

Note

Cleaning of Alumina Fouled with Bovine Serum Albumin by the Combined Use of Gaseous Ozone and Alkaline Electrolyzed Water

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Alumina (Al_2O_3) particles fouled with bovine serum albumin (BSA) were cleaned with alkaline electrolyzed water and NaOH solutions of different pHs. The efficiency and rate of BSA desorption during batchwise and continuous cleaning with alkaline electrolyzed water depended on the solution pH, i.e., the hydroxide ion concentration. Pretreatment of BSA-fouled Al_2O_3 with 0.3% (v/v) gaseous ozone markedly facilitated BSA desorption during subsequent cleaning with alkaline electrolyzed water, depending on the length of pretreatment. The results suggest that the efficiency of cleaning with gaseous ozone and alkaline electrolyzed water (pH 11.7) in combination is comparable to that of cleaning with high-pH NaOH solutions (pH > 13.0) alone.

Key words : Alkaline electrolyzed water/Ozone/Cleaning/Protein fouling.

The control of microorganisms in food products and on surfaces of food-handling equipment is essential to provide consumers with wholesome and safe products. Products are often biologically contaminated from equipment surfaces, and therefore food residues that serve as nutrients for microorganisms on food-contact surfaces should be removed by cleaning after each processing operation. In the food and dairy industry, alkaline detergents and hypochlorites have so far been the most widely used chemicals in cleaning and sanitizing operations (Clegg, 1962; Mitchell, 1981; Twomey, 1968).

Recently, electrolyzed water has been receiving attention as a novel disinfectant and cleaning solution. When NaCl solution is electrolyzed through a membrane, acidic electrolyzed water (pH 2 to 3), containing hypochlorous acid (HOCl) which is generated from the reaction of Cl_2 and H_2O , is generated at the anode site, while alkaline electrolyzed water (pH 11 to 12) is generated at the cathode site. Acidic electrolyzed water has been demonstrated to have bactericidal activity against various types of bacteria

(Nakagawara et al., 1998; Ueda et al., 1999) and has been used for medical, agricultural, and industrial purposes. On the other hand, practical use of alkaline electrolyzed water is presently undefined and limited to preliminary cleaning before the disinfecting operation with acidic electrolyzed water. It is expected that for the purpose of cleaning, the alkalinity of alkaline electrolyzed water is not sufficient to solubilize organic fouling deposits on the equipment surface. However, the efficiency of cleaning with alkaline electrolyzed water is still not fully understood.

In our previous paper (Takehara et al., 2000), we reported that the efficiency of the alkali cleaning of alumina (Al_2O_3) fouled with bovine serum albumin (BSA) increased with increasing pH of the NaOH solution over the pH range of 12.0 to 13.5. Furthermore, it was found that pretreatment of BSA-fouled Al_2O_3 with gaseous ozone markedly accelerated the BSA removal during the subsequent alkali cleaning, even when a low-pH NaOH solution was used. This finding stimulated our interest in the application of alkaline electrolyzed water in combination with ozone pretreatment for cleaning operations.

The objectives of this study were to compare the ability of alkaline electrolyzed water and NaOH

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solutions of different pHs to remove protein from equipment surfaces, and to examine the effectiveness of the combined use of gaseous ozone and alkaline electrolyzed water for cleaning purposes in a laboratory model system. We used BSA as a model protein and Al_2O_3 particles as a model material having a hard surface, such as ceramic membrane and metallic materials, because all metal surfaces are covered with thin oxide layers under atmospheric conditions. Gaseous ozone at 0.3% (v/v) was used for the pretreatment of BSA-fouled Al_2O_3 particles.

α - Al_2O_3 particles (Sumicorundum AA-5; lot YM6601) were supplied by Sumitomo Chemical Co. Ltd. (Tokyo); their specific surface area and mean diameter were $0.5 \text{ m}^2/\text{g}$ and $4.6 \text{ }\mu\text{m}$, respectively. BSA (fraction V; lot M7G9556) was obtained from Nacalai Tesque Inc. (Kyoto). All other chemicals were of analytical grade and were purchased from commercial sources. Alkaline electrolyzed water was generated at the cathode site by electrolysis of 0.1% NaCl solution through a membrane using an electrolyzed water generator (Super Oxseed Labo; Amano Corp, Yokohama). The pH of the alkaline electrolyzed water was controlled by electrolysis time over the pH range of 10.5 to 11.7. Alkaline electrolyzed water and NaOH solutions of different pHs were freshly prepared before use. Ozone was generated from pure oxygen (99.999%, v/v) by a laboratory silent discharge ozonizer (Model 961; Ohnit Co. Ltd., Okayama) equipped with an ozone monitor (EG-2001; Ebara Jitsugyo Co. Ltd., Tokyo).

