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Silver Dispersed Stainless Steel with Antibacterial Property

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Kawasaki Steel has developed Ag-bearing stainless steels having an antibacterial property, namely an effect of suppressing the growth of bacteria, in response to the recent tendency to emphasize hygiene and cleanliness. In an antibacterial activity test, Ag-bearing stainless steels showed antibacterial activity with respect to O-157, MRSA, *Salmonella* as well as *Escherichia coli* and *Staphylococcus aureus*. In a cyclical corrosion test, Ag-bearing stainless steels showed the same corrosion resistance as Ag-free stainless steel. It was found that Ag-bearing stainless steel has both excellent antibacterial property and corrosion resistance.

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Silver Dispersed Stainless Steel with Antibacterial Property*


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1 Introduction

Since the incident of the mass O-157 infection in 1996, the consciousness of hygiene safety in food preparation staffs has been rapidly raised among their customers. Furthermore, food poisoning caused by *Staphylococcus aureus* and hospital infection by MRSA frequently occurred in 2000 and public concern about sanitary management systems in enterprises and hospitals has also risen. In response to such social circumstances, many antibacterial products[1~3] such as sanitation fixtures, plastic products and articles of clothing have been developed in various fields. (Here in this article, "antibacterial" is defined as "suppression of bacteria proliferation" and is differentiated from "sterilization".) Although stainless steel has been positively used in many places requiring sanitary management including kitchens and food processing factories because of its excellent corrosion resistance and feeling of surface cleanliness, antibacterial activity is not provided in ordinary stainless steel.[4] Furthermore, when film coatings containing antibacterial materials are applied to stainless steel, as is done with other antibacterial products[2], the film is removed through polishing and press working and also through daily washing and as a result, the antibacterial property deteriorates.

Nakamura et al.[4] found that an antibacterial effect against *Escherichia coli* (*E. coli*) etc. is produced in stainless steel by adding copper by an amount of 1.5~4.0 mass%. It has been reported by Matsuyama et al.[5] that an addition of a large amount of copper results in a deterioration of the corrosion resistance, therefore, it has been considered difficult to simultaneously maintain both antibacterial and corrosion resistance at a satisfactory level with Cu-bearing stainless steel. Other than Cu, various elements such as Ag, Zn and Ni have antibacterial properties. Among these elements, however, an element considered to have a strong antibacterial property is Ag.[3] Since the solubility of Ag into α-Fe is extremely low[6] and is only 0.00 02 atomic%, the effect of Ag addition to stainless steel had not been studied at all.

Kawasaki Steel paid special attention to the excellent antibacterial property of Ag and engaged in developing stainless steel that helps the antibacterial property while maintaining the original properties of stainless steel, particularly the corrosion resistance. As a result, it has been found that by adding Ag of about 0.04 mass% to stainless steel and dispersing Ag uniformly and finely in the stainless steel, stable antibacterial properties are produced and corrosion resistance equivalent to that of Ag-free stainless steel can be simultaneously secured. This paper describes the various properties of Ag-bearing stainless steel as well as the mechanism of the appearance of antibacterial activity in stainless steel of this

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Table 1 Chemical compositions of steels used

(mass%)

	C	Si	Mn	P	Cr	Ni	Ag
Ag-bearing austenitic stainless steel	0.05	0.30	1.00	0.03	18.2	8.5	0.042
Ag-bearing ferritic stainless steel 1	0.06	0.25	0.65	0.03	16.2	—	0.039
Ag-bearing ferritic stainless steel 2	0.01	0.25	0.45	0.03	17.7	—	0.040
SUS304 (Conventional stainless steel)	0.06	0.31	1.02	0.03	18.1	8.5	—

type.

2 Test Methods

2.1 Materials Tested

Kawasaki Steel succeeded in developing antibacterial Ag dispersed type austenitic and ferritic stainless steels. **Table 1** shows the chemical compositions of the Ag-bearing stainless steels and of Ag-free stainless steel, SUS 304, for the purpose of comparison. Among these, antibacterial and corrosion properties of the austenitic stainless steel are described in this paper as an example. Ag-bearing steel and SUS 304 steel, after casting by continuous casting, were cold rolled to be finished 0.7 mm thick sheets, through hot rolling, hot rolled plate annealing, cold rolling, recrystallization annealing and acid cleaning and then were subjected to antibacterial activity and corrosion resistance testing.

