Ferrous Ferric Chloride Water Decreases Attachment of Bacteria to Surfaces — Scanning Electron Microscopy Observation and Force-Volume Microscopy Measurement of Titanium Surfaces Immersed in Ferrous Ferric Chloride Water©

Tadao Fujimori
Institute for Biological Process Research, Akatsuka Garden Co., Ltd., 1868-3 Takanoo-cho, Tsu, Mie 514-2293, Japan
Email: ffctf@akatsuka.gr.jp

INTRODUCTION

Akatsuka Garden Company has continued research and development on various solutions which accelerate plant growth and activate physiological functions of plants since 1984. We have focused our attention on the behavior of iron ions in water and interaction of iron ions and water. Based on that research we developed a new water improvement device named "Ferrous Ferric Chloride (FFC®) ceramic balls" (Sugi and Yamashita, 1991) in 1995. Water treated with FFC ceramic balls (called "FFC water") possesses specific biological effects such as stimulation of plant growth, especially root growth (Hasegawa et al., 2006). In animals, FFC also possesses stimulative effects on cell growth (Hirobe, 2007). The FFC ceramic balls have been utilized by users in many different fields in primary and secondary industries. Those users obtained many advantages from the utilization of FFC ceramic balls — for example, productivity enhancement, cost reduction, decrease in amount of agricultural chemicals required, etc. (Yokomizo, 2011). In addition, many users have realized that utilization of FFC ceramic balls for production facilities and their water distribution systems can decrease the harmful biofilm formation which can cause contamination and deterioration of facilities and the clogging of drainage pipes. We considered that these beneficial phenomena could be due to physicochemical origins of the FFC water's antifouling property against bacterial attachment. Therefore we established collaborative research with Harvard University from 2004 until today. In this paper, I introduce some of the effects of FFC water to prevent or hinder bacterial attachment using titanium surfaces for this research.

MATERIALS AND METHODS

The control solution was prepared by dissolving 0.22 g of $(NH_4)_2SO_4$, 0.12 g of KH_2PO_4, 0.23 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of $CaCl_2 \cdot 2H_2O$, and 0.05 g of BBL™ yeast extract in 1 L of deionized water followed by autoclave sterilization. The FFC solution was prepared as described for the control solution, but with deionized water first equilibrated with four FFC ceramic balls under vigorous stirring for 1 h.

Sterilized titanium squares (0.5×0.5 cm^2, thickness of 0.5 mm) were immersed in the control or FFC solutions with and without *Pseudomonas aeruginosa* inoculation at 25 °C and 120 rpm. The squares with the inoculation were removed after 4 days of incubation, and the squares without the inoculation were removed after 2 days of incubation. The density of attached *P. aeruginosa* cells to the surfaces with the inoculation was examined by scanning electron microscopy (SEM). Surface forces (repulsive and adhesion forces) of the titanium squares with and without the inoculation were examined by force-volume microscopy (FVM) (Na et al., 2010).

RESULTS AND DISCUSSION

The SEM observations showed that the densities of attached *P. aeruginosa* were 6.2 (±1.3) × 10^5 cells/cm^2 for surfaces immersed in the FFC solutions compared to 8.7(±0.8) × 10^6 cells/cm^2 for surfaces immersed in the control solution (Figs. 1, 2a). Parallel measurements by FVM demonstrated that regions of elevated interfacial repulsion covered 72±2 % of the surfaces immersed in the FFC solutions, compared to 26±2 % for immersion in the control solutions (Fig. 2b). Additionally, the FVM measurements also indicated that the upper fifth percentile of surface adhesion was 1784±40 pN for surfaces immersed in the FFC solutions compared to 2284±40 pN for the control solutions (Fig. 2c). These results suggested that more extensive regions of elevated interfacial repulsion as well as of decreased surface adhesion provided an explanation for the lower density of attached cells observed for the surfaces immersed in the FFC solutions compared to the control solutions.

Our previous experiments showed that the lower densities of attached cells after using FFC water were observed for surfaces of not only titanium but also steel, plastic, and glass. Thus, we consider that the utilization of FFC ceramic balls for plant production facilities and its water distribution systems may be a worthwhile strategy for preventing or at least hindering bacterial attachment and biofilm formation.