The BSA-fouled Al_2O_3 particles were prepared at 40°C as described previously (Takehara et al., 2000). The amount of adsorbed BSA (Γ) was $4.0 \pm 0.1 \text{ mg}/\text{m}^2$, suggesting a monolayer adsorption (Fukuzaki et al., 1996; Urano and Fukuzaki, 2000). Before alkali cleaning, a 2-g aliquot of the BSA-fouled Al_2O_3 particles was treated with 0.3% (v/v) gaseous ozone at room temperature and approximately 0% relative humidity (RH) for 8 to 120 min as described previously (Takehara et al., 2000).

Batchwise cleaning was conducted by introducing a 1-g portion of BSA-fouled Al_2O_3 particles and 5 ml of NaOH solution (pH 9.2 to 13.7) or 5 ml of alkaline electrolyzed water (pH 10.5 to 11.7) into 25-ml glass vial, which was then laid on its side in a water bath at 40°C and reciprocally shaken (140 oscillations per min) for 2 h. After being shaken, the vial was centrifuged at $2300 \times g$ for 10 min. The BSA concentration in the supernatant obtained, i.e., the amount of BSA removed (R), and the amount of BSA still remaining on the Al_2O_3 (S) were measured by the Lowry-Folin method as described previously (Lowry et al., 1951; Urano and Fukuzaki, 1997;). The efficiency of BSA

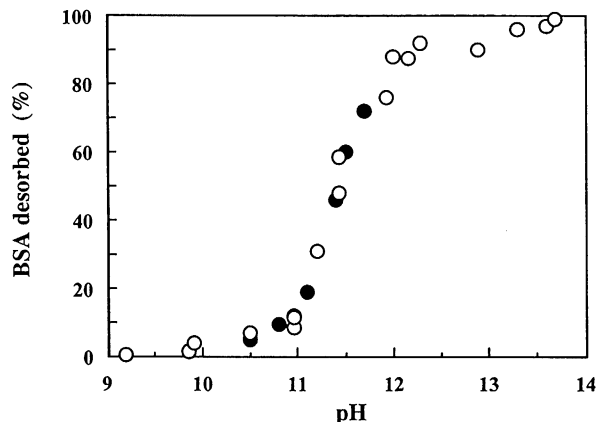


FIG. 1. Effect of pH on the desorption of BSA from Al_2O_3 when cleaned with a NaOH solution and alkaline electrolyzed water. Batchwise cleaning was conducted in a 25-ml glass vial for 120 min at 40°C with shaking (140 oscillations per min). Symbols: ○, cleaning with a NaOH solution; ●, cleaning with alkaline electrolyzed water.

desorption was expressed as the percentage of R to Γ $\{(R/\Gamma) \times 100\}$.

Continuous cleaning was conducted by introducing a 1-g portion of ozone-treated and non-ozone-treated BSA-fouled Al_2O_3 particles into a stainless steel column ($4 \text{ mm } \phi \times 50 \text{ mm}$) held vertically in a column oven at 40°C and then by feeding alkaline electrolyzed

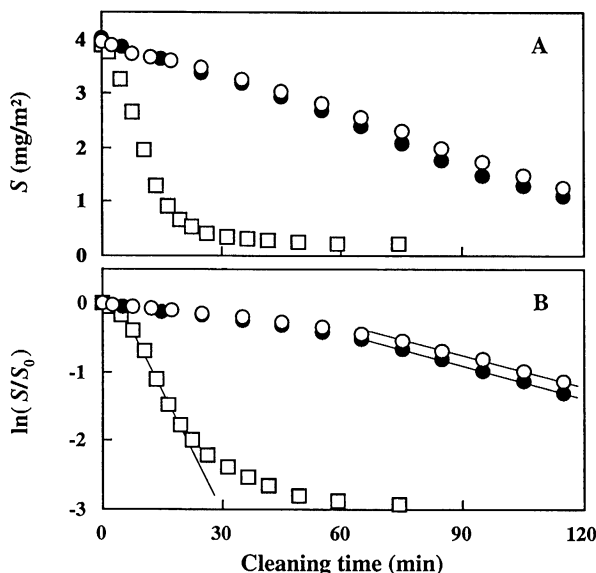


FIG. 2. Time courses of BSA desorption from Al_2O_3 surfaces during continuous cleaning with NaOH solutions and alkaline electrolyzed water. (A) S versus t , (B) $\ln(S/S_0)$ versus t . Continuous cleaning was conducted in a stainless steel column at 40°C for 120 min by continuously feeding the NaOH solution or alkaline electrolyzed water from the bottom of the column at a space time of 1.25 min. Symbols: ○, NaOH solution of pH 11.8; □, NaOH solution of pH 12.8; ●, alkaline electrolyzed water of pH 11.7.