2.2 Antibacterial Activity Test

The shake-flask method[7] was adopted for evaluation of the antibacterial activity of metallic ions. Concretely speaking, bacterial test solution was prepared to make a content of *E. coli* about 10^7 cfu/mℓ (cfu: colony forming unit) using a nutrient broth diluted to 1/500. The broth was made up by dissolving 5.0 g of flesh extract, 5.0 g of NaCl and 10.0 g of peptone in 1 000 mℓ of purified water and its pH was adjusted to 7.0~7.2. Various metallic ions of a required amount were added to 15 mℓ of the thus prepared test solution and after *E. coli* was grown by shaking the solution for 24 h, the number of bacteria was counted by the agar plate method. The metallic ions of Ag^+, Cu^{2+} and Ni^{2+} were added in the form of $AgNO_3$, $CuCl_2$ and $NiCl_2$ respectively.

Evaluation of the antibacterial property of the stainless steels was carried out according to JIS Z 2801 (2000) "Antibacterial processed products—Antibacterial activity test method and antibacterial effect".[8] According to the definition in JIS, a product is regarded as antibacterial if the number of bacteria on the antibacterial product after 24 h is less than 1% of that on the comparison products. In this test, SUS 304 steel was used as the comparison product and the rate of cell reduction defined by the following Eq. (1) was used in order to make it easy to recognize the appearance of the antibacterial effect. When adopting this rate of cell reduction, the antibacterial effect is regarded to have appeared when the rate is larger than 99%.

(Rate of cell reduction)
= {(Number of viable bacteria on Ag-free steel)
− (Number of viable bacteria on Ag-bearing steel)}
/ (Number of viable bacteria in Ag-free steel)
× 100 (%) ··············· (1)

2.3 Corrosion Resistance

The corrosion resistance was evaluated using a saltwater spray based acceleration test and an electrochemical test. In the saltwater spray accelerated test, a salt drying and humidifying composite cyclic corrosion test was performed such a way that one cycle consists of spraying 5.0 mass% NaCl solution for 0.5 h, of drying test pieces at 60°C for 1 h, and keeping them for 1 h at the atmosphere of more than 95% in relative humidity. The test was performed for 10 or 20 cycles. The electrochemical test was carried out to draw and measure an anodic polarization curve (JIS G 0579) while using a sweeping potential at a rate of 0.02 V/min in a 5.0 mass% sulphuric acid solution.

3 Test Results

3.1 Antibacterial Property of Various Metallic Ions

The antibacterial properties of three metallic ions, Ag, Cu and Ni, were compared by means of the shake-flask

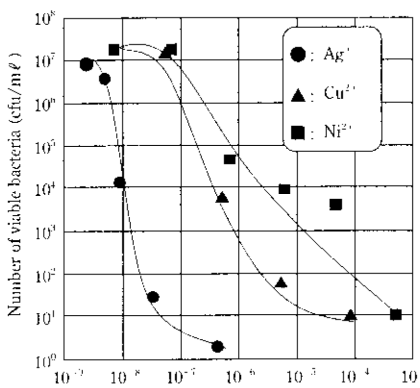

Fig. 1 Effect of concentration of metallic ion on number of viable bacteria after the shake-flask test

method. The relationships between the number of viable bacteria of *E. coli* after 24 h of growth and the concentration of metallic ions added to the bacterial test solution are shown in **Fig. 1**. When evaluating the results according to a standard similar to that in JIS[8], the antibacterial effect is judged to have appeared if the number of viable bacterial after 24 h is less than 10^4 cfu/mℓ. The concentration of metallic ions showing appearance of antibacterial effect is 1×10^{-8} mass% for Ag^+ whereas that of Cu^{2+} is 1×10^{-6} mass% and that of Ni^{2+} is 8×10^{-6} mass%. From these results, by comparing the antibacterial effect at the same value of concentration, it has become clear that the antibacterial activity of Ag^+ is about 100 times stronger than that of Cu^{2+} and about 800 times stronger than that of Ni^{2+}.

