J Mater Sci (2007) 42:5118–5122
DOI 10.1007/s10853-006-0603-9

Antibacterial property of Ce-bearing stainless steels

**Hemin Jing · Xinqiang Wu · Yongqian Liu ·
Manqi Lu · Ke Yang · Zhimin Yao · Wei Ke**

Received: 9 January 2006 / Accepted: 20 June 2006 /

Abstract Rare earth (RE) elements inhering in low toxicity have been widely used in biomedicine. The RE elements can also improve the hot workability, corrosion resistance and mechanical properties of stainless steels. However, the antibacterial mechanisms of the RE elements are still under discussion and the antibacterial property of RE-bearing stainless steels has not yet been investigated or reported so far. In this paper several Ce-bearing stainless steels were prepared, the microstructure of the steels was examined, and the antibacterial property of Ce-bearing stainless steels was investigated. It was found that Ce-rich zones were precipitated in the Ce-bearing stainless steels and the volume fraction of the Ce-rich zones increased with increasing Ce content. The Ce-bearing stainless steels showed excellent antibacterial property when the amount of Ce added was above a critical value. Compared to the conventional Cu-bearing antibacterial stainless steels, the Ce-bearing stainless steels investigated presently exhibited good antibacterial ability without any thermal aging treatment. The antibacterial mechanism of Ce-bearing stainless steels is also discussed.

Introduction

With the advances of science and technology, people pay increasing attention to the health of their living environments. As a result, many kinds of antibacterial materials have been developed during past several decades, which can be divided into two categories. One is the organic antibacterial materials. The typical examples are some organic coatings [1]. Another is the inorganic antibacterial materials. The typical examples are Cu-bearing antibacterial stainless steels [2]. However, the antibacterial effects of the organic coatings usually cannot last for a long time, while a special thermal aging treatment is required to obtain a good antibacterial property for the Cu-bearing stainless steels.

In the past, rare earth (RE) elements have been extensively used in the fields of steel and medicine due to their special optical, electric and magnetic properties. It is well-known that the RE elements can play a significant role in de-sulfurizing, de-oxidizing and inclusion-control in the process of steelmaking, by which the performances of stainless steels such as thermoplasticity, hot workability and corrosion resistance can be obviously improved [3, 4]. Moreover, the RE elements have been found to be low toxicity with good antibacterial, anticancer and anti-HIV bioactive properties [5]. For examples, for cerium (Ce) in actinium RE family, valence 3 cerous salts have been found to be poisonous to bacteria and epiphytes. They can effectively kill G-bacterial when used as therapy medicine in burn cure [6]. They can play a role in immunity accommodation and prevent the blood-poisoning resulted from mass burn [7]. Now the RE is also used to produce fertilizer, antiseptics for agriculture

H. Jing (✉) · X. Wu · Y. Liu · M. Lu · K. Yang ·
Z. Yao · W. Ke
Environmental Corrosion Center, Institute of Metal Research, Chinese Academy of Sciences, Shenyang 110016, P.R. China
e-mail: hmjing@imr.ac.cn

Present Address:
H. Jing
Anhui Key Laboratory of Metal Materials and Processing, School of Materials Science and Engineering, Anhui University of Technology, Maanshan 243002, P.R. China
e-mail: jinghemin999@yahoo.com.cn

production, feedstuff additive, and so on [8–10]. However, the antibacterial mechanisms of the RE elements are still under discussion and the antibacterial property of RE-bearing stainless steels has not yet been investigated or reported previously. In the present study, the rare earth element Cerium has been used as the antibacterial element to add to the alloy, and the antibacterial property of several Ce-bearing stainless steels was investigated. The related antibacterial mechanism of Ce-bearing stainless steels is also discussed.

Materials and methods

Material preparation

The Ce-bearing stainless steels used in the present study were melted in 2 kg vacuum induction furnace, whose nominal compositions are shown in Table 1. A commercial stainless steel 00Cr18Ni9 was also included in the present study for comparison purpose. The cast ingots of the Ce-bearing stainless steels were solution-treated at 1,030 °C for 30 min.

