Materials Science and Engineering C 51 (2015) 356–361

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Anti-biofilm formation of a novel stainless steel against *Staphylococcus aureus*

Li Nan [a], Ke Yang [a], Guogang Ren [b,*]

[a] Institute of Metal Research, Chinese Academy of Sciences, Shenyang 110016, China
[b] University of Hertfordshire, Hatfield AL10 9AB, UK

ARTICLE INFO

Article history:
Received 20 August 2014
Received in revised form 8 January 2015
Accepted 9 March 2015
Available online 11 March 2015

Keywords:
Cu-bearing stainless steel
S. aureus
Antibacterial
Biofilm

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a bacterium frequently found proliferating on metal surfaces such as stainless steels used in healthcare and food processing facilities. Past research has shown that a novel Cu-bearing 304 type stainless steel (304CuSS) exhibits excellent antibacterial ability (i.e. against *S. aureus*) in a short time period (24 h.). This work was dedicated to investigate the 304CuSS's inhibition ability towards the *S. aureus* biofilm formation for an extended period of 7 days after incubation. It was found that the antibacterial rate of the 304CuSS against sessile bacterial cells reached over 99.9% in comparison with the 304SS. The thickness and sizes of the biofilms on the 304SS surfaces increased markedly with period of contact, and thus expected higher risk of bio-contamination, indicated by the changes of surface free energy between biofilm and the steel surfaces. The results demonstrated that the 304CuSS exhibited strong inhibition on the growth and adherence of the biofilms. The surface free energy of the 304CuSS after contact with sessile bacterial cells was much lower than that of the 304SS towards the same culture times. The continuously dissolved Cu^{2+} ions well demonstrated the dissolution ability of Cu-rich precipitates after exposure to *S. aureus* solution, from 3.1 ppm (2 days) to 4.5 ppm (7 days). For this to occur, a hypothesis mechanism might be established for 304CuSS in which the Cu^{2+} ions were released from Cu-rich phases that bond with extracellular polymeric substances (EPS) of the microorganisms. And these inhibited the activities of cell protein/enzymes and effectively prevented planktonic bacterial cells attaching to the 304CuSS metal surface.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Stainless steel is widely used in a variety of manufacturing, engineering, and whole spectrum of industries, including energy, chemical engineering, medical devices, healthcare and environmental protection, marine, building construction, and agro-food systems [1,2]. However, within the healthcare and food-agro industry, the current publications or reports are more concentrating on the biofilm formation ability on materials such as plastics, fabrics, cloths and metals [2–8]. Many research papers have been published in order to demonstrate the antibacterial abilities of the Cu-bearing stainless steels (304CuSS) [9–11], which inhibit and weaken the biofilm formation particularly in the applications of healthcare and medical devices. It is a surprisingly scare that there is no research paper that could be found published across the entire agro-food and medical processing and packaging facilities.

Some bacteria can live and breed well on the surfaces of general materials such as plastics, ceramics and metals. Particularly, normal stainless steel products strongly support the bacterial growth in the biofilm formation for an extended period of time, which greatly increases the risk of bacterial cross-contamination, such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)/*Methicillin-resistant Staphylococcus aureus* (*MRSA*), etc. Because of these, it is estimated that each year in the United States, approximately 9000 deaths are associated with healthcare, environmental related food contaminations [12]. Many studies have been focused on removing the pathogenic bacteria formed on stainless steels, as summarized in Table 1 [13–19]. However, these technologies cannot maintain their inhibition ability against sessile bacteria for a prolonged period, because a bacterial adhesion to a solid surface can lead to biofilm formation. And the ability of the biofilm formation is depending on not only the physiochemical properties of the bacterial cell surface, but also the substrate's chemical composition and physical properties underneath them as well [20], mainly including the substrate surface free energy, ionic charges, hydrophobicity, roughness, as well as the type of bacterial proteins presented [21]. Wherein, the surface energy is well governed by van der Waals and electrostatic forces [22].

The emphasis on eco-friendly environment and healthy food products is a pivotal movement to allow food manufacturers to consider this novel and antibacterial stainless steel owning to the limitations of the commercial SS on the uses of chemical disinfectants, chemical preservatives and other preservation methodologies (freezing and

* Corresponding author.
E-mail address: g.g.ren@herts.ac.uk (G. Ren).

http://dx.doi.org/10.1016/j.msec.2015.03.012
0928-4931/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1
Removing technologies of common pathogenic bacteria on the surfaces of stainless steels.

Methods	Common pathogenic bacteria	References
Chemical sanitizer	*Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Cronobacter sakazakii*	[13,14]
Natural bactericidal agents	*Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli O 157:H7*	[17,19]
Surface treatment	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*	[15,18]
Biological competition	*Listeria monocytogenes*	[16]

canning, etc.) [23]. Therefore the prevention of bio-contamination is crucial to various application environments.

S. aureus, a typical Gram-positive *coccus*, is one of the most common pathogens associated with serious foodborne diseases and has long been considered as a major issue in public health risks [24,25]. It is often found on the surfaces of stainless steels used in the food processing industry worldwide [26,27]. More importantly the antibiotic-resistant or disinfectant resistant bacteria can easily form new pathogenic *S. aureus* (e.g. MRSA), which is a devastating concern causing serious problem in the clinical medicine, hospitals and agro-food industries [28–30].