3.2 Properties of Ag-bearing Steel

3.2.1 Result of antibacterial activity tests by official organizations

Figure 2 shows the results of antibacterial activity

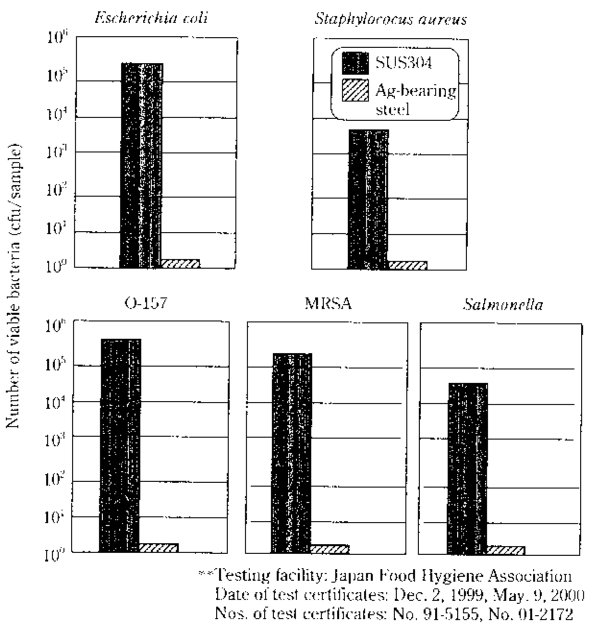

Fig. 2 Results of antibacterial test** of 0.04 2 mass% Ag-bearing steel

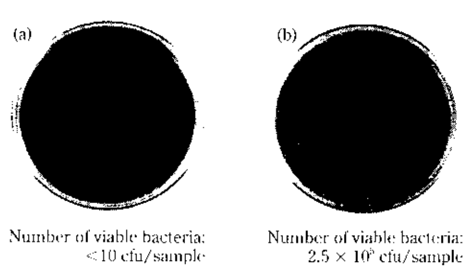

Photo 1 Example of culture plates of (a) Ag-bearing steel and (b) SUS304 after antibacterial test (*Escherichia coli*)

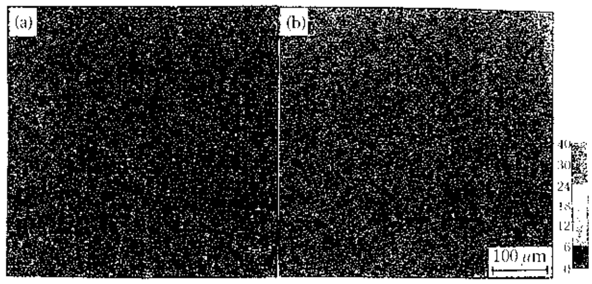

Fig. 3 Analysis of Ag on (a) surface and (b) plane polished by 2 μm of Ag-bearing steel

tests on the Ag-bearing stainless steel and SUS304 stainless steel carried out by the Japan Food Hygiene Association. **Photo 1** shows example antibacterial activity test results. The Ag-bearing steel exhibited antibacterial effects not only against *E. coli* and *Staphylococcus aureus* but also against O-157 and MRSA which is a bacterium causing hospital infections. The number of viable bacteria after the 24 h test duration was less than 10 cfu/mℓ (a rate of cell reduction > 99.9%) for any bacteria. Furthermore, the antibacterial effect against *E. coli* was investigated with the Ag-bearing steel after its surface was polished 2 μm using a piece of emery paper. The antibacterial property remained unchange, showing a rate of cell reduction exceeding 99.9%.

Upon confirming the above results, the dispersion conditions of Ag ions on the surface layer of the Ag-bearing steel and on the plane polished by 2 μm were analyzed using an electron microanalyzer. The results are shown in **Fig. 3**. The parts shown in red in the figure are the regions where the Ag-concentration is high. It can be indicated that Ag is finely and uniformly dispersed on the surface layer as well as on the inner plane (after being polished 2 μm).

