (e.g, pK_b of ε -NH_3^+ of lysine is 10.5). Protonated free amino groups are likely to be readily chlorinated by OCl^-, owing to the greater tendency of the amino groups to combine with anions (the first step in eq. 8). In this context, Koukouraki and Diamadopoulos (2003) reported that the amount of trihalomethane formed during the chlorination of nitrified wastewater increased by 2-fold for every 1.25 unit rise in pH from 6.0 to 8.5. Besides, the peptide bond become more susceptible to the Cl^+ attack at the high alkaline pH region (>11.5) since the electron density of the N atom increases by deprotonation. These findings suggest that the electophilic action of Cl^+ derived from OCl^- toward nucleophilic N atoms on BSA molecules can enhance the removal of BSA.

The active species involved in the removal of pectin was also OCl^-, whereas significant amounts of pectin were removed during the cleaning with NaOCl in the pH range of 3.0 to 6.5, where available chlorine in the NaOCl solution exists predominantly as HOCl (Fig. 2). This result suggested that the action of NaOCl in pectin removal was available in the forms not only of OCl^-, but also of HOCl to some extent. The difference between the OCl^- dependence in the removal of BSA and in that of pectin could probably be ascribed to the reactivity of their molecules with OCl^- and to their molecular structures. Pectin is composed mainly of α -1,4 linked D-galacturonic acid units with varying degrees of methylated carboxyl groups (methoxyl groups). Since pectin contains no amino groups, thiol groups, and unsaturated double bonds on the molecule, the reactivity of pectin with OCl^- appears to be relatively low. In fact, the chromatogram of the pectin removed by the cleaning with NaOCl showed a peak at the same t_R as that corresponding to native pectin although no evidence was obtained in the present study to reject the occurrence of the fragmentation of pectin molecule (Fig. 6B). It is thought that OCl^- reacts with the methoxyl groups ($-COOCH_3$) of galacturonic acids and converts

them into carboxyl groups (−COOH). The formation of free carboxyl groups on the pectin molecule leads to the increases in polarity and the solubility of pectin in aqueous solution. In addition, OCl^- ions, like as OH^-, are likely to strongly adsorb onto most metal oxide surfaces. It has been reported that oxoacids, such as HPO_4^{2-} and OCl_4^- ions, could replace base OH groups on Al_2O_3 particles by the ligand exchange phenomenon (Fukuzaki et al., 2002; Schulthess and Sparks, 1986). Attractive forces between pectin and positively charged Al_2O_3 surfaces are considered to be electrostatic interactions and hydrogen bonds between polar groups ($-COO^-$, −OH) on the pectin and base OH groups on Al_2O_3, like as on positively charged stainless steel surfaces (Takehara and Fukuzaki, 2002). Although there is insufficient data in our study to discuss the mechanism of the action of NaOCl at solid-liquid interfaces, it is speculated that OCl^- ions might adsorb onto basic sites on Al_2O_3 surfaces and partially displace or cleave the adsorbing groups on pectin from Al_2O_3 surfaces. Presumably, this displacement process by OCl^- might also partly contribute to the BSA removal.

The OCl^- concentration had a significant influence on both the efficiency and the rate of the cleaning. It was found that the k^{OCl} values for the removal of BSA or pectin increased exponentially with OCl^- concentration (Fig. 5). In most cleaning processes, unlike reactions occurred in liquid phase, reactions between cleaning chemicals and soils occur at solid-liquid interfaces. The penetration (diffusion) of OCl^- into the adsorbed layers of BSA or pectin is one of important factors governing the cleaning efficiency. The profile of OCl^- concentration within the adsorbed layers is a function of the OCl^- concentration in the bulk solution. In this study, the acceleratory threshold concentration of OCl^- for BSA removal was found to be 60 mg/l (Fig. 1A). A mass transfer barrier within the adsorbed layers appears to be the rate-limiting factor. The occurrence of considerably long lag phases during continuous cleaning of BSA-fouled Al_2O_3 particles (Fig. 3) suggests a mass transfer barrier imposed by the dried compact BSA layer. On the other hand, pectin is negatively charged at pHs greater than 2.7 and it has by far the more extended conformation than BSA (Takehara and Fukuzaki, 2002). Therefore, OCl^- and OH^- can readily diffuse into the expanded structure of pectin molecule even in the acidic pH region, giving shorter lag phases as shown in Fig. 4. The estimated k^{OH} and k^{OCl} values for pectin removal were larger by one order of magnitude than those for BSA removal. These findings imply that the adsorbed pectin is more susceptible to the cleaning actions of OCl^- and OH^- than BSA and/or that the relative binding strength of pectin on Al_2O_3 surfaces was lower than that of BSA. As discussed above, the reactions of OCl^- with BSA or pectin molecules are complicated and multiple. It is thought that an increase in OCl^- concentration enhanced the rate of the removal of BSA or pectin exponentially, presumably by changing the reaction equilibrium, by accelerating the reaction kinetics, and by increasing the solubility of reaction products.

Besides the dissociation of HOCl to OCl^-, another role of OH^- is to facilitate the mass transfer of OCl^- into the adsorbed layers of BSA or pectin. Hydroxyl ions give rise to dissociation (deprotonation) of functional groups on BSA, pectin, and Al_2O_3 surfaces and to make negative charges of their surfaces. The increase in the net negative charge of BSA or pectin leads to the higher degrees of swelling of the molecule and hence an expanded adsorbed layer. This allows easier access for OCl^- solution to diffuse into the adsorbed layers and to reach the contact surfaces between BSA or pectin and Al_2O_3 surfaces, resulting in facilitation of the attack of Cl^+ derived from OCl^-. BSA has a compact globular conformation due to the intramolecular interactions sustaining its tertiary structure in aqueous solution and forms a close-packed monolayer on Al_2O_3 particles (Urano and Fukuzaki, 2000). An elevated OH^- concentration also accelerates the dispersion of decomposed fragments into the aqueous phase by overcoming intramolecular interactions on BSA. On the other hand, low OH^- concentrations ($pH<6$) are not able to disperse the decomposed fragments even though undissociated HOCl or a minor OCl^- decomposes the BSA molecule partially. It is true that pretreatment of BSA-fouled Al_2O_3 particles by low concentrated gaseous ozone (<0.1%, v/v) results in the partial decomposition of BSA, but only small amounts of the decomposed BSA fragments can be removed by the subsequent rinse treatment with 10^{-3} M KNO_3 solution of pH 5.9 (Urano and Fukuzaki, 2001). It is reasonable that relatively higher OH^- concentrations are required for the expansion of BSA with a high internal stability than the more open-structured pectin.

The results presented here provide useful information concerning the cleaning and sterilizing operations based on the NaOCl solution. The concentration of dissociated OCl^- is a key factor determining the cleaning efficiency of the NaOCl solution, especially for proteinaceous soils. This implies that the optimal pH region of the cleaning activity of NaOCl differs from that of its germicidal activity. The results also confirmed the general concept that the combined use of hypochlolites and caustic alkali can provide an adequate cleaning treatment.

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