Cleanability of Stainless Steel Surfaces as Influenced by Heat Treatment

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The cleanability of heat-treated 316L stainless steel particles treated at various temperatures of 100 to 500°C was studied in a plug-flow column fed by a 0.1M NaOH solution. Bovine Serum albumin (BSA) was used as the model fouling agent. Heat treatment resulted in the enrichment of iron in passive films on stainless steel particles depending on temperature. The degree of surface hydroxylation and the apparent surface charge density (σ_app) of stainless steel particles decreased markedly with increasing heating temperatures. The saturation amounts of BSA adsorbed (Γ_sat) were larger on the particles heated at higher temperatures. No correlation was observed between the Γ_sat and σ_app values. With increasing heating temperatures, the rate of BSA desorption from stainless steel particles decreased gradually in the initial and later stages of cleaning, resulting in larger amounts of BSA remaining on the particles at the end of 120-min of cleaning. The susceptibility to BSA adsorption and the cleanability were found to be correlated with the iron content of the passive films on stainless steel particles. It could be suggested that the decrease in the cleanability was probably due to the formation of iron-enriched passive films with lower degrees of surface hydroxylation of stainless steel particles due to heat treatment.

Key words: Cleanability/Stainless steel/Heat treatment/Iron-rich passive film/Affinity for protein adsorption.

INTRODUCTION

Austenitic stainless steels are widely used not only as construction materials for process plants in the biotechnology, pharmaceutical, and food industries but also as implant materials in the medical area (EHEDG, 1993a; Suzuki, 2002). These alloys fulfill many requirements regarding corrosion resistance, mechanical and thermal strength, longevity, and ease of fabrication. The chromium in these alloys produces their highly stain-resistant characteristics through the formation of chromium-rich oxide films on the surfaces. The passive film on stainless steel is self-healing as long as it has enough oxygen and water if damaged chemically or mechanically. In addition to the above attributes, the cleanability of stainless steel equipment is a matter of great concern in hygienic plant operations. One approach to improve the cleanability is to provide the smoothness to the product-contact surfaces by mechanical polishing and electropolishing (EHEDG, 1993b, 1993c). On the other hand, the nature of passive film on stainless steel varies widely depending on the surface polishing conditions (Asami and Hashimoto, 1979; Warzee, 1965). Besides the surface physical characteristics, the chemical properties of the stainless steel surface can also influence fouling and cleaning processes because the adsorption and desorption behaviors of fouling materials are physicochemical events at solid-liquid interfaces (Fukuzaki et al., 2001a, 2001b; Karlsson et al., 1999, 1998). However, the influence of the surface chemical composition of stainless steel on the cleanability has not been fully understood.

Equipment and cookware made of stainless steel
are often exposed to high-temperature conditions. For instance, inner surfaces of heat exchangers, evaporators, steam-fed piping systems, tanks, vessels, and implant devices are frequently heated at various temperatures in the liquid and gas phases during manufacturing, cleaning, sterilization, or machining processes. It has been reported that discoloration of stainless steel occurred after being brought into contact with high-temperature steam (Suzuki, 2002; Suzuki et al., 1998). The authors have found that the surface charge property of stainless steel changed after heat treatment under atmospheric conditions (Fukuzaki et al., 1998). These heat-induced surface variations are apparently accompanied by a variation in the surface chemical composition of stainless steel, which may in turn affect the magnitude of the forces of interaction between the stainless steel and fouling materials. Among fouling phenomena, adsorption of the protein onto a contact solid surface takes place spontaneously and often causes a severe problem for most biological fluid-handling equipment (Lalande et al., 1985; Norde, 1986; Wahlgren and Arnebrant, 1991). Therefore, knowledge of the surface chemical properties of heat-treated stainless steel is required to reduce the susceptibility of stainless steel to protein adsorption and improve its cleanability.

The aim of this study is to quantitatively investigate the fouling and the cleanability of heat-treated stainless steel at various temperatures under a normal atmosphere. To focus the discussion on physicochemical features of the adsorption and desorption behaviors, we used nonporous stainless steel particles and bovine serum albumin (BSA) as the model fouling agent. The cleaning experiment was conducted in a plug-flow column fed by 0.1M NaOH solution, where the hydrodynamic forces are extremely low. This system is well suited for investigating the influence of the physicochemical binding of BSA on desorption behavior. The curve of BSA desorption was kinetically analyzed as a function of time. The cleanability of the heat-treated stainless steel particles was discussed in relation to the iron content in the passive film on the particles and the binding strength of BSA.