water (pH 11.7) or NaOH solution (pH 11.8 and 12.8) from the bottom of the column at a flow rate of 0.3 ml/min (space time=1.25 min) (Takehara et al., 2000). The alkali eluent was fractionally collected, and its BSA concentration was measured by the Lowry-Folin method. The difference between the measured concentrations of ozone-treated and non-ozone-treated BSA due to the oxidation of Lowry-positive sites, i.e., tryptophan, tyrosine, and peptide bonds, was amended by comparing the standard curves for non-ozone-treated and ozone-treated BSA. After continuous cleaning, the particles were withdrawn from the column and S_r was determined by the Lowry-Folin method as described above.

Figure 1 shows the effect of pH of the NaOH solution and alkaline electrolyzed water on the efficiency of BSA desorption from the Al_2O_3 surfaces during batch cleaning. In the case of cleaning with NaOH solution, the efficiency of BSA desorption was considerably low at pHs lower than 11, but above which it increased gradually with increasing pH and reached about 98% at pH 13.7. The removal of BSA by cleaning with alkaline electrolyzed water also depended on pH. The relationship between the efficiency of BSA desorption and pH obtained for cleaning with alkaline electrolyzed water over the pH range of 10.5 to 11.7 almost coincided with that obtained for cleaning with the NaOH solution. These results indicated that the ability of alkaline electrolyzed water to remove BSA from Al_2O_3 depended on pH, i.e., hydroxide ion concentration.

Figure 2A shows time course changes in the amount of residual BSA (S) on Al_2O_3 surfaces during continuous cleaning with NaOH solutions of pH 11.8 and 12.8, and with alkaline electrolyzed water of pH 11.7. During 75 min of cleaning with the NaOH solution of pH 12.8, BSA was readily desorbed with a short lag time and approximately 95% fraction of adsorbed BSA was removed ($S_r=0.21$ mg/m²). On the other hand, BSA desorption occurred very slowly and similar desorption rates were observed when using the NaOH solution of pH 11.8 and alkaline electrolyzed water. Figure 2B shows semilogarithmic graphs of the data in Fig. 2A, in which the ordinate is the natural logarithm of the ratio of S at any time t to the amount of BSA at time 0 ($S_0 = \Gamma$). Assuming that the rate of BSA desorption is directly proportional to S , as proposed by Jennings (1965), the following first-order equation will become valid:

$$-dS/dt = kS \quad (1)$$

where k is the first-order desorption rate constant. On integration and rearrangement, eq. 1 gives the following:

$$\ln(S/S_0) = -kt \quad (2)$$

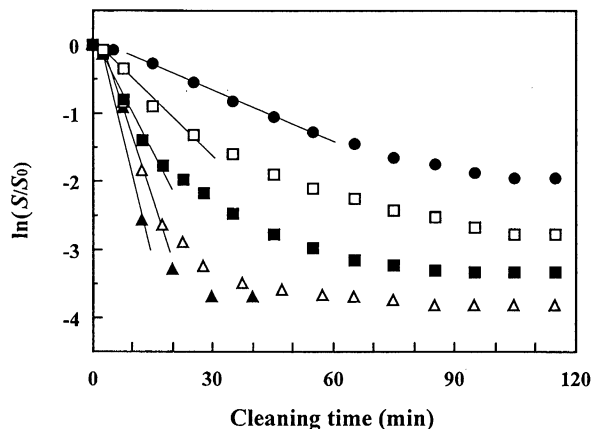


FIG. 3. Time courses of BSA desorption from Al_2O_3 surfaces during continuous cleaning with alkaline electrolyzed water combined with ozone pretreatment. Before cleaning, BSA-fouled Al_2O_3 particles were pretreated with 0.3% (v/v) gaseous ozone for 8 (●), 15 (□), 30 (■), 60 (△), or 120 min (▲) at room temperature and ca. 0% RH. Cleaning conditions are given in the legend to Fig. 2.

so that the graph of $\ln(S/S_0)$ versus t is expressed as a straight line. However, for instance, the whole curve of BSA desorption during cleaning with the NaOH solution of pH 12.8 was not described by simple first-order kinetics. This suggests that there exists different types of conformations of BSA on Al_2O_3 surfaces. Nevertheless, the linear portion of BSA desorption occurs in the initial stage of cleaning, indi-

TABLE 1. The k and S_r values obtained for cleaning with alkaline electrolyzed water (pH11.7) with and without ozone pretreatment, and for that with NaOH solutions of different pHs.