Microstructural examination

The distribution of Ce element on the polished specimen surface was measured using an EPMA 1610 microprobe. A linear analysis method was used to measure the volume fraction of Ce-rich zones in the stainless steels.

Antibacterial test

The specimens for antibacterial tests were 50 mm in diameter and 3 mm in thickness. They were mechanically polished with SiC paper of 800 grit and then cleaned with alcohol before antibacterial tests. *Escherichia coli* ATCC 8099 (*E. coli*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*) were used as the testing bacteria in the present antibacterial tests. *Escherichia coli* belongs to Gram-negative bacteria, they are common clinical bacterial isolates and can be used as indicators of fecal pollution in environment and food sanitation. *Staphylococcus aureus* belonged to Gram-positive bacteria and exists in the nature such as air, water, soil, article, human being's skin and so on. Some pathogenic *Staphylococcus* bacteria exist on the skin or in the nasopharynx. They are the common and main sources of cross infection in hospital [11]. Also *Staphylococcus bacteria* are a common reason for the food poisoning. The probability of food poisoning would decrease by reducing the amount of the *S. aureus* on the stainless steels that people always touch [2]. The concentrations of *E. coli* and *S. aureus* used in the present study were 8.1×10^5 and 9×10^5 colony forming units (cfu)/ml, respectively. The bacteria were cultured at 37 °C for 24 h using the conventional agar pour plate method. The number of living bacteria (NLB) after antibacterial tests was measured by the microcolony culture counting method. Each test was repeated three times and the NLB average was calculated. Table 2 showed the results of antibacterial tests: rates were calculated for each type of material using the following formula

$$\text{Antibacterial rate}\,(\%) = \frac{\mathrm{NLB_{CSS}} - \mathrm{NLB_{ASS}}}{\mathrm{NLB_{CSS}}} \times 100\%, \tag{1}$$

where $\mathrm{NLB_{CSS}}$ is the NLB for the 00Cr18Ni9 stainless steel after antibacterial tests, $\mathrm{NLB_{ASS}}$ is the NLB for the Ce-bearing stainless steels after antibacterial tests.

Results

Microstructure of Ce-bearing stainless steels

Figure 1 shows the mapping morphologies of Ce in the Ce-bearing stainless steels. When the Ce content was 0.01 wt.%, no obvious Ce-rich zones were observed on the surface of the stainless steel (Fig. 1a). When the Ce content was increased to 0.11 wt.%, the Ce-rich zones having small size and dispersive dot-like morphology appeared (Fig. 1b). When the Ce content was increased to 3.25 wt.%, a large amount of Ce-rich zones having dendrite-like morphology were observed

Table 1 The nominal compositions (wt.%) of the stainless steels used presently

Steels	Ce	C	Si	Mn	Ni	Cr	Others	Balance
Ce-1	0.01	≤0.03	≤0.80	≤1.50	8.00–12.00	17.00–19.00	(Mo, Nb, Ti, V, Zr, Co,B) ≤ 1.0	Fe
Ce-2	0.11	≤0.03	≤0.80	≤1.50	8.00–12.00	17.00–19.00	(Mo, Nb, Ti, V, Zr, Co, B) ≤ 1.0	Fe
Ce-3	3.25	≤0.03	≤0.80	≤1.50	8.00–12.00	17.00–19.00	(Mo, Nb, Ti, V, Zr, Co,B) ≤ 1.0	Fe
00Cr18Ni9	–	≤0.03	≤0.50	≤0.50	8.00–12.00	17.00–19.00	–	Fe

Table 2 The results of antibacterial tests for the stainless steels

Steels	*E. coli* (8.1×10^5 cfu/ml)		*S. aureus* (9×10^5 cfu/ml)	
	Mean number of cfu	Antibacterial rate (%)	Mean number of cfu	Antibacterial rate (%)
Ce-1	710	12.3	400	74.0
Ce-2	9	98.9	13.5	99.1
Ce-3	8	99.0	8.5	99.4
00Cr18Ni9	810	–	1,540	–

on the surface of the stainless steel (Fig. 1c). Figure 2 is the X-ray analysis results of the Ce-bearing stainless steels. Clearly, the Ce-rich zones in the stainless steels were composed of $CeFe_7$ and pure Ce. Table 3 shows the volume fraction of Ce-rich zones in the stainless steels.

