Adsorption of Colloidal Iron by Bacteria

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The adsorption of iron from a positive-iron sol by species of seven bacterial genera was examined by electron microscopy. All species precipitated the iron from the sol, and the bacterial cells became encrusted with iron. This was related to iron deposition in surface water supplies.

The accumulation of ferric iron by microorganisms in water can lead to plugging or fouling of water pipes in treatment plants and in reticulation systems. This results in economic loss and inconvenience caused by shut-down of equipment and pipelines for cleaning. Many of these problems have been attributed to the activities of bacteria like Gallionella and Sphaerotilus, poorly defined “iron bacteria,” and a number of algae. In some instances the deposition of iron may arise from enzymatic mechanisms that bring about the oxidation of ferrous iron in solution. However, it may also result from non-enzymatic reactions followed by adsorption of the iron to the surface of the microbial cell. For example, Pringsheim (4) proposed that the precipitation of dissolved iron by algae results from oxidation of ferrous iron by oxygen generated during photosynthesis to hydrated ferric oxide which is adsorbed to the algal cells. Alexander (1) has suggested that encrustation may also arise from passive adsorption of cationic iron that is already oxidized. As a large proportion of iron that is transported in water may be in the form of a colloid (5), the present study was undertaken to examine the interaction between selected bacteria and a colloidal iron preparation.

MATERIALS AND METHODS

Bacterial cultures. Cultures of Caulobacter sp., Micrococcus sp., Pseudomonas fluorescens, Mycobacterium phlei, Escherichia coli, Klebsiella pneumoniae, and Corynebacterium pseudodiphtheriticum were obtained from the culture collection of the Microbiology Department, University of Queensland. With the exception of Caulobacter sp., the cultures were grown at 30°C for 48 hr in a liquid medium of the following composition: 0.5% yeast extract (Difco) and 0.5% peptone (Difco). Caulobacter sp. was grown in 0.1% peptone water at 30°C for 5 days. After incubation, washed suspensions of the bacteria were prepared in distilled water. Each suspension was adjusted to an absorbance reading of 0.75 at 540 nm.

Ferric iron sol. The positive-ferric-iron sol was made by the method described by Weiser (6), and chloride was removed by dialysis against distilled water for 4 days. A stable, deep red-brown colored sol resulted. Prior to mixing with the bacterial cell suspensions, the concentrated iron sol preparation was diluted 1:10 with distilled water. The diluted sol was amber in color.

Adsorption of iron. Equal amounts of the bacterial suspensions and diluted iron sol were mixed and incubated at 30°C for 2 hr. The appearance of the mixtures was observed every 20 min. During this time the bacterial suspensions and colored sol had precipitated out, leaving a clear supernatant and a red-brown deposit of bacteria and iron. The pH of the mixture was 5.25. The deposit was washed by centrifugation in distilled water and finally was resuspended in this medium for electron microscopy.

Electron microscopy. The bacteria-iron preparations were put onto 200-mesh copper grids of carbon-coated nitrocellulose film and examined using a Philips EM 300 electron microscope. Staining of the bacteria prior to electron microscopy was not performed. Preparations were photographed using Agfa Scientia 22D50 film.

RESULTS AND DISCUSSION

All of the bacteria tested formed a precipitate with the positive-iron sol. Although no effort was made to follow the rate of the reaction, observations made at 20-min intervals during the 2-hr incubation period indicated that the precipitate was formed more quickly by some bacterial suspensions than others. For example, precipitation was complete after 20 min with suspensions of Pseudomonas fluorescens and Mycobacterium phlei, whereas the supernatant retained some of the ferric iron sol color, even after 2 hr of incubation in the case of Corynebacterium pseudodiphtheriticum and Klebsiella pneumoniae. This aspect is being examined in more detail.

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Fig. 1. Electron micrographs of ferric iron sol and bacteria encrusted with iron after reaction with sol. A, Ferric iron sol; B, Klebsiella pneumoniae; C, Micrococcus sp.; D, Escherichia coli.
Fig. 2. Electron micrographs of Corynebacterium pseudodiphtheriticum after reaction with positive-ferric-iron sol. A, Cell aggregate encrusted with iron; B, Higher magnification of cell in (A) marked with arrow.
Fig. 3. Electron micrographs of bacteria encrusted with iron after reaction with positive-ferric-iron sol. A, Pseudomonas fluorescens; B, Mycobacterium phlei; C, Caulobacter sp.; D, rosette of Caulobacter sp. with common holdfast marked by arrow.
It is of significance that all of the bacteria could be easily visualized in the electron microscope without requiring negative staining. Figure 1a shows the electron-dense appearance of the iron sol in the electron microscope. Figures 1b, c, and d and Fig. 2 and 3 show the appearance of the bacteria-iron suspensions. All of the bacteria became heavily encrusted with the iron. The stalk of Caulobacter sp. (Fig. 3c) and the holdfast of the same bacterium (Fig. 3d) show considerable adsorption of the colloidal iron.

The mechanism of adsorption is not known. However, the results of this study indicate that many types of bacteria could cause iron problems in surface water supplies through adsorption of colloidal iron that is transported in the water in an oxidized form. Positively charged colloidal iron does occur in surface waters and is formed when iron is precipitated (3). Some of the bacteria used in this study are not likely to be found in water supplies but were included because they were representative of a particular type of surface morphology, for example, the presence of a capsule in the case of Klebsiella pneumoniae.

Colonization of the inner surface of the pipes by bacteria followed by precipitation of the colloidal iron on the bacterial cells could lead to iron-plugging of water pipe lines. Zapfe (7) has suggested that the sticky nature of precipitated iron facilities attachment to pipe walls and the combination of precipitate and bacteria forms the basis of the iron deposit. In connection with the formation of ferro-manganese concretions, Kalinenko (2) claims that the primary concretion results from colloidal precipitations of iron and manganese created by the biochemical activity of the bacteria. The involvement of the classical iron bacteria would not be necessary.

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LITERATURE CITED

1. Alexander, M. 1971. Microbial ecology. John Wiley & Sons, Inc., New York.
2. Kalinenko, V. O. 1946. The role of bacteria in the formation of ferro-manganese concretions. Mikrobiologiya 15:384-389.
3. Lowman, F. G. 1961. Iron and cobalt in ecology. Proc. 1st Nat. Symp. Radioecol. Fort Collins, Colo., Reinhold, New York, p. 561-567.
4. Pringsheim, E. G. 1949. The filamentous bacteria Sphaerotilus, Leptothrix, Cladothrix, and their relation to iron and manganese. Phil. Trans. Roy. Soc. London Ser. B 233:453-482.
5. Shapiro, J. 1964. Effect of yellow organic acids on iron and other metals in water. J. Amer. Water Works Ass. 56:1062-1082.
6. Weiser, H. B. 1935. Inorganic colloid chemistry Vol. II. The hydrous oxides and hydroxides. J. Wiley & Sons, Inc., New York.
7. Zapfe, C. 1931. Deposition of manganese. Econ. Geol. 26:799-832.