Staphylococcal biofilm formation is one of the critical processes in the control of bacterial inhibition by the disinfectant and antibacterial materials. This biofilm formation comprises two steps, beginning with the initial attachment to a contact surface, followed by the accumulation of multi-layered cell clusters — namely intercellular adhesion [31]. Once the formation of biofilm occurs, the resistance of bacteria towards antibacterial material or agent is enhanced. According to the work of Pastoriza et al. [32], the establishment of the poison food caused by *S. aureus* depends on the ability of the strain to survive on a colonized substrate, multiply under a variety of conditions and produce organic EPS. This is a complex mixture of biomolecules, such as proteins, humic-like substances, polysaccharides, uronic acid, lipids and glycoproteins, which surround the bacterial cell [33]. Therefore, fewer biofilms on the stainless steel substrate may reduce the risk of bio-contamination towards healthcare and food processing facilities.

Previous papers on the 304CuSS showed it a strong antibacterial ability against *S. aureus* with the antibacterial rate over 99.9% within 24 h [34–36]. However, there has been no work on whether or not the 304CuSS can mitigate the formation of the biofilm after an extended period of exposure to *S. aureus*. Therefore, the objectives of this work are to investigate the adhesion behavior of *S. aureus* on the surface of the 304CuSS by using a standard direct plate counting (JIS Z 2801-2000 or GB/T 21510-2008). The Cu^{2+} release from the 304CuSS was examined by inductively coupled plasma-mass spectrometer (ICP-MS), contact angle measurement for surface free energy (SFE), and fourier transformed infrared spectroscopy (FTIR) for identifying the variations of functional groups after sessile bacteria were contacted with steels, and morphologies of the bacteria were observed by a scanning electron microscope (SEM).

2. Material and methods

2.1. Sample preparation

The samples used in this study were the 304CuSS (0Cr18Ni9Cu3.8) and the 304SS (0Cr18Ni9) and they were solution treated at 1040 °C for 30 min followed by a water quenching process. And then the samples were aged at 700 °C for 6 h followed by air-cooling. These heat treatments allowed the 304CuSS to have a strong bactericidal ability resulting from a certain amount of saturated Cu-rich precipitates [34] that have been formed in the 304CuSS, as shown in Fig. 1 [37]. The size of samples was 10.0 mm in diameter and 5.0 mm in thickness,

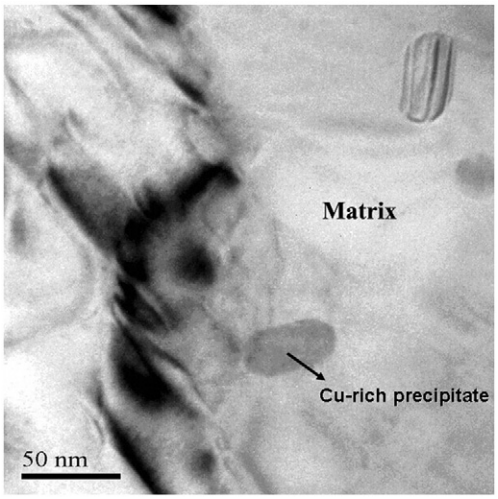

Fig. 1. Cu-rich precipitates from a Cu-bearing Stainless steel matrix after the thermal/heat treatment [37].

and they were grounded by a series of grit SiC papers (400, 600, 800 and 1000). The samples were then sterilized by an autoclave at 120 °C for 20 min before tests.

2.2. Bacterial culture and direct plate counting

A Gram-positive *S. aureus* ATCC25923 was selected and its solution was incubated overnight at 37 °C, and then diluted into the Luria-Bertani (LB) medium as a standard approach [38] with a concentration of 3.0×10^5 CFU/ml. The number of adhered bacteria on samples was determined by a direct plate-counting method (JIS Z 2801-2000). To fit into this standard test, the steel samples were placed first into a 24-well plate, and then 1 ml of the LB medium that incubated bacterial solutions was dropped into each well of the plate for co-culture. These plates were incubated at 37 °C for 2, 4, and 7 days separately to facilitate the biofilm formation.

After removing the bacterial cells with the sterile distilled water for three times, adhered bacteria were then swabbed and serially diluted onto LB plates. Bacterial colonies were counted and results were expressed in log (CFU/ml). Three parallel samples were used for different time points.

2.3. Contact angles and surface free energy analysis

Contact angle was measured by using the Sessile-Drop methodology by a contact angle analyzer (OCA-20, Dataphysics, Germany). The SFE of a solid can be calculated by the following expression [39]:

$$\gamma_L(1+\cos\theta) = 2\sqrt{\gamma_S^d\gamma_L^d} + 2\sqrt{\gamma_S^p\gamma_L^p} \quad (1)$$

where the $\boldsymbol{\gamma}_L$ is an experimentally determined surface free energy of the liquid, θ is the contact angle, $\boldsymbol{\gamma}_S^d$ is the dispersion component of the surface free energy of the solid, γ_L^d is the dispersion component of the surface free energy of the liquid, $\boldsymbol{\gamma}_S^p$ is the polar component of the surface free energy of the solid, and $\boldsymbol{\gamma}_L^p$ is the polar component of the surface free energy of the liquid.

The shapes of the droplets on the stainless steels were calculated both the left and right contact angles from the shapes of the droplets with an accuracy of $\pm 0.1°$. The planktonic bacteria liquids, LB medium and other substances were removed through washing with sterile distilled water for 15 s three times, and the average value of the contact angle at four points on the surface was regarded as the mean value of the contact angle for each treated sample at 25 °C [40,41]. Contact

Download English Version:

https://daneshyari.com/en/article/7869723

Download Persian Version:

https://daneshyari.com/article/7869723

Daneshyari.com