Results of antibacterial tests

Table 2 shows the results of antibacterial tests for the Ce-bearing stainless steels and 00Cr18Ni9 stainless steel against *E. coli* and *S. aureus*. When the Ce content was 0.01 wt.%, the stainless steel exhibited a certain antibacterial ability compared to the 00Cr18-Ni9 steel. When the Ce content was increased to 0.1 wt.%, the stainless steel exhibited an excellent antibacterial ability. When the Ce content was increased to 3.25 wt.%, the stainless steel showed a similar antibacterial ability compared to the stainless steel with 0.1 wt.% Ce. The above results suggested a critical value of Ce content may exist in the range of 0.01–0.1 wt.% in the stainless steels, above which the antibacterial ability of the stainless steel will be improved abruptly. As stated previously, the main difference in the Ce-bearing stainless steels was the volume fraction of Ce-rich zones (Fig. 1 and Table 3). It is, thus, believed that these dispersive Ce-rich zones played a key role on the antibacterial ability of the stainless steels.

Discussion

It is well-known that some metal elements such as silver (Ag), copper (Cu), and zinc (Zn) inhere in good antibacterial property. Much work has been done previously on the antibacterial mechanisms of these metal elements. Several possible antibacterial mechanisms have been proposed as follows [12–19]:

(1) *Electrical field effects*. Metal ions can kill bacteria by destroying their cell walls and cell membranes because metal ions having stronger reduction can extract the electrons from proteins of bacteria, causing their cytoplasm to run off and oxidizing their cell nucleus;
(2) *Freezing effects of protein*. The metal ions can penetrate into the cell and freeze the protein of bacteria by reacting with the mercapto radical (–SH) of bacteria, thus destroy the activity of the synzyme and prevent the bacteria from reproducing;
(3) *Damage effects of enzyme system*. Metal ions can damage the enzyme system and normal metabolism of bacteria;
(4) *Catalysis effects*. Under the action of light, some metal ions such as Ag^{n+} and Ti^{n+} can activate oxygen in air or water and produce hydroxyl (–OH) and active oxygen ions (–O–). Due to their strong oxidation and reduction ability, (–OH) and (–O–) can damage the (–SH) on the dyhydrogen enzyme of bacteria and in turn restrict the reproducing of bacteria.

However, little work has been focused on the antibacterial mechanism of the RE elements previously. The investigations of Muroma [20] showed that the RE ions, depending on their concentration, may restrict or promote the growth of bacteria. About 0.1–10 mmol/L Ln^{3+} can restrict their growth, while 0.01 mmol/L Ln^{3+} promoted the growth of bacteria. Recently Hou et al. [21] also reported that the RE ions had two-way effects on the growth of some tissues of creatures and plants,

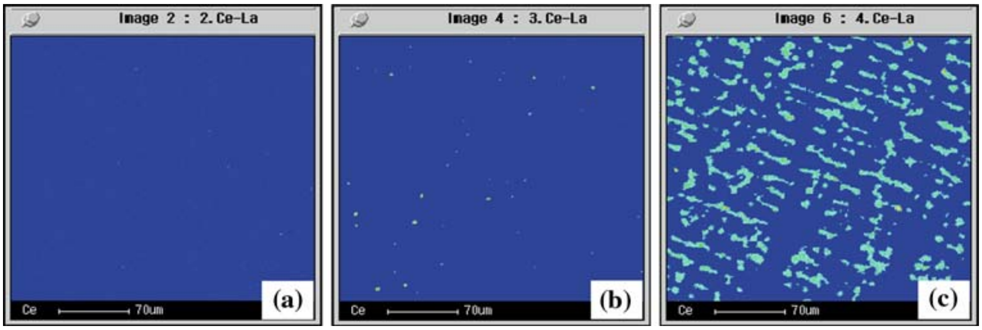

Fig. 1 The mapping morphologies of Ce in the Ce-bearing stainless steels (**a**) 0.01 wt.% Ce; (**b**) 0.11 wt.% Ce; (**c**) 3.25 wt.% Ce