3.2.2 Effect of cultivation time on the antibacterial effect

The antibacterial effect determined by ordinary antibacterial activity tests is evaluated based on the number of viable bacteria after 24 h cultivation of a bacteria solution. However, it is preferable that the antibacterial effect under an actual environment appear as quickly as possible. Therefore, the effect of cultivation time after preparation of an *E. coli* bacteria solution on the appearance of the antibacterial effect was investigated. The relationship between the number of viable bacteria of *E. coli* and the cultivation time is shown in **Fig. 4**. With the Ag-bearing stainless steel, it was confirmed that the number of viable bacteria decreased in less than 1 h after preparing the bacteria solution and the rate of cell reduction exceeded 99% after 3 h showing that the antibacterial effect had already succeeded. On the other hand, with the SUS304 steel tested for comparison, the number of viable bacteria increased with time and reached about 10^6 cfu after 24 h.

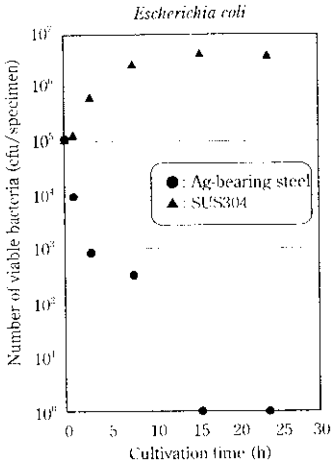

Fig. 4 Effect of cultivation time on number of viable bacteria

3.2.3 Corrosion resistance property

The external appearance of the specimens after the cyclic corrosion test (10 cycles) is shown in **Photo 2**. It is clear that the Ag-bearing steel has a corrosion resistance equivalent to that of SUS 304 steel. Furthermore, the anodic polarization curves of Ag-bearing steel and SUS 304 steel in 5 mass% sulphuric acid solution are shown in **Fig. 5**. It can be observed that the Ag-bearing steel shows nearly equal polarization behavior to that of SUS 304 steel. It is clear from the above that Ag addition of a small amount (about 0.04 mass%) to SUS 304 steel has almost no effect on the corrosion resistance.

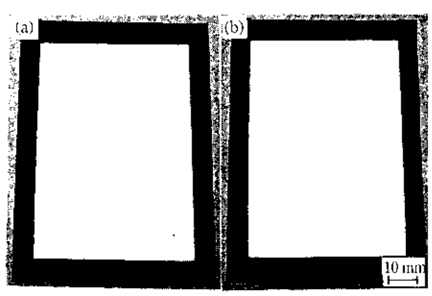

Photo 2 Appearance of (a) Ag-bearing steel and (b) SUS304 after cyclic corrosion test of 20 cycles

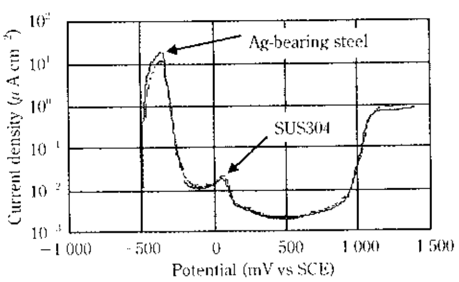

Fig. 5 Polarization curves in 5 mass% H_2SO_4

4 Mechanisms of Antibacterial Activity Appearance and Maintaining Corrosion Resistance of Ag-bearing Steel

The results of tests described in the foregoing chapter showed that it is possible for the Ag-bearing antibacterial stainless steel to have satisfactory antibacterial properties and corrosion resistance at the same time. This chapter discusses the mechanism of the appearance of the antibacterial properties by Ag-addition as well as the mechanism of maintaining corrosion resistance even with Ag-addition.