Caustic solution	Ozone (0.3%, v/v) pretreatment time (min)	k (min ⁻¹)	S_r (mg/m ²)
Alkaline electrolyzed water (pH11.7)	—	0.014	1.27
	8	0.025	0.54
	15	0.060	0.25
	30	0.128	0.14
	60	0.166	0.09
NaOH solution	120	0.242	0.09
pH 11.8	—	0.015	1.11
pH 12.8	—	0.102	0.21
pH 13.1 ^a	—	0.138	0.13
pH 13.5 ^a	—	0.240	0.12
pH 13.7 ^a	—	0.240	0.10

BSA-fouled Al_2O_3 particles were pretreated with 0.3% (v/v) gaseous ozone at room temperature for 8 to 120 min. For cleaning conditions, see the legend to Fig. 2.

^aReported by Takehara et al. (2000).

cating that BSA desorption in this phase apparently follows first-order kinetics. Similarly, the desorption curves during the cleaning with the NaOH solution of pH 11.8 and alkaline electrolyzed water also had linear portions in parts. The slope of the linear portion is considered to be a measure sufficient to assess the cleaning rate (Jennings, 1965; Nagata et al. 1995; Sarkar and Chatteraj, 1996). Therefore, we evaluated the k from the slope of the linear portion of each desorption curve as a measure of the cleaning rate. The k values obtained for cleaning with the NaOH solution of pH 11.8 and 12.8, and alkaline electrolyzed water (pH 11.7) were 0.014, 0.102, and 0.015 min^{-1} , respectively (see Table 1). Apparently, the rate of BSA desorption during cleaning with alkaline electrolyzed water also depended on pH.

The effect of pretreatment with 0.3% gaseous ozone for 8 to 120 min on the efficiency of cleaning with alkaline electrolyzed water was examined. Figure 3 shows the curves of BSA desorption from Al_2O_3 surfaces during continuous cleaning with alkaline electrolyzed water (pH 11.7) combined with ozone pretreatment. Pretreatment of BSA-fouled Al_2O_3 with ozone was found to markedly facilitate the desorption of BSA. The effect of ozone pretreatment on the rate of BSA desorption clearly depended on the length of pretreatment. In addition, S_r value decreased depending on the length of pretreatment. The effect of ozone pretreatment on facilitation of BSA desorption can probably be attributed to the partial decomposition of the adsorbed BSA molecules by ozone, which in turn could accelerate the swelling and dispersion of peptized fragments in alkaline electrolyzed water (Takehara et al., 2000).

For all the desorption curves in Fig. 3, the k values were also evaluated from the slopes of the linear portions of the desorption curves in the initial stage of cleaning. The k and S_r values obtained for cleaning with gaseous ozone and alkaline electrolyzed water in combination are summarized in Table 1. By conducting ozone pretreatment for 120 min, a higher k value of 0.24 min^{-1} was achieved even with alkaline electrolyzed water of pH 11.7. The S_r value also decreased from 1.27 (non-ozone-treatment) to 0.09 mg/m^2 by lengthening the pretreatment to 60 and 120 min. We reported that the k and S_r values obtained for cleaning with NaOH solution of pH 13.1 to 13.7 alone under the same conditions as this study were between 0.14 and 0.24 min^{-1} and between 0.13 and 0.10 mg/m^2 , respectively (Takehara et al., 2000) (see Table 1). These values were in good agreement with the k and S_r values obtained for cleaning with alkaline electrolyzed water combined with ozone pretreatment for 30 to 120 min. Thus, ozone pretreatment

greatly improved the efficiency of cleaning of BSA-fouled Al_2O_3 with alkaline electrolyzed water.

In a laboratory model system, it was suggested that the efficiency of cleaning of BSA-fouled Al_2O_3 with the combined use of gaseous ozone and alkaline electrolyzed water is comparable to that of cleaning with high-pH NaOH solutions ($\text{pH} > 13.0$) alone. Although application of ozone pretreatment is limited by ozone-resistant equipment material, alkaline electrolyzed water appears to be available for the cleaning of protein-fouled surfaces when used in combination with gaseous ozone.

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