Bacterial Reduction of Ferric Iron in a Stratified Eutrophic Lake

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The rate of release of Fe(II) from anoxic lake sediments was lower in the presence than in the absence of nitrate. The reduction of Fe(III) by the sediments had a temperature optimum of 30 °C and was inhibited by HgCl₂, suggesting that the process was largely biological in nature. Of the iron sources tested with cultures of anaerobic iron-reducing bacteria, FeCl₃ was the most readily reduced and goethite the least. Reduction was faster in the presence of a chelating agent and was suppressed by the addition of NO₃⁻, ClO₃⁻, MnO₂, Mn₂O₃ and O₂. An iron-reducing chemoorganotroph, tentatively identified as a member of the genus *Vibrio*, was isolated. Physical contact between the bacterium and iron particles was essential to ensure maximum rates of Fe(III) reduction but > 30% of the activity appeared to be associated with extracellular components. Although Fe(III) reduction by whole cells and cell-free extracts was decreased in the presence of electron transport inhibitors, the molar growth yield of the organism was unaffected by the presence of Fe(III). It is assumed that the organism used the Fe(III) as a hydrogen sink. A second organism, an anaerobic facultative chemolithotroph, appeared to conserve energy by the reduction of Fe(III). Biomass yield (measured as ATP) was greater in the presence of Fe(III), and the organism was able to use H₂ as a source of reducing power.

INTRODUCTION

Much of the ecological work on iron reduction has been concerned with bacterial activity in waterlogged soils. Of the genera isolated which were able to reduce Fe(III) (Ottow & Glathe, 1971), many were facultative anaerobes and possessed dissimilatory nitrate reductases. These enzymes were implicated in the process (Ottow & Munch, 1978) particularly since nitrate reductaseless mutants were unable to reduce Fe(III) (Ottow, 1970). The reduction of Fe(III) by organisms possessing nitrate reductase was suppressed by the addition of NO₃⁻ or MnO₂, whereas those which did not possess the enzyme were inhibited only by MnO₂. Munch & Ottow (1977) suggested that a specific ferrireductase was responsible for reduction in the latter. Lascelles & Burkes (1978) concluded that Fe(III) was reduced by *Staphylococcus aureus* by electron transport chain components which preceded cytochrome *b*, but which were preferentially oxidized by a nitrate reductase system. On the other hand it has been proposed that the apparent effect of NO₃⁻ in Fe(III) reduction may be due, under certain circumstances, to reoxidation of the Fe(II) by the NO₂⁻ formed (Obueke *et al.*, 1981). Munch & Ottow (1982) demonstrated that physical contact was necessary for bacterial electron transport to ferric iron, yet extracellular reduction of Fe(III) took place in aerobic cultures of a marine diatom (Anderson & Morel, 1980).

Electron transport phosphorylation coupled to Fe(III) reduction is thermodynamically feasible given the redox potential of the Fe³⁺/Fe²⁺ couple, and a ΔG° of $-228.3 \text{ kJ mol}^{-1}$ for the reaction $2\text{Fe}^{3+} + [\text{H}_2] \rightarrow 2\text{Fe}^{2+} + 2\text{H}^+$ (Thauer *et al.*, 1977) yet few attempts have been

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; TPTZ, 2,4,6-tripridyl-1,3,5-triazine.

made to determine whether bacteria obtain energy from the process. Takai & Kamura (1966) reported that bacteria isolated from a waterlogged paddy soil grew five times faster in the presence of $\text{Fe}(\text{OH})_3$ than in its absence, and that growth was proportional to the amount of $\text{Fe}(\text{II})$ produced. They did not, however, include any results which supported these statements. Balashova & Zavarzin (1979) isolated a pseudomonad which was both a facultative anaerobe and a facultative chemolithotroph. This organism reduced $\text{Fe}(\text{III})$ with H_2 as the electron donor, and plate counts were 10^3 times higher after 10 d incubation in the presence of $\text{Fe}(\text{III})$ than in its absence. In a study with cell-free extracts, Lascelles & Burke (1978) showed that the inhibitors HOQNO and azide did not directly affect the reduction of $\text{Fe}(\text{III})$ by membrane fractions.