MATERIALS AND METHODS

Materials and chemicals

Nonporous 316 L stainless steel particles were obtained from The Nalaco Corp. (Tokyo); their specific surface area and mean diameter were 0.38 m²/g and ca. 9 μm, respectively. Before use, stainless steel particles were washed in acetone, then in ethanol with sonication, and dried at 150°C for 4 h. Each 20-g aliquot of stainless steel particles was heated in a furnace under a normal atmosphere at various temperatures from 100°C to 500°C for 4 h. After the heat treatment, the particles were transferred into a 50-ml grass centrifugation tube and washed with 30ml of deionized water five times by centrifugation (420 × g for 1 min). After being washed, the particles were dried at 40°C for 24h. Crystalline BSA (Lot M9B4259) was purchased from Nacalai Tesque Inc. (Kyoto) and used as received. All other chemicals were of analytical grade and purchased from commercial sources.

Adsorption of BSA

Adsorption of BSA onto stainless steel particles was conducted in the presence of 10⁻⁶M KNO₃ at 40°C and at the apparent point of zero charge, pzcapp, of BSA (pH 5.0) as described previously (Takahashi and Fukuzaki, 2003). To examine the saturation amount of the BSA adsorbed (Γsat), the initial concentration of 5 g/l of BSA was used. For cleaning experiments, in order to unify the initial amount of BSA adsorbed (Γo) on each of the heat-treated particles, the initial concentration of BSA of 0.1 g/l was used, resulting in the Γo of ca. 1.3 mg/m². The amount of BSA adsorbed was determined as the sum of the amount of BSA removed and that of BSA still remaining on the particles (Sr) after batchwise treatment with 0.1M NaOH for 2 h with shaking (140 oscillations per min) as described previously (Fukuzaki et al., 2001a). BSA in solution or on stainless steel particles was determined by Lowry-Folin method as described previously (Fukuzaki et al. 2001a).

Cleaning experiment and kinetic analysis

The cleaning experiment was conducted at 40°C in a plug-flow column (4mm φ × 50mm) fed by 0.1M NaOH solution (space time: 2 min) for 120 min as described previously (Takahashi and Fukuzaki, 2003). After a 120-min cleaning operation, the particles were then withdrawn from the column and the amount of BSA remaining on the particles (E') was determined. The curve of BSA desorption was obtained by plotting the logarithm of the amount of residual BSA against cleaning time. To analyze the nonlinear curve of BSA desorption, the concept that two first-order reactions occur independently and simultaneously during the cleaning process was used (Urano and Fukuzaki, 2002):

\[ \ln \Gamma = \ln(\exp(\ln \Gamma_o - k't) + \exp(\ln \Gamma_o - k''t)) \]

where Γ is the amount of adsorbed BSA at any given time t, Γ_o and Γ_o (including Γ_o) are the amounts of a faster-desorbing BSA (BSA') and a slower-
desorbing BSA (BSA\textsuperscript{+}) at zero time, respectively; $k_1$ and $k_2$ are the desorption rate constants of BSA\textsuperscript{+} and BSA, respectively. The kinetic constants were calculated by a data-fitting procedure based on a nonlinear least-squares regression method (Urano and Fukuzaki, 2002).

Analyses

The apparent surface charge density ($\sigma_{\text{app}}$) and the apparent point of zero charge ($pzc_{\text{app}}$) of stainless steel particles were determined by potentiometric titration (Fukuzaki et al., 2001a). The isotherm for water vapor adsorption onto stainless steel particles was determined volumetrically at 25°C by using an automatic adsorption apparatus equipped with a data-processing computer (Fukuzaki et al., 2001a). The chemical composition of the outermost surfaces of stainless steel particles was determined by X-ray photoelectron spectroscopy (JPS-9200; JEOL Ltd., Tokyo) using a monochromatized AlK\textsubscript{x} radiation at 300 W (12 kV). For the analysis, stainless steel particles were molded into a plate (13 mm diameter × 1 mm thickness) by compression (20 MPa, 2 min). To compensate for sample charging during analysis, all the binding energies were referred to the carbon 1s signal at 284.7 eV.