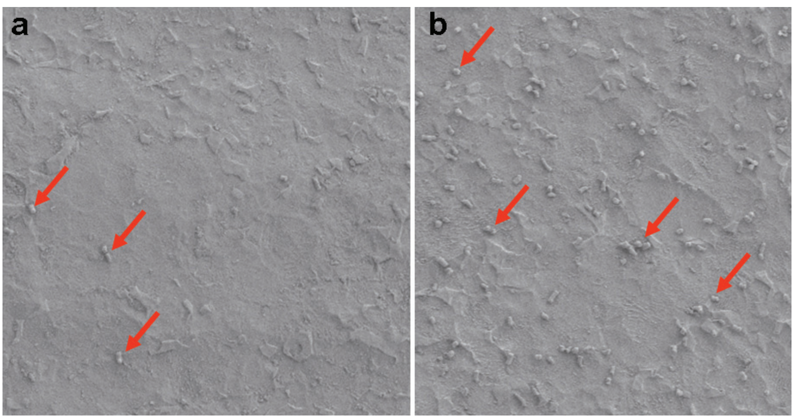

Figure 1. Scanning electron microscopy (SEM) image of titanium surfaces immersed in (A) ferrous ferric chloride (FFC) or (B) control solutions for 4 days. Prior to immersion SEM images showed clean surfaces. Image size: 40 × 40 μm^2.

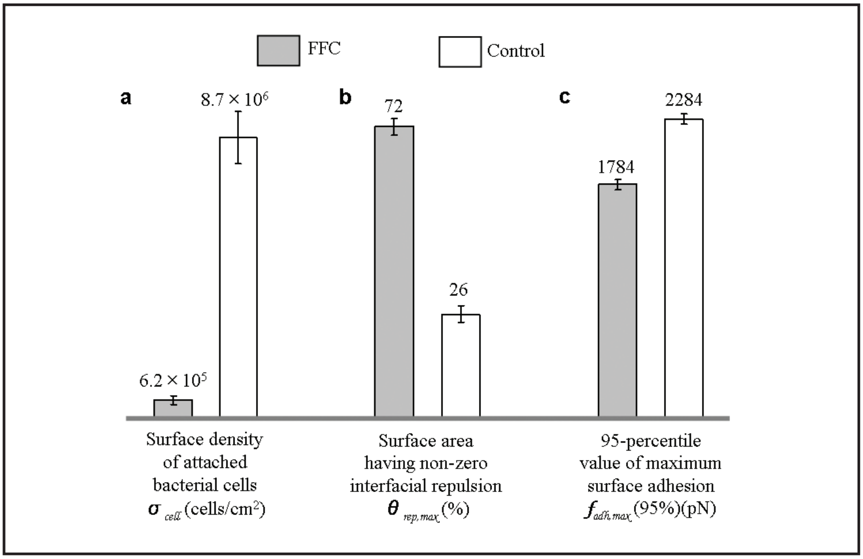

Figure 2. Comparison for ferrous ferric chloride (FFC) and control solutions of (A) surface cell density, (B) surface area having non-zero interfacial force, and (C) the 95-percentile value of maximum surface adhesion.

LITERATURE CITED

Hasegawa, S., A. Meguro, M. Shimizu, T. Nishimura, and **H. Kunoh.** 2006. The ceramic bead that is suitable for a carrier of plant-rooting accelerator, *Streptomyces* sp. MBR-52. Actinomycetologica 20:23–29.

Hirobe, T. 2007. Ferrous ferric chloride stimulates the proliferation and differentiation of cultured keratinocytes and melanocytes in the epidermis of neonatal mouse skin. J. Health Sci., 53:576–584.

Na, C., C.J. McNamara, N.R. Konkol, K.A. Bearce, R. Mitchell, and **S.T. Martin.** 2010. The use of force-volume microscopy to examine bacterial attachment to titanium surfaces. Ann. Microbiol. 60:495–502.

Sugi, J., and **S. Yamashita.** 1991. The method obtaining ferrous ferric chloride. Bull. Soc. Sea. Water Sci. Jpn. 42:11 (in Japanese).

Yokomizo, I. 2011. FFC de Keiei ha Kasseika suruka. The Food Industry. 53(17):57–62 (in Japanese).