The reduction of $\text{Fe}(\text{III})$ occurs in eutrophic lakes (Mortimer, 1941, 1942) and the dynamics of the process have been studied in some detail (Davison *et al.*, 1981; Verdouw & Dekkers, 1980). The upward transport of $\text{Fe}(\text{II})$ by turbulence and its oxidation to hydrous ferric oxide on contact with oxygenated water produces a distinctive turbidity profile in the hypolimnion. The shape and movement of the turbidity peak provides valuable information on the balance between turbulence and reducing conditions (Davison *et al.*, 1981) as well as the location of certain bacterial populations (Jones, 1981). The mechanism of $\text{Fe}(\text{III})$ reduction, however, requires investigation and this paper examines the distribution and physiology of iron-reducing bacteria in a stratified eutrophic lake.

METHODS

Sampling site and procedure. Samples were taken from Blelham Tarn (English Lake District: $54^\circ 24' \text{ N}$, $2^\circ 59' \text{ W}$) and all field procedures were as described by Jones *et al.* (1982) and Jones (1981).

Core tube experiments. Sediment cores were taken from the profundal zone of Blelham Tarn before the onset of summer stratification. The water above the sediment was analysed for major ions, particularly potential electron acceptors. The water was then removed and replaced, with minimum disturbance of the sediment, by a chemically defined salts solution based on the above analysis. This contained ($\mu\text{mol l}^{-1}$ in distilled H_2O): NaHCO_3 , 320; K_2HPO_4 , 0.25; CaCl_2 , 250; MgSO_4 , 110; $(\text{NH}_4)_2\text{SO}_4$, 10; KNO_3 , 70. A second solution, in which the KNO_3 was replaced by $70 \mu\text{mol KCl l}^{-1}$, was also prepared to determine the effect of NO_3^- on $\text{Fe}(\text{III})$ reduction. Unstoppered sediment cores were incubated for 16 h at 8°C and the overlying water was replaced once again by the chemically defined solutions. It was hoped that this treatment would establish relatively reproducible gradients of electron acceptors within the sediment while allowing the experiment to start with overlying water of known composition. The tubes were then stoppered after inserting magnetic stirring vanes (Jones, 1976). The upper stopper contained two wide-gauge syringe needles, one for removal of water samples and the other attached to a reservoir of the minerals solution which had been degassed and maintained under a slight pressure of N_2 . The cores were incubated at 20°C and the water stirred gently immediately before removal of small ($\leq 1\%$ of the volume of overlying water) samples for chemical analysis.

Chemical analysis. Nitrate and $\text{Mn}(\text{II})$ were determined according to Mackereth *et al.* (1978) and $\text{Fe}(\text{II})$ with TPTZ (Standing Committee of Analysts, 1977).

Enumeration of bacteria which reduced or deposited $\text{Fe}(\text{III})$. The semi-solid media and procedures used were as described by Jones (1983).

Isolation of $\text{Fe}(\text{III})$ -reducing bacteria. Primary enrichments in semi-solid agar media were subcultured on to plates of solid medium from which $\text{Fe}(\text{II})$ had been omitted. Immediately after visible growth of a colony was detected, a small portion of agar adjacent to it was removed and tested for the presence of $\text{Fe}(\text{II})$ with TPTZ. Colonies which showed the strongest reaction were subcultured into liquid medium. These cultures, designated mixed enrichment cultures at this stage, were transferred several times from liquid to solid media of the same composition as the medium used for isolation, and were periodically checked for purity on medium which also contained 100 mg l^{-1} each of peptone and yeast extract. Although they originated from single colonies, several passages through this process were necessary to ensure purity of the cultures, particularly those of the H_2 -utilizing anaerobes. The bacteria were treated as strict anaerobes and were cultured using a serum vial modification of the Hungate technique (Latham & Wolin, 1978). The gas mixtures were purified by passage through commercially available oxygen traps which had previously been shown to reduce O_2 levels sufficiently to allow growth of methanogens (Jones *et al.*, 1982). Hydrogen utilization was detected as a drop in pressure and by gas chromatographic analysis of the head space.

