

BACTERIAL REDUCTION OF AMORPHOUS AND CRYSTALLINE IRON OXIDES

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In flooded soils with sufficient amount of easily decomposable organic matter, the reduction of ferric oxides is to be ascribed to the activity of aerobic and anaerobic bacteria.

In model experiments with nitrate- and/or manganese and iron oxide-reducing bacteria (*Bacillus polymyxa*, nit⁺ and *Clostridium butyricum*, nit⁻) the manganese or iron reduction was repressed by nitrate so far the organism was equipped with nitrate reductase (*B. polymyxa*). If not (*C. butyricum*), manganese and iron oxide reduction were not affected by nitrate in the media, and nitrate remained unchanged although Mn (II) or Fe (II) accumulated and completely reduced conditions (rH = 0) were recorded. Apparently, nitrate, Mn (IV) and Fe (III) are reduced directly and enzyme-specifically rather than indirectly as a consequence of reducing metabolites and/or a lowered Eh, so that reduction of ferric oxides is to be considered as an anaerobic respiration.

The enzymatical transfer of hydrogen from the bacterial cells to the external ferric acceptor needs an intimate contact between the practically insoluble ferric oxides and the bacterial cells. The influence of separation of cells from Fe (III)-oxides was studied in model experiments by including synthetic hematite in dialytic bags (pores < 20 Å), incorporated in a glucose-mineralsalt-broth (pH > 7), inoculated with one of the chosen strains of iron reducing bacteria, and incubated anaerobically (Table I).

Furthermore, the bacterial reduction of iron oxides is specific in view of oxide form, as observed in the course of the reduction of mixtures of amorphous and ⁵⁹Fe-labelled crystalline Fe (III) - oxides (Tables II and III). The amorphous forms were used rather than the crystalline forms as hydrogen acceptors according to their energy of formation.

In flooded soils and in sites of intensive mineralisation, iron reduction should be considered as a direct biochemical rather than a chemical process.

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Table I : Separation of hematite particles from *B. polymyxa* S55 and *C. butyricum* S22a and its influence on iron reduction, pH and rH under anaerobic conditions.

| Incubation days ¹⁾ | <i>B. polymyxa</i> | | | | | | <i>C. butyricum</i> | | | | | |
|-------------------------------|--------------------|-----|--------------|-------------------------|-----|--------------|---------------------|------|--------------|-------------------------|-----|--------------|
| | mixed | | | separated ²⁾ | | | mixed | | | separated ²⁾ | | |
| | pH | rH | Fe(II) µg/ml | pH | rH | Fe(II) µg/ml | pH | rH | Fe(II) µg/ml | pH | rH | Fe(II) µg/ml |
| Start ³⁾ | 7,3 | 19 | 2,0 | 7,4 | 27 | 2,0 | 7,3 | 19 | 2,0 | 7,4 | 27 | 2,0 |
| 2 | 5,7 | 0,2 | 22,8 | 5,3 | 0,5 | 2,0 | 5,0 | 0 | 30,0 | 4,2 | 0 | 2,0 |
| 5 | 6,2 | 3,5 | 81,0 | 3,7 | 1,2 | 2,0 | 4,5 | 10,8 | 425,0 | 4,2 | 7,0 | 4,0 |
| 9 ⁴⁾ | 7,1 | 8,8 | 59,8 | 5,8 | 1,9 | 8,0 | 4,5 | 16,8 | 375,0 | 4,2 | 9,8 | 5,5 |
| 12 | - | - | - | - | - | - | 4,5 | 16,8 | 375,0 | 4,2 | 9,8 | 5,5 |

- (1) Under anaerobic conditions ($N_2/CO_2 = 9/1$) at 30°C.
- (2) Fe_2O_3 powder in dialysis tubes (regenerate cellulose, pores diameter : 15-20 Å).
- (3) Sterile controls at the end of incubation time : pH = 6.6, rH = 18, Fe (II) concentration = 2.0 µg/ml.
- (4) At this stage the incubation was interrupted since the dialysis membrane was about to be destroyed through the cellulolytic activity of *B. polymyxa*.

Table II : Differentiation of Fe (II) formation by the iron reducing bacteria *C. butyricum* S22a from a mixture of non-crystalline iron oxides (gley soil : $Fe_o/Fe_d = 0,78$) and ^{59}Fe -labelled hematite.

| Incubation days | $Fe(II)_t$ | $^{59}Fe(II)$ | $Fe(II)_t$ - $^{59}Fe(II)$ | $^{59}Fe(II)$ in % of $Fe(II)_t$ | $^{59}Fe_d/Fe_d$ total % | non-labelled Fe(II) in % of Fe_d from gley soil (470 mg gley=4,1 mg Fe) | $^{59}Fe(II)$ in % from ^{59}Fe -hematite (30 mg hematite=20,9 mg Fe) |
|------------------|------------------------|---------------|----------------------------|----------------------------------|--------------------------|---|---|
| | mg Fe / 500 mg mixture | | | | | | |
| Start | 0,02 | 0 | 0,02 | - | 83,5 | 0,5 | 0 |
| 4 | 1,78 | 0,02 | 1,76 | 1,4 | 90,0 | 42,9 | 0,1 |
| 8 | 3,00 | 0,10 | 2,90 | 3,2 | 92,2 | 70,1 | 0,5 |
| 13 ¹⁾ | 3,07 | 0,13 | 2,94 | 4,2 | 92,5 | 70,8 | 0,6 |

- (1) Maximum Fe (II) concentration in solution was reached after 13 incubation days at 30°C, under anaerobic atmosphere ($N_2/CO_2 = 9/1$).

Table III : Differentiation of Fe (II) formation by the iron reducing bacteria *C. butyricum* S22a from a mixture of non-crystalline iron oxides (gley soil : $Fe_o/Fe_d = 0,78$) and ^{59}Fe -labelled goethite (1).

| Incubation days | $Fe(II)_t$ | $^{59}Fe(II)$ | $Fe(II)_t$ - $^{59}Fe(II)$ | $^{59}Fe(II)$ in % of $Fe(II)_t$ | $^{59}Fe_d/Fe_d$ total % | non labelled Fe(II) in % of Fe_d from gley soil (470 mg gley=4,1 mg Fe) | $^{59}Fe(II)$ in % from ^{59}Fe -goethite (30 mg goethite=9,26 mg Fe) |
|-----------------|------------------------|---------------|----------------------------|----------------------------------|--------------------------|---|---|
| | mg Fe / 500 mg mixture | | | | | | |
| Start | 0,01 | 0 | 0,01 | - | 70,7 | 0,3 | 0 |
| 4 | 1,45 | 0,21 | 1,24 | 14,5 | 79,5 | 29,9 | 2,1 |
| 8 | 2,75 | 0,23 | 2,52 | 8,4 | 81,9 | 61,4 | 2,3 |
| 13 | 3,22 | 0,32 | 2,90 | 9,9 | 84,2 | 70,1 | 3,2 |

- (1) Natural goethite from Ward's Company, Rochester, USA.