Electrochemical Regeneration of Fe(III) To Support Growth on Anaerobic Iron Respiration

Naoya Ohmura,* Norio Matsumoto, Kazuhiro Sasaki, and Hiroshi Saiki
Department of Bio-Science, Central Research Institute of Electric Power Industry, 1646 Abiko, Abiko-city, Chiba 270-1194, Japan

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Here we describe artificial help for the respiratory electron flow supporting anaerobic growth of Thiobacillus ferrooxidans through exogenous electrolysis. Flux between H2 and an anode through cells was accomplished with electrochemical regeneration of iron. The electrochemical help resulted in a 12-fold increase in yield compared with the yield observed in its absence.

In 1964, Kinsel et al. discovered that the chemolithotrophic bacterium Thiobacillus ferrooxidans grew on ferrous iron electrochemically generated by an electrode (7). In this system, the electrode provided electrons to the aerobic respiratory chain of the bacterium via iron (3, 5, 12, 13). However, as a practical matter, this electrochemical help is available to only a few iron oxidizers that derive energy for growth from aerobic iron respiration. On the other hand, anaerobic iron respiration has been found in many eubacteria and archaeabacteria (4, 6, 8, 15, 16, 18). These organisms are able to derive energy for growth from iron reduction mediated by various electron donors (9, 10, 14). If microorganisms could respire on ferric iron electrochemically generated by an electrode, then the electrode would be accepting electrons from a respiratory chain via iron and would support growth of bacterial cultures by regenerating a terminal electron acceptor in the total electron flow comprising anaerobic iron respiration. It is conceivable that such a novel system could facilitate culture of many iron reducers.

Apparatus for electrolytic cultivation. Electrolytic cultivation under potentiostatic conditions was carried out with an apparatus comprised of catholyte and anolyte baths separated by a cation-exchange membrane (type A-201; Asahi Chemical, Tokyo, Japan), into which carbon and platinum mesh electrodes (10 by 70 mm), respectively, were inserted (Fig. 1). The anolyte bath was filled with 100 ml of Fe2(SO4)3-containing medium, while the catholyte bath was filled with medium that was identical to the anolyte bath medium except that it lacked Fe. Each medium contained (per liter of distilled water) 133 mg of (NH4)2SO4, 41 mg of K2HPO4, 490 mg of MgSO4·7H2O, 9 mg of CaCl2·2H2O, 52 mg of KCl, 1 mg of ZnSO4·7H2O, 2 mg of CuSO4·5H2O, 1 mg of MnSO4·H2O, 0.5 mg of Na2MoO4·2H2O, 0.5 mg of CoCl2·6H2O, 1 mg of Na2SeO3·10H2O, and 1 mg of NiCl2·6H2O. Forty-eight grams of ferric sulfate hydrate (60 to 80% of the ferric sulfate content) was added to 1 liter of the medium, and the pH of the medium was adjusted to 2.0 with 6 N H2SO4. An Ag-AgCl reference electrode situated in the anolyte bath between the anode and the cation-exchange membrane was used to control the anodic potential (12). The entire apparatus was placed in an airtight box (width, 30 cm; depth, 30 cm; height, 25 cm) and incubated at 30°C with an atmosphere containing 80% H2 and 20% CO2 at a pressure of 250 kPa. After the anolyte bath was inoculated with T. ferrooxidans JCM 7811 obtained from the Japan Collection of Microorganisms, the electrodes were connected to a potentiostat, and the potential driving the electrolysis was maintained at 400 mV. Culture samples were anaerobically taken from a port in the airtight box, which was connected to the electrolytic apparatus. Cell numbers were determined directly by counting with a phase-contrast microscope at a magnification of ×400. The concentrations of Fe3+ were determined by the phenanthroline method as described previously (12). The total concentration of iron was also determined by the same method after reduction of iron by NH2OH·HCl. The Fe3+ content was calculated by subtracting the Fe2+ content from the total iron content.

