Influence of Colloidal Iron on the Respiration of a Species of the Genus Acinetobacter

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Washed suspensions of Acinetobacter sp. isolated from water caused the precipitation of iron from a suspension of colloidal ferric iron at pH 6.0 and 7.6. Iron-encrusted cells of the bacterium formed large aggregates. The amount of iron removed from the colloidal preparation in the form of aggregates was from 21 to 52% at pH 7.6 and 49% at pH 6.0 by the bacterial cells. Endogenous respiration rates of the iron-encrusted cells were from 32 to 72% lower than the rates for unencrusted cells. Respiration rates, measured polarographically in the presence of glucose, were also greatly reduced by the coating of iron on the cells.

Previous studies have shown that certain bacterial genera can precipitate ferric iron from a stable positive sol so that the cells became encrusted with iron (2). Because similar species of iron are known to exist in surface waters, this precipitation reaction was suggested to be important in the formation of iron deposits associated with plugging and fouling of water pipes. Apart from such considerations, it is also of importance to know what effect iron encrustation may have upon the length of time the bacterial cells remain viable within the encrustations, particularly when one is concerned with the isolation and identification of the bacteria involved.

Inorganic and organic colloids are known to affect biochemical activities of microorganisms. In some instances (1, 8) the effect has been a reduction in biochemical activity of the microbe, whereas in others (4, 7) a stimulation of activity has been reported.

The aim of the present study was to examine the interaction of cells of an Acinetobacter sp. isolated from water and an iron sol preparation. In particular, the effect of iron encrustation upon the respiration of the bacterium was studied.

MATERIALS AND METHODS

Organism and cultural conditions. The bacterium was a species of the genus Acinetobacter previously isolated from water (3). The bacterium was grown in filter-sterilized medium of the following composition: 0.25 g of K$_2$HPO$_4$, 0.20 g of MgSO$_4$, 7H$_2$O, 0.01 g of CaCl$_2$, 2H$_2$O, 0.001 g of FeCl$_3$, 6H$_2$O, 3.00 g of NH$_4$NO$_3$, and 10 g of glucose per liter of distilled water. To provide cell suspensions for respiration studies, 1-liter amounts of the above medium in 2-liter Erlenmeyer flasks, provided with forced aeration, were inoculated and incubated for 48 h at 30 C. The cells were harvested by centrifugation at 5,300 x g, washed twice with 0.025 M phosphate buffer, and resuspended in the same buffer (pH 6.0 or 7.6) so that the suspension had an absorbance of 0.70 when determined at 540 nm.

Ferric iron sol. The colloidal iron preparation was made as described previously (10), except that, after dialysis of the concentrated preparation, it was diluted 1:10 with appropriate 0.025 M phosphate buffers to give two preparations, pH 6.0 and 7.6. To prepare samples of cells encrusted with iron, the following mixtures were made in 250-ml Erlenmeyer flasks: washed cell suspension (112.5 ml) and iron sol preparation (12.5 ml). Mixtures of the same proportions of cells and the appropriate phosphate buffer were also made to provide cell suspensions free of colloidal iron. The mixtures were incubated for 20 h at 30 C, after which the respiration rates of the suspensions were studied by polarography. Samples were also taken for electron microscopy.

Respiration studies. The polarographic method for the determination of oxygen uptake by the bacterial suspensions was based on a method reported earlier (6) and employed a recording polarograph (Metrohm Polarecord type E261R, Metrohm Ltd., Switzerland) and a dropping mercury electrode. Dissolved oxygen determinations were performed by using a constant applied potential of -0.5 V. The reaction vessel was kept in a water bath maintained at 30 C. The reaction mixture, consisting of 38 ml of cell suspension (approximately 10$^9$ cells/ml) and 1.0 ml of a 0.02% aqueous solution of gelatin (to suppress the maximum in the first polarographic wave for oxygen), was placed in the polarograph vessel and aerated for 10 min. After aeration, the endogenous oxygen uptake was recorded. When a suitable length of recording had been obtained (usually 6 min), 1 ml of glucose solution containing 100 mg of glucose was added and mixed, and the recording of oxygen uptake in the presence of substrate was made for a period of 12 min.

Electron microscopy. The bacteria-iron prepara-
tion was washed by centrifugation in distilled water and suspended in this medium. Samples of the suspension were put onto 200-mesh copper grids which had been covered with carbon-coated nitrocellulose film and examined by using a Philips EM 300 electron microscopy. Staining of the bacteria prior to electron microscopy was not performed, since it was found previously (2) that the iron-encrusted cells were electron dense enough to be visualized without staining.

Chemical analysis. The amounts of iron in the colloidal preparation and in the supernatant above the bacteria-iron aggregates (after centrifugation at 1,200 rpm for 5 min in a bench-top angle head centrifuge) were determined spectrophotometrically (9).