Measurement of bacterial growth. The dry weight of pure cultures was determined and corrected for the quantity of $\text{Fe}(\text{III})$ present after three washings with distilled water. Before measuring the turbidity of liquid cultures $5 \mu\text{l}$ of $10 \text{ mol l}^{-1} \text{ HCl}$ was added to 1 ml of culture to dissolve completely the ferric oxy-hydroxides. The addition of acid did not affect the turbidity of cultures grown in the absence of $\text{Fe}(\text{III})$. Very good linearity was obtained between

dry weight and turbidity determinations. ATP was determined by a sensitive modification of the luciferin-luciferase procedure (Jones & Simon, 1977). Samples were injected directly either into boiling Tris buffer pH 7.8, or into an equal volume of ice-cold 10% (w/v) TCA containing 4 mmol EDTA l⁻¹; the latter were diluted to 50-fold in buffer before analysis. Direct counts of bacteria were made by epifluorescence microscopy (Jones & Simon, 1975). When glucose was used as substrate its concentration in the growth medium was determined by conversion to gluconate 6-phosphate with stoichiometric production of NADPH (Boehringer Mannheim Test Kit, code no. 139106). Molar growth yields were determined both as the final dry weight obtained from a given concentration of glucose, and as the relationship between increase in turbidity and glucose utilization during the early exponential phase of growth.

Cell-free extracts. Cells were harvested anaerobically by centrifugation at 6000g for 30 min, and the pellet was resuspended and washed in 50 mmol l⁻¹ Tris/HCl buffer, pH 7.2. The cells were finally resuspended in 10 ml of buffer, treated for 2 min in an ultrasonic disintegrator set to an amplitude of 12 µm, and then centrifuged at 10000 g for 30 min. The supernatant was used as a crude cell-free extract either immediately or after storage on ice under N₂. The protein content of the extract was determined by the Coomassie Brilliant Blue dye binding technique (Bradford, 1976). The reaction mixture (final pH 6.8) used to measure Fe(III) reduction by cell-free extracts contained: enzyme preparation (100 vols); MgCl₂, 1 mol l⁻¹, K₂HPO₄, 1.2 mol l⁻¹ (1 vol); ADP, 200 mmol l⁻¹ (1 vol); NADH, 300 mmol l⁻¹ (1 vol); FeCl₃, 500 mmol l⁻¹ (1 vol). The following inhibitors were added as × 100 strength solutions; the final concentrations (mol l⁻¹) were: rotenone, 10⁻⁵; HOQNO, 10⁻⁴; ClO₃⁻, 5 × 10⁻³; antimycin A, 10⁻⁴ [or 0.15 µg (mg protein)⁻¹]; NaN₃, 10⁻³ and 10⁻⁵; DNP, 10⁻⁴; CCCP, 10⁻⁶. These inhibitors were also used to treat growing cultures of Fe(III)-reducing bacteria.

Effect of direct contact on bacterial iron reduction. Bacteria were grown in standard medium in 100 ml serum vials. In control vials FeCl₃ was incorporated in the medium to a final concentration of 0.4 mmol l⁻¹. In the test vials the FeCl₃ was contained in a dialysis bag (Visking tubing 6 mm diameter, 0.32 mm wall thickness and nominal mol. wt cut off about 10000). The amount of FeCl₃ in the dialysis bag was equivalent to a final concentration of 0.4 mmol l⁻¹ in the 50 ml of medium in the vial. The FeCl₃ was made up in complete medium, set to pH 6.8 and allowed to flocculate overnight before the dialysis bags were introduced into vials. The possibility of extracellular reduction of Fe(III) was tested by removal of bacteria at the end of an experiment by centrifugation at 5000 g for 45 min at 4 °C followed by filtration of the supernatant through 0.2 µm pore size polycarbonate filters (Nuclepore). The cell-free medium was then incubated anaerobically with FeCl₃ added to a final concentration of 0.4 mmol l⁻¹. All manipulations were carried out in the anaerobic handling chamber.