RESULTS

Characterization of heat-treated stainless steel particles

When stainless steel particles were heated at temperatures above 300°C, the color (silver) of the particles turned light bronze (300°C), bronze (400°C), and violet (500°C). These interference colors suggested variations in the chemical composition and thickness of passive films on the particles. Table 1 shows the atomic composition of main metal components of the passive films on heat-treated stainless steel particles at various temperatures. Iron, chromium, and oxygen were detected as the main components of the passive films on all the heat-treated particles. Significant differences were observed in the contents of iron in the surfaces. With increasing heating temperatures from 100 to 500°C, the iron content increased from 6 to 11 atom%. Figure 1 shows the XPS spectra of the passive films on heat-treated stainless steel particles. For all the particles, the peaks of the Fe 2p\textsubscript{3}/2 spectra and the Cr 2p\textsubscript{3}/2 spectra were approximately fitted with Fe\textsuperscript{3+}-oxide (Fe\textsubscript{2}O\textsubscript{3}) at 710.8 ± 0.1 eV and with Cr\textsuperscript{3+}-oxide (Cr\textsubscript{2}O\textsubscript{3}) at 576.6 ± 0.1 eV, respectively (Rossi and Elsener, 1995). The signal at 706.8 eV, which is assigned to Fe in the metallic state, disappeared gradually with increasing heating temperatures. The peaks of the O 1s spectra of all the particles were situated at binding

![FIG. 1. XPS spectra of Fe 2p\textsubscript{3}/2 (A), Cr 2p\textsubscript{3}/2 (B), and O1s (C) of the passive films on heat-treated stainless steel particles at various temperatures. Spectra: (a) 100°C; (b) 200°C; (c) 300°C; (d) 400°C; (e) 500°C.](image-url)
energy values between 530.0 ± 0.2 eV of M-O-M oxygen ("M" denotes metals) and 531.3 ± 0.25 eV of M-OH oxygen (Rossi et al., 2000). It was observed that the peak of the O1s signal was shifted gradually to the binding energy assigned to oxygen in M-O-M with increasing heating temperatures up to 500°C. These results indicated that the transformation of M-OH oxygen into M-O-M oxygen and the formation of Fe2O3-enriched passive films on stainless steel particles occurred depending on the heating temperature.

To evaluate the variation in the degree of surface hydroxylation of heat-treated stainless steel particles, isotherms for water vapor adsorption on the particles were measured. Figure 2 shows the monolayer saturation amounts of water vapor adsorbed, as expressed in volume per square meter ($V_m$) and in molecule per square nanometer ($N_m$), determined by the BET analysis of the isotherms. With increasing heating temperatures from 100 to 500°C, the $V_m$ decreased gradually from 0.31 to 0.21 m$^3$/m$^2$, corresponding to the $N_m$ of 7.5 to 5.1 molecules/nm$^2$. This indicated that the number of adsorption sites for water vapor, i.e., OH groups, on stainless steel surfaces decreased through the condensation dehydration at higher temperatures. Assuming that one H$_2$O molecule is adsorbed on two surface OH groups, the number of adsorption sites on heat-treated particles at 100 and 500°C were estimated to be approximately 15 and 11 OH groups/nm$^2$, respectively, which corresponds to approximately 25 and 18 μmol/m$^2$.

Figure 3 shows the $\sigma_{app}$ curves of heat-treated stainless steel particles as a function of pH. The $\sigma_{app}$ of the particles decreased over the pH range of 4 to 10 depending on temperature. These changes were due to the removal of surface OH groups on stainless steel particles by condensation dehydration during heat treatment, as well as to the variation in the surface chemical composition.

The specific surface area of heat-treated stainless steel particles at 100 to 500°C ranged between 0.37 to 0.42 m$^2$/g, suggesting that surface roughness changed slightly. However, in the case of BSA with a molecular size of the nm order, unlike microorganism cells with a size of the μm order, the possible influence of surface roughness of heat-treated particles on the adsorption and desorption behaviors of BSA is expected to be negligible.