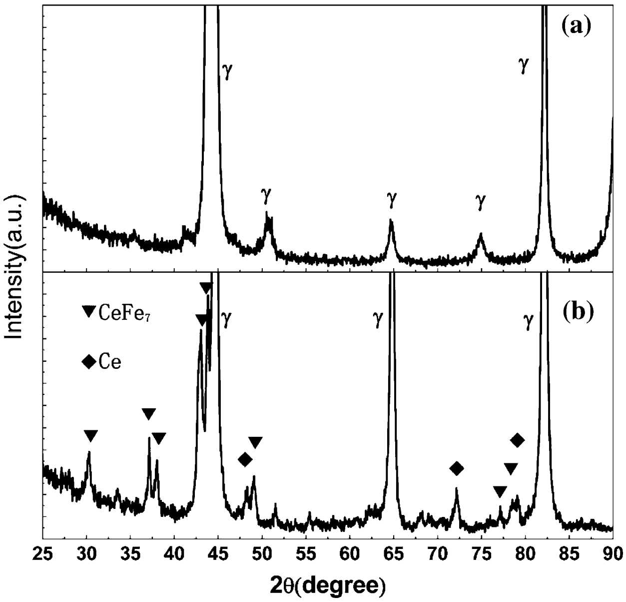

Fig. 2 X-ray analysis results of Ce-bearing stainless steels. (**a**) 0.01 wt.% Ce; (**b**): 3.25 wt.% Ce

Table 3 The volume fraction of Ce-rich zones in the stainless steels

Steels	Ce-1	Ce-2	Ce-3
Volume fraction of Ce rich zones (%)	≈0	1.7	19.7

including promoting effects at low concentration and retarding effects at high concentration. In practice, some RE oxides or RE salts are usually added into the inorganic antibacterial agents containing TiO_2, ZnO, Ag, Cu and Zn [22–24] to further improve their antibacterial ability. The present experimental results (Table 2) indicated that the Ce-bearing stainless steels had stronger antibacterial ability than the Ce-free 00Cr18Ni9. Moreover, the more the cerium content in the stainless steel was, the stronger the antibacterial ability of the steels appeared. It is believed that Ce may have the similar antibacterial effects as those metal elements mentioned above. The Ce-ions can be dissolved into the water from the Ce-rich zones on the surface of the stainless steels (Fig. 1), and thus come into contact with the bacteria on the surface of steels. Then these Ce-ions may kill bacteria through various mechanisms mentioned above. Moreover, the RE ions (belonging to hard acid) are difficult to be polarized, they have strong affinity with oxygen and nitrogen atoms (belonging to hard alkali) and can meet different ligand species. So the RE ions can replace some metal ions (such as Ca^{2+}) in the cells of bacteria to form ligands with the organic function clusters of bacteria [25], thus affecting the vital processes of bacteria.

The formation of the Ce-rich zones in the stainless steels could be traced back to the steelmaking process. During solidification, solute atoms, such as C, Si, Ce, and so on, are accumulated into the bulk melt (liquid) continuously at solid/liquid interfaces. Along with the advance of the solid/liquid interfaces, these solutes gradually concentrate in the residual melt in dendrites or grain boundary areas. These areas rich in solutes can remain until the end of solidification. The intermetallics $CeFe_7$ or even pure Ce (Figs. 1, 2) may precipitate in the interdendritic regions either in the residual melt if the solubility product of Ce and Fe is exceed, or in the solid following steelmaking processes due to relatively low solubility of Ce in iron (less than 0.048 wt.% at room temperature [26] and less than 0.1 wt.% at 900 °C [27]).

In addition, it should be noted that the Ce-bearing stainless steels investigated presently exhibited good antibacterial ability only after conventional solid solution treatments. In the steelmaking process of stainless steels, the solution treatment is usually employed to homogenize the metallographic structure and to improve the mechanical property. In view of the cost of steelmaking, this is believed to be a potential merit for Ce-bearing antibacterial stainless steels compared to the conventional Cu-bearing antibacterial stainless steels that need not only conventional solid solution treatment but also optimal temperature and duration of thermal aging treatments to obtain effective and enough precipitated antibacterial phases [2, 17].