RESULTS

Iron reduction in sediments and by bacteria

The reduction and reprecipitation of Fe(III) in a stratified eutrophic lake produces a characteristic turbidity profile. Such a profile, and most probable number counts of iron-depositing and iron-reducing bacteria in the water column, are shown in Fig. 1. The population of reducers was the larger by almost an order of magnitude, averaging about 500 bacteria ml⁻¹, but the difference may merely reflect the efficiency of recovery on the two media used. A more detailed examination of the water-borne population about the turbidity peak showed that counts of iron reducers at the peak were three to four times higher than those either side. The numbers were decreased by 60–90% if NO₃⁻ was incorporated in the medium. Iron-depositing bacteria were more numerous (3.9 × 10⁴ ml⁻¹) in the oxidized littoral sediment than in the anoxic profundal mud (2.7 × 10⁴ ml⁻¹) whereas, as might be expected, the reverse was true of bacteria which reduced Fe(III) (counts of 1.3 × 10⁴ ml⁻¹ and 8.2 × 10⁴ ml⁻¹, respectively, in the two zones). The rate of release of Fe(II) from profundal sediments was measured in sealed sediment cores (Fig. 2). The Fe(II) concentration did not begin to rise appreciably until the oxygen was depleted (70 h), when a release rate of 2.2 µmol l⁻¹ h⁻¹ was observed. The presence of NO₃⁻ in the overlying water resulted in a slower release of Fe(II) until all the NO₃⁻ had been respired anaerobically (190 h). The NO₃⁻ may have been involved in any of the three mechanisms mentioned in the Introduction, but its effect on the *E_h* and the chemical release of iron should not be discounted. The release of Mn(II) was relatively small and did not continue with time.

To determine the relative importance of the biological and chemical mechanisms for the reduction of Fe(III) we measured the effect of temperature and a biological poison on Fe(II) release from diluted profundal sediment. The presence of a clear temperature optimum (Fig. 3), and significant inhibition by HgCl₂, suggested that the process was largely biological in nature.

Fig. 1

Fig. 2

Fig. 1. Distribution of temperature (○), dissolved oxygen (●), turbidity (□), numbers of Fe(III)-depositing (△) and Fe(III)-reducing (▲) bacteria with depth in the water column of Blelham Tarn on 30 July 1981. The 95% confidence limits of the log counts are ± 0.30 .

Fig. 2. Release of Fe(II) from sediment in sealed cores in the presence (●) and absence (○) of NO₃⁻ in the overlying water. Also shown is the removal of NO₃⁻ (□) and the accumulation of Mn(II) (△). The cores were taken from Blelham Tarn before the onset of summer stratification and incubated at 20 °C.

Fig. 3. Effect of temperature on the release of Fe(II) from profundal sediments of Blelham Tarn. Samples were incubated in serum bottles for 23 h (●) and 96 h (○) under an atmosphere of 95% N₂/5% CO₂. The horizontal lines indicate Fe(II) release at 30 °C over the same time periods after treatment with HgCl₂ at a final concentration of 100 mg l⁻¹.

Table 1. *Effect of electron acceptor and electron donor on Fe(III) reduction in mixed enrichment cultures of sediment bacteria*

Gas phase . . .	Rate of Fe(II) formation*			
	80% H ₂ /20% CO ₂		95% N ₂ /5% CO ₂	
	Present	Absent	Present	Absent
Organic C and N† . . .				
Fe(III) source‡				
FeCl ₃	100	4.5	83	0.3
FePO ₄	76	1.3	68	0.2
Fe ₂ O ₃	58	1.1	41	ND
Goethite	40	1.1	21	ND

ND, Not detectable.