RESULTS
Preliminary experiments aimed at determining the stability of the iron sol preparation at different pH values and in the presence of 0.025 M phosphate showed that the sol was stable for at least 22 h at 30 C at pH 6.0 and 7.6. However, at pH 6.8 and 7.2 the sol was not stable and formed a heavy brown precipitate within 22 h. Therefore, respiration studies were carried out at either pH 6.0 or 7.5.

When the washed cell preparations were mixed with the stable colloidal ferric iron preparation, heavy red-brown aggregates of bacteria and iron were formed which settled out of suspension during the incubation period of 20 h. Electron microscopy of the aggregates showed the bacterial cells to be coated with iron particles (Fig. 1).

Table 1 shows the results obtained for the respiration rates of the washed bacterial suspensions. The coating of iron was found to reduce the endogenous respiration and the respiration rate of the cells in the presence of glucose at both pH values.

To determine how much colloidal iron was removed from suspension in the bacterial aggregates, iron in the original colloidal preparation and in the supernatant above the bacteria-iron aggregates was determined (Table 2). At pH 6.0 the amount of iron removed in the bacterial aggregate was 49%, whereas at pH 7.6 the amounts removed were 21 and 52%. No change in pH was detected at either pH at the end of each experiment.

DISCUSSION
The results obtained in the present study are similar to those obtained by others (1, 8), in that respiratory activity of the bacterium was greatly reduced when aggregated with colloidal materials. However, increased shaking rate in

![Fig. 1. Electron micrograph of cell aggregate of Acinetobacter sp. with iron encrustation, unstained.](image)
TABLE 1. Effect of colloidal iron encrustation on respiration of washed cell suspensions of Acinetobacter sp. in the presence of glucose

| Expt | pH | Oxygen uptake (µl/h) | Iron-encrusted cell suspension | Reduction in respiration rate (%) |
|------|----|---------------------|-------------------------------|----------------------------------|
|      |    | Cell suspension     |                               |                                  |
| 1    | 7.6| 955 (750)           | 546 (273)                     | 43 (64)                          |
| 2    | 7.6| 2,524 (477)         | 955 (156)                     | 62 (72)                          |
| 3    | 7.6| 1,364 (955)         | 614 (648)                     | 55 (32)                          |
| 4    | 6.0| 473 (273)           | 136 (136)                     | 71 (50)                          |
| 5    | 6.0| 546 (409)           | 477 (238)                     | 13 (42)                          |

*Endogenous respiration values shown in parentheses.

Table 2. Precipitation of iron from a stable colloidal ferric iron preparation by washed cells of Acinetobacter sp.

| Expt | pH | Conc of iron (µg/ml) | Iron precipitated with bacterial cells (%) |
|------|----|---------------------|-----------------------------------------|
|      |    | Original colloidal prep* | Supernatant after aggregation |
| 1    | 7.6| 170                 | 81                                      |
| 2    | 7.6| 220                 | 174                                     |
| 3    | 6.0| 220                 | 113                                     |

*Concentration was adjusted to allow for addition of bacterial suspension.

The Warburg respirometer increased the respiration rate of aggregates of Bacillus subtilis and clay mineral. Although other factors may have been involved, it seems likely that equilibrium between gas-phase oxygen and the bacteria-clay suspensions was not reached at the slower shaking rates, thereby limiting respiration. This is a problem commonly overlooked in respirometric studies, where the assumption is made that equilibrium is established between the liquid and gas phase with respect to oxygen concentration. In the present study, where direct measurement of dissolved oxygen was carried out, diffusion into the reaction mixture is not relevant.

Inhibition of endogenous respiration of the Acinetobacter sp. by the coating of iron could have been due to unavailability of oxygen or inability to eliminate waste products through the iron barrier. This has already been suggested (1) in the case of B. subtilis-clay mineral aggregates. Also, the inhibition of respiration of the Acinetobacter sp. encrusted with iron in the presence of glucose may have been due to lowered diffusion rates of the glucose to the bacterial cells through the iron barrier. In addition, unavailability of oxygen and waste product accumulation could have limited respiration.

In view of the greatly reduced respiration rate due to iron encrustation, iron deposits in pipelines or other parts of water supply systems formed by bacteria such as the one used in this study are most likely to consist chiefly of dead bacterial cells, heavily encrusted with iron, that have no access to energy substrate or facility for excretion of wastes. Isolation of the bacteria in pure culture would be difficult due to the preponderance of dead cells. Colonization of the dead regions of the deposits by anaerobic bacteria could introduce the further problem of corrosion through the establishment of differential aeration cells (5).

The results show that large amounts of iron can be entrapped among bacterial cells and removed from colloidal suspension in the form of bacteria-iron aggregates. Whereas the respiration rates with iron-encrusted cell suspensions were in general greatly reduced, there was considerable variation among the results of the various experiments with respect to the extent of the reduction. Also, there was variation in the amount of iron precipitated with the bacterial cells (Table 2). Such differences are difficult to explain, but could possibly be related to slight differences in the physiological ages of the cells used and to differing surface characteristics such as variable aggregate size and the rate at which the aggregates are formed.

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