Growth on electrolytic respiration. T. ferrooxidans is generally considered to be an autotrophic bacterium that can grow aerobically on soluble ferrous iron or sulfur compounds (2). This bacterium nevertheless exhibited chemolithoautotrophic growth under strictly anaerobic conditions through reduction of Fe3+ using H2 as an electron donor. Growth of the bacterium proved to be strongly related to the reduction of Fe3+, eventually yielding a cell density of 8.4 × 108 cells/ml (Fig. 2a). During a 74-h incubation period, the Fe3+ added was reduced completely to Fe2+, after which growth entered a stationary phase (Fig. 2b). Growth resumed, however, upon application of potential-controlled electrolysis, which regenerated Fe3+ by oxidizing Fe2+ at the anode. Throughout cultivation, the concentration of Fe3+ was kept between 30 and 50 mM by passage of 10.0 to 15.5 mA of current, and the final cell density after 142 h of electrolysis was 1010 cells/ml (Fig. 2a and b). Thus, electrolysis resulted in a 12-fold increase in cell density compared with the cell density achieved in the absence of electrolysis. On the other hand, no growth occurred in the absence of iron or H2, whether current was applied or not. In addition, no reduction occurred in the presence of iron and H2 under electrolysis conditions without cells (data not shown).

To confirm that the observed increase in bacterial growth was a consequence of electrolytic respiration, in another batch of cells electrolysis was initiated 35 h after inoculation, when
the cells were still growing logarithmically (Fig. 2a and d). With
the assistance of electrolytic respiration, the cells grew for
130 h to a density of $7.1 \times 10^9$ cells/ml. In the absence of
electrolysis, by contrast, growth stopped after 71 h, when all of
the Fe$^{3+}$ had been reduced to Fe$^{2+}$, and the cell density was
only $7.5 \times 10^8$ cells/ml (Fig. 2a and c). Apparently, the anode
was able to effectively serve as a terminal electron acceptor
supporting anaerobic bacterial respiration, with iron mediating
the transfer of electrons from the bacterial respiratory chain to
the electrode.

The potential for oxidation of Fe$^{2+}$ at the anode was kept
constant at 400 mV, which was sufficient to sustain oxidation of
Fe$^{3+}$ in the medium (12). At the same constant potential, H$_2$

Figure 1: Schematic diagram illustrating the concept behind electro-
chemical regeneration of an electron acceptor for anaerobic respira-
tion.

The schematic diagram in Fig. 1 summarizes our concept of
bacterial cultivation driven by electrochemical regeneration of
an electron acceptor for respiration. The electron flux begins
between H$_2$ and the bacterial cell. The bacterium oxidizes H$_2$
an aerobically and then transfers the accepted electrons to
Fe$^{3+}$ in its respiratory chain. The Fe$^{2+}$ generated is oxidized by
the anode, completing the electron flux from H$_2$ to the elec-
trode through the bacterium. The oxidation of Fe$^{2+}$ regener-
ates Fe$^{3+}$ capable of accepting additional electrons. With re-
spect to the total electron flow, the anode can support anaerobic respiration of the bacterium using iron as an elec-
tron mediator.

For the past 35 years, bacterial cultivation using electrodes
has been discussed in terms of electrochemical regeneration of
Fe$^{2+}$ as the electron donor for aerobic growth of T. ferro-
oxidans (3, 5, 7, 12, 13). On the other hand, the concept of
regenerating an electron acceptor for anaerobic respiration is
novel and may be useful for culturing numerous as-yet-unk-
known organisms, since conventional isolation techniques are
suitable for culturing only a small percentage of the species in
an environmental sample (1, 19). Indeed, because the elec-
trode would be able to oxidize a number of soluble iron com-

Figure 2: Chemolithoautotrophic growth of T. ferrooxidans strain JCM 7811 mediated by electrochemical regeneration of an electron acceptor
for anaerobic iron respiration. (a) Cell density with and without electrolysis. Electrolysis was applied to cultures at different times (35 to 178 h [▲]
and 97 to 239 h [■]). The arrows indicate when electrolysis was started. No electrolysis was applied to a control culture (○). (b, c, and d) Iron
concentrations and electric currents in the cultures described above. (b) Culture electrolysed at 97 to 239 h; (c) control culture; (d) culture
electrolysed at 35 to 178 h. Symbols: ○ and ▲, time-dependent changes in Fe$^{3+}$ and total iron concentrations, respectively; □, current driving the
electrolysis. All values are averages based on duplicate determinations with independent cultures.
plexes at neutral pH, anaerobic respiration and growth of a wide variety of both Bacteria and Archaea could be supported (9, 10, 14). Although iron-reducing bacteria have recently been isolated from various sediments, the deep subsurface, groundwater, and hydrothermal vents (4, 6, 8, 15, 16), electrolytic cultivation should provide another approach for isolating additional iron reducers that respire anaerobically on metals (9, 10, 14) or nitrogen compounds (17). Thus, electrochemically driven growth has the potential to be a highly productive approach for accelerating bacterial degradation of organic materials, including toxic chemicals, some of which are capable of serving as electron donors that support bacterial respiration (11).

REFERENCES