* Expressed as a percentage of that obtained with FeCl₃ in the presence of organic C and N and under a H₂/CO₂ atmosphere.

† Added as glucose 100 mg l⁻¹, peptone 100 mg l⁻¹, yeast extract 10 mg l⁻¹.

‡ At 0.4 mmol l⁻¹.

The residual activity after addition of HgCl₂ at 30 °C was of the same order as that observed in control samples at 90 °C, indicating that both methods provided similar estimates of the chemical component.

The profundal sediment was a major sink for iron, with a concentration of about 50–60 mg g⁻¹ (approximately equivalent to 2.5–3.0 g l⁻¹ of wet sediment) in the top 1 cm, and therefore we chose to examine the population of Fe(III)-reducing bacteria in the sediment, where the larger quantities of the potential electron acceptor were found. Although these cultures were obtained from single colonies they were designated as mixed enrichments (see Methods) until they had been subjected to several passages through a variety of liquid and solid media. This was particularly important in the case of the H₂-utilizing anaerobes, which were difficult to separate from associated chemoorganotrophs. All the sources of Fe(III) provided were reduced by the bacteria (Table 1), the rates being much faster (30–100 µmol l⁻¹ d⁻¹) in the presence of organic C and N. The effect of the iron source was the same on both the chemoorganotrophic and chemolithotrophic enrichments, in that FeCl₃ provided the most readily reduced form of iron and goethite the least. The FeCl₃ will hydrolyse immediately on addition to the liquid medium, and will then flocculate over a longer time period, the rate being governed, to some degree, by the ionic strength of the medium. To provide the Fe(III) in a more readily available, i.e. soluble, form, it is necessary to incorporate a chelating agent. Addition of citrate to the medium stimulated reduction by 70–220% depending on the organism involved, but we chose to omit chelating agents in experiments where growth on defined carbon sources was required. The mixed enrichments were finally tested for their response to alternative electron acceptors in the presence of equimolar concentrations of Fe(III). The reduction of Fe(III) was suppressed by NO₃⁻ (35–95%), ClO₃⁻ (45%), MnO₂ (64–93%), Mn₂O₃ (75%) and O₂ (> 99%).

Iron reduction by chemoorganotrophic bacteria

Enrichment cultures in which glucose was the source of carbon and reducing power yielded a pure culture of a Gram-negative facultative anaerobe which actively reduced Fe(III). The bacterium was fermentative in Hugh & Leifson medium, a rod 0.8–1.0 µm wide and 1.5–2.0 µm long, and it possessed a single polar flagellum. It was catalase and oxidase positive, and sensitive to O/129. It was therefore tentatively assigned to the genus *Vibrio*. The optimum temperature for Fe(III) reduction was 30 °C and the rate of reduction was dependent on Fe(III) concentration. Below 100 µmol Fe(III) l⁻¹ the response to concentration was curvilinear, but above this inflexion activity increased in a linear manner from 25 to 140 µmol l⁻¹ d⁻¹ Fe(II) produced at 1000 µmol Fe(III) l⁻¹, which was the highest concentration tested. This was interpreted in terms of a form of saturation kinetics at the lower concentration, with the linear response being attributed to increased frequency of contact between bacteria and the larger number of Fe₂O₃ particles at the higher concentrations. The need for direct contact was tested by separating the

Fig. 4. Growth, as measured by ATP biomass (○) and direct counts (●), and Fe(III) reduction (□) by a freshwater *Vibrio* sp. The organisms were grown in serum vials in a shaking water bath at 20 °C, with glucose (5 mmol l⁻¹) as sole C and energy source. The gas phase was 95% N₂/5% CO₂.