The mechanisms of antibacterial activity appearance with inorganic metallic antibacterial materials can be classified into that by the action of metallic ions[9-11] and that by the action of active oxygen generated on the metal surface.[12] Among these, the mechanism of the appearance of antibacterial activity by the action of metallic ions is considered to consist of the following three cases:

(1) Metallic ions eluted from the material's surface are absorbed onto surface of cells and damage the cell membrane and solid structure of its proteins.

(2) Metallic ions taken into cells through active transport cause enzyme inhibitation through reaction with various enzymes represented by an SH group or functional disorder due to reaction with DNA.

(3) Some active oxygen is generated during the process of reaction with enzyme destroying cells.

Based on a comparison between the antibacterial effect of various kinds of metallic ions against *E. coli* shown in Fig. 1, it was shown that Ag^+ exhibits an antibacterial effect even when present in a very small amount of about 1×10^{-8} mass% (0.01 mass ppm). This was clarified by the following experiment, testing the amount of Ag that actually elutes as ions from the Ag-bearing steel. Eleven 5 cm square specimens were prepared. One specimen was used in an antibacterial activity test to obtain the number of viable bacteria and the other 10 specimens were used in inoculation tests and then each cultures were recovered after cultivating for 24 h. The amount of Ag in each recovered cultures

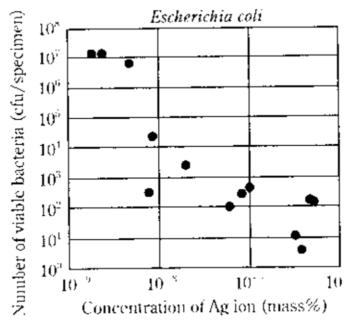

Fig. 6 Relation between concentration of Ag ion and antibacterial activity

were quantitatively analyzed by atomic absorption analysis. The relationship between the number of viable bacteria of *E. coli* after 24 h and the amount of eluted Ag ions converted to the number per sample is shown in **Fig. 6**. The figure shows that the number of viable bacteria decreases as the concentration of eluted Ag increases. A concentration of eluted Ag ions exceeding 1×10^{-8} eluted Ag necessary for appearance of antibacterial activity agrees well with the test results using metallic ions. From the result described above, it has been shown that Ag^+ is eluting from the Ag-bearing steel in an amount required for causing the antibacterial effect and at the same time, it can be concluded that the mechanism of the appearance of antibacterial activity is mainly the very small amount of Ag^+ eluted from the steel.

It is indispensable for the appearance of the antibacterial property that Ag elute from the steel as ions. Generally speaking, the excellent corrosion resistance of stainless steel is maintained by the presence of a passive film on the steel sheet surface. At the part where the antibacterial metal elutes from the steel plate, thereby producing the antibacterial effect, it is highly probable that the passive film is deteriorated or destroyed. Nevertheless, the Ag-bearing steel indicates a stable antibacterial property as well as a corrosion resistance equivalent to that of Ag-free steel. This is mainly because the required amount of eluted Ag can be secured by the addition of a very small amount of Ag since Ag has a greater antibacterial effect than other antibacterial metals, and the unstable part of the passive film is very small because Ag added is uniformly and finely dispersed.

5 Conclusions

Based on the results of studying the effect of Ag addition on the antibacterial properties and corrosion resistance of stainless steel, Kawasaki Steel developed Ag dispersed stainless steels simultaneously possessing stable antibacterial properties and a corrosion resistance equivalent to that of Ag-free stainless steel.

(1) The Ag-bearing stainless steel exhibits its antibacterial effect against *E. coli* and *Staphylococcus aureus* as well as against O-157 and MRSA. In addition, the corrosion resistance is equivalent to that of Ag-free stainless steel. This is believed to be because the unstable part in the passive film is minimal because the added Ag ions are uniformly and finely dispersed.

(2) The antibacterial property of Ag-bearing stainless steel is considered to be produced by a very small quantity of Ag^+ eluting the steel surface acting on bacteria cells.

(3) Ag-bearing stainless steels are being agressively used at present for washing vessels, kitchen facilities, western style tableware and for interior finishing materials for hospitals and are expected to be used more widely in the future in various fields where sanitary management is required.

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