The stainless steels have been widely used in biomedicine for many years [28, 29] and the RE elements including Ce have also been used in medicine for many years. It is thus believed that the Ce-bearing stainless steels investigated presently may not have any toxicity to human being. However, detailed toxicity tests need to be performed in future work. The Ce-bearing antibacterial stainless steels may find their potential applications in many fields such as medical apparatus and instruments, food machines, toilet facilities, containers of water or beverage, installations in public places, and so on.

Conclusions

Adding Ce to the stainless steels can improve the antibacterial property of steels. To obtain a good antibacterial ability, the amount of Ce added must be above a critical value. For the present study, the critical results to be in the range of 0.01–0.11 wt.%. The antibacterial ability of Ce-bearing stainless steels can be attributed to the Ce-rich zones precipitated in the steels. It is believed that the RE ions can kill bacteria by destroying the normal vital processes of bacteria such as metabolism, reproducing and breathing. Compared to the conventional Cu-bearing antibacterial steels, the Ce-bearing stainless steels exhibited good antibacterial ability without thermal aging treatments, cutting down the steelmaking process and cost.

Acknowledgements This study was supported by the Innovation Fund (CX0008-6) of Institute of Metal Research, Chinese Academy of Sciences.

References

1. Yokota T, Tochihara M (2001) Kawasaki Steel Rep 33:88
2. Hong IT, Koo CH (2005) Mater Sci Eng A393:213
3. Wang L, Du T, Wang Y (2003) J Chinese Rare Earth Soc 21:491
4. Lin Q, Ye W, Chen N, Liu Y (1997) J Chinese Rare Earth Soc 15:228
5. Evans CH (1990) Biochemistry of the lanthanides. Plenum Press, New York
6. Yang J (1988) J Chinese Hosp Pharm 8:424
7. Ni J (1995) Bioinorganic chemistry of rare earth elements. Science Press, p 47 (in Chinese)
8. Yang Q, Xu J, Gao F, Song H (1989) Chin Pat 1032281A
9. Mu K, Zhang F, Wang C, Zhang W, Hu L (1997) J Chinese Rare Earth Soc 21:1
10. Wei Y (2005) Chin Pat 1586270A
11. Lu D (2001) Medical microbiology, 5th edn. People's Sanitation Press, Beijing, p 93, p 115 (in Chinese)
12. Marsh PB (1945) Phytopathology 35:54
13. Troger R (1958) Arch Microbiol 29:430
14. Fuhrmann GF (1968) Biochem Biophys Acta 163:325
15. Stardey RL (1973) J Gen Micro 98:217
16. Ishiguro F, Nishikawa T, Amano R, Koyanagi T (1999) Materia Japan 38:64
17. Yokota T, Tochihara M (2001) Kawasaki Steel Rep 33:88
18. Nakamura S (1997) Nisshin Steel Rep 76:48
19. Qokubo N (1998) Nisshin Steel Rep 77:69
20. Muroma A (1958) Ann Med Exp Biol Fenn 36:1
21. Hou A, Qu S, Huang W, Liu Y (2003) Acta Phys Chim Sin 19:134
22. Jin Z, Liang J, Wang J (2002) Chin Pat 1086109C
23. Tu M, Liu X, Jiang Y, Zhang B (2003) Chin Pat 1454481A
24. Bo W, Li Z, Wang S, Chen Y (2002) Chin Pat 1370940A
25. Huo C, Zhang D, Liu J, Zhang T (2000) Acta Chim Sin 60:1065
26. Zhang F, Gu L, Qu L, Liu W, Yan Y (1987) ACTA Metall Sin 23:A503
27. Chuang YC, Wu CH, Shao ZB (1987) J Less-Common Metals 136:147
28. Black J (1999) Biological performance of materials. Marcel Dekker, New York
29. Brown BH, Smallwood RH, Barber DC, Lawford PV, Hose DR (1999) Medical physics and biomedical engineering. Institute of Physics, Bristol, Philadelphia