bacteria and the source of Fe(III) with a dialysis membrane. The rate of Fe(II) production ($\mu\text{mol l}^{-1} \text{ h}^{-1}$, $\pm 95\%$ confidence limits) decreased from 5.9 ± 0.8 to 1.04 ± 0.29 , but this does not provide conclusive proof of a need for physical contact, since the membrane may also prevent passage of extracellular components responsible for reduction, as well as being permeable to small quantities of FeCl₃ before hydrolysis/flocculation occurred. In an experiment in which the culture reduced Fe(III) at a rate of $6.1 \pm 0.96 \mu\text{mol l}^{-1} \text{ h}^{-1}$, centrifuged and filtered spent medium reduced Fe(III) at $2.2 \pm 0.8 \mu\text{mol l}^{-1} \text{ h}^{-1}$. This showed that extracellular components could account for $>30\%$ of the reduction observed in a whole cell suspension.

Reduction of Fe(III) by the *Vibrio* sp. was closely correlated with growth, as measured by ATP biomass, and to a lesser degree with cell numbers (Fig. 4). There was good agreement between turbidity, protein content and dry weight of cells and a calibration curve was used to determine cell yield in future experiments. Growth was linear with glucose concentration up to 5 mmol l⁻¹, at which point an inflexion in the growth curve was observed. The molar growth yield was unaffected by the presence of Fe(III) as was the final dry weight of the culture. In three separate experiments the molar growth yields in the presence of Fe(III) were 15.8, 31.6 and 24.3 g dry wt mol⁻¹, and in its absence 14.6, 29.2 and 24.3 g dry wt mol⁻¹, respectively. This suggested that the *Vibrio* sp. used Fe(III) as a hydrogen sink, i.e. to allow reoxidation of NADH rather than for energy conservation via electron transport phosphorylation. The effect of selected inhibitors was then tested on both whole cells and cell-free extracts of the bacterium. The reduction of Fe(III) by cell-free extracts was linear with time up to 6 h, although a large chemical component (30–90% of activity) was observed in control treatments during the first hour of incubation. Measurements were therefore made between 1 and 1.5 h incubation. The specific activity of extracts prepared under rigorously controlled anaerobic conditions [$1.6 \mu\text{mol Fe(III)}$ reduced (mg protein)⁻¹ h⁻¹] was significantly higher than that of aerobic extracts ($0.07 \mu\text{mol mg}^{-1} \text{ h}^{-1}$) but the extraction yield was much less in the absence of O₂. This presumably reflected the inefficiency of ultrasonic disruption under anaerobic conditions (Hughes *et al.*, 1971). The range of activities obtained with aerobic extracts was 0.03 – $0.13 \mu\text{mol mg}^{-1} \text{ h}^{-1}$. Inhibitors of electron transport, nitrate reductase, ATPase and ionophores all reduced the yield of Fe(II) in cell-free extracts although rotenone and antimycin A did not affect the activity of whole cells (Table 2). Negative results with whole cells are not, however, easily interpreted, since they may merely reflect inability to reach the site of activity within the cell. Although some inhibition of ATP production was observed in cell-free extracts, the analyses were unsatisfactory. This we attributed, in part, to interference of the chemiluminescent reaction by such large quantities of iron. Therefore, although we were able to demonstrate the involvement of an electron transport system in Fe(III) reduction there was no evidence of energy conservation, in terms of growth yield, from the whole cell experiments.

Table 2. *Inhibition of Fe(III) reduction in whole cells and cell-free extracts of a freshwater Vibrio sp.*

Inhibitor	Percentage inhibition*	
	Whole cells	Cell-free extracts
Rotenone	0	61–52
HOQNO	89	50–100
Antimycin A	0	74–100
ClO ₃ ⁻	—	58–60
NaN ₃	36	56–66
DNP	70	16
CCCP	34	6–79

—, Not determined.

* The effect on whole cells was measured over 24 h and on cell-free extracts over 0.5 h. The control rates of Fe(III) reduction [$\mu\text{mol (mg protein)}^{-1} \text{ h}^{-1}$] were: whole cells, 0.873; cell-free extracts, 0.105.