**Adsorption of BSA**

Experiments were conducted to examine the $\Gamma_{sat}$ on each of the heat-treated stainless steel particles at various temperatures. To minimize the effects of electrostatic interaction between BSA molecules on their adsorption, BSA adsorption was conducted at the $pzc_{app}$ of BSA, i.e., pH 5.0, where all the particles were positively charged (see Fig. 3). Figure 4 shows the $\Gamma_{sat}$ values on all the heat-treated particles. Compared with the $\Gamma_{sat}$ (3.7 mg/m$^2$) on the particles heated at 100°C, slightly smaller $\Gamma_{sat}$ values were obtained on the particles heated at 150 and 200°C. On the other hand, the $\Gamma_{sat}$ tended to increase gradually at heating temperatures above 200°C although the $\sigma_{app}$ value decreased markedly as shown in Fig. 3. The $\Gamma_{sat}$ appears to be not correlated with $\sigma_{app}$ value at pH 5.0.

On all the heat-treated particles, adsorbed BSA
FIG. 4. \( \Gamma_{\text{sat}} \) and \( S_r \) values for heat-treated stainless steel particles treated at various temperatures. The adsorption experiment was conducted at 40°C and at pH 5.0. The error bars represent the deviations of the three replicate experiments. Symbols: \( \bigcirc \), \( \Gamma_{\text{sat}} \); \( \triangle \), \( S_r \).

FIG. 5. Curves characterizing BSA desorption from heat-treated stainless steel particles at various temperature during cleaning with 0.1 M NaOH. The cleaning experiment was conducted at 40°C in a plug-flow stainless steel column installed vertically. Symbols (heating temperature): \( \blacksquare \), 100°C; \( \bigcirc \), 150°C; \( \bullet \), 200°C; \( \triangle \), 300°C; \( \blacktriangle \), 400°C; \( \bigdiamond \), 500°C.

Molecules showed irreversibility in adsorption after repetitious rinsing with 10^{-3} M KNO_3 solution, whereas desorption of the molecules was observed after batchwise treatment with 0.1M NaOH solution with shaking. The amount of BSA remaining on the particles (\( S_r \)) after the NaOH treatment also increased with increasing heating temperatures from 200 to 500°C.

### Cleanability of heat-treated stainless steel particles

In order to facilitate the comparison of the cleanability, the \( \Gamma_0 \) values for each of heat-treated stainless steel particles at various temperatures were almost unified at 1.3±0.1 mg/m² because the rate of BSA desorption depends on \( \Gamma_0 \) values (Takahashi and Fukuzaki, 2003). Figure 5 compares curves characterizing BSA desorption from the heat-treated particles during cleaning with 0.1M NaOH solution. Significant differences in cleanability between the heat-treated particles at lower temperatures (100 to 200°C) and those at higher temperatures (400 and 500°C) were evident during the overall cleaning operation. High-temperature heating resulted in lower rates of BSA desorption and in large amounts of \( \Gamma_0 \) in comparison to low-temperature heating. The solid lines in Fig. 5 were derived from the computer analysis using eq. 1 based on a nonlinear regression method. The estimated constants of eq. 1 for each curve are summarized in Fig 6. For the heat-treated particles at 100 to 200°C, the values of all the constants were almost equivalent to each other, whereas they differed much from the constants obtained for the particles treated at 400 and 500°C. The proportion of \( \Gamma_0^s \) to \( \Gamma_0 \) became large at temperatures above 300°C (Fig. 6A). The \( k^s \) and \( k^t \) values for heat-treated particles at 400 and 500°C were approximately 1.2-
fold and 1.5-fold as small as those for heat-treated particles at 100 to 200°C, respectively (Figs. 6B and 6C). These results indicated that the cleanability of stainless steel particles decreased by high-temperature heating treatments.

**DISCUSSION**

The results showed that cleanability of stainless steel particles was influenced by heat treatment depending on temperature. The heat-treated stainless steel particles at 400 and 500°C were found to have higher affinity for BSA adsorption and to be less cleanable than the particles treated at 100 to 200°C. The surfaces of heat-treated particles at higher temperatures were characterized by the formation of Fe₂O₃-enriched passive films, low degrees of surface hydroxylation, and low σ_app values. Apparently, differences in cleanability between the heat-treated particles at lower and higher temperatures were probably due to the variation in the above-mentioned surface characteristics of the particles induced by heat treatment.

The Γ_sat on heat-treated stainless steel particles ranged between 3.1 and 4.2 mg/m², corresponding to less than a monolayer coverage of BSA adsorbed, which amounts to approximately 4 mg/m² for a closely packed monolayer (Norde and Giacomelli, 2000). In addition, BSA was irreversibly adsorbed on all the particles. Thus, the BSA molecules (BSA⁺ plus BSA⁻) were suggested to be directly on surfaces of all the heat-treated particles. In the temperature range of 100 to 200°C, where little variation in the surface chemical composition occurred, the Γ_sat decreased slightly in proportion as the σ_app of the particles decreased at pH 5.0. BSA is adsorbed on positively charged stainless steel surfaces (M-OH₂⁺) through varying numbers of carboxyl groups on the molecule (Fukuzaki et al., 1998). In our previous studies, reducing the positive σ_app values of stainless steel particles, with or without the enrichment of chromium, made the particles less prone to BSA adsorption (Fukuzaki et al., 2001a, 2001b). Therefore, the decrease in Γ_sat could be interpreted as arising from the decrease in the positive σ_app of stainless steel particles at pH 5.0. On the other hand, the Γ_sat and Sₙ increased gradually with increasing heating temperatures from 200 to 500°C although the σ_app of the particles decreased successively. Thus, no correlation was observed between Γ_sat and σ_app, as well as between Sₙ and σ_app, in the temperature range of 100 to 500°C. These findings indicated that the σ_app value is not necessarily a predominant factor determining the Γ_sat value when the surface chemical composition varies considerably. It is of interest to note that the increase in the Γ_sat was correlated with the iron content, i.e., Fe₂O₃, in passive films on the heat-treated particles (see Table 1).

It is also noted that the cleanability of heat-treated particles depended on the iron content in the passive films. Kinetic analysis of curves of BSA desorption showed that the k⁺ and k⁻ for the Fe₂O₃-enriched passive films formed on the particles at higher temperatures were lower than those for the particles treated at lower temperatures. In addition, the proportion of a slower-desorbing BSA⁺, i.e., Γ⁺, and hence Γ, on stainless steel particles increased by high-temperature heating treatments. In addition, the Γ⁺ values were in good agreement with the S values. In the present study, the cleaning experiment was carried out in a plug-flow column, where the line velocity of the NaOH solution was approximately 0.33 mm/s (Takahashi and Fukuzaki, 2003). The hydrodynamic forces and shearing stresses in the column were extremely low. Under these conditions, the primary contribution of the NaOH solution is to minimize the physicochemical binding forces between BSA and stainless steel surfaces. Therefore, the cleanability can reflect the magnitude of the forces to hold BSA on stainless steel surfaces. It is reasonable that Fe₂O₃-enriched passive films have higher affinity for BSA adsorption than the particles treated at lower temperatures even though the positive σ_app values were low. It is believed that adsorption of proteins on hydrophilic stainless steel surfaces is electrostatic in nature, whereas carboxyl groups on the proteins are suggested to play an important role in non-electrostatic interactions in excess of the coulombic term, i.e., covalent or coordinate bonding, (Sakiyama et al., 1999; Urano and Fukuzaki, 2002). It has been reported that carboxyl groups of small organic acids can be adsorbed on goethite (α-FeOOH) by inner sphere complexes (Filius et al., 1997; Parfitt et al., 1977; Tejedor-Tejedor et al., 1992). Inner sphere complexes are characterized by direct coordination of one or more oxygen atoms of carboxyl groups to the Fe atoms of goethite, giving a larger binding strength per bond than electrostatic interaction, i.e., outer sphere complexes. At present, we are unable to draw definite conclusions, but it is conceivable that the enrichment of Fe₂O₃ in the passive films might allow BSA to strongly interact with stainless steel surfaces, resulting in larger Γ⁺ and hence lower cleanability.

Apart from the enrichment of Fe₂O₃ in the passive films, lower degrees of surface hydroxylation on stainless steel particles may influence the susceptibility of heat-treated surfaces to the cleaning action of