Table 3. *Reduction of Fe(III) by a two-membered consortium and a pure culture of a H₂ utilizing anaerobe*

Culture conditions*	Consortium			Pure culture		
	Fe(II) produced ($\mu\text{mol l}^{-1}$)	ATP yield (nmol l^{-1})		Fe(II) produced ($\mu\text{mol l}^{-1}$)	ATP yield (nmol l^{-1})	
		+ Fe(III)	– Fe(III)		+ Fe(III)	– Fe(III)
H ₂ only	28	0.04	0.05	14	0.05	0.05
H ₂ + acetate	41	0.29	0.05	7	0.08	0.05
H ₂ + yeast extract	60	1.26	0.47	38	0.16	0.08
N ₂ + acetate	6	0.05	—	7	0.04	—
N ₂ + yeast extract	40	0.84	—	27	0.15	—

—, Not determined.

* The consortia were incubated for 14 d and pure cultures for 28 d at 30 °C.

Iron reduction by mixed and pure cultures of a chemolithotrophic bacterium

It was extremely difficult to obtain pure cultures of anaerobic, chemolithotrophic bacteria which reduced Fe(III). An enrichment culture in which H₂ was the source of reducing power, and CO₂ the source of C, reduced Fe(III) (the only potential electron transport acceptor in the medium). After several sub-cultures through solid and liquid media two organisms remained, which, together, reduced Fe(III) more rapidly than did the iron-reducing member of the consortium when it was eventually obtained in pure culture. This organism was a small Gram-negative cocco-bacillus, 0.9 to 1.2 μm long. It was a facultative anaerobe and a facultative autotroph. Growth was enhanced by the presence of yeast extract but has been so slow, or has not occurred on conventional media that further characterization has not been possible. A greater biomass yield (measured as ATP) of both mixed and pure cultures was obtained in the presence of Fe(III) than in its absence (Table 3) on media to which yeast extract or acetate had been added. In addition to the increased biomass yield in the presence of Fe(III), larger quantities of Fe(II) and ATP were produced if the gas phase was H₂/CO₂. This suggested that hydrogen could act as major, if not sole source of reducing power for the reaction.

DISCUSSION

The results obtained with sealed sediment cores showed that such systems could be used as crude models of hypolimnetic processes. The absence of significant release of Mn(II) supported the suggestion by Davison (1981) that the sediment is not a major source of supply of this metal to the hypolimnion. The process of Fe(III) reduction has been shown to be largely biological in nature, and the activity of bacterial isolates was affected by the presence of NO₃⁻, ClO₃⁻ and higher manganese oxides. The results with ClO₃⁻ as well as NO₃⁻ imply a direct involvement of

nitrate reductase in at least some of the organisms. In others, where ClO_3^- sensitivity was less, the inhibitory effect of NO_3^- could have been due to its capacity to divert electron flow (Lascelles & Burke, 1978). The suppression by higher manganese oxides is comparable with a separate reductase system such as those reported in soil organisms (Munch & Ottow, 1977). The higher rates of reduction obtained with FeCl_3 may have been due to the smaller particle size of the hydrolysed product and may more closely resemble rates of reduction of the natural particles in the lake (Tipping *et al.*, 1981). Physical contact also plays a part in the reduction process although the enclosure of the Fe(III) in a dialysis bag did not, on this occasion, reduce activity to the degree observed by Munch & Ottow (1982). The extracellular reduction of Fe(III) has been reported elsewhere as an alternative mechanism to the use of siderophores for the mobilization of iron (Anderson & Morel, 1980).

The experiments with cell-free extracts and whole cells of the fermentative bacterium, tentatively assigned to the genus *Vibrio*, did not provide conclusive proof of energy conservation associated with Fe(III) reduction. No increase in molar growth yield was observed in the presence of Fe(III) and it was assumed that the iron was used as a hydrogen sink, to allow the regeneration of NAD via electron transport not linked to ATP production. A facultatively anaerobic and chemolithotrophic bacterium was also isolated from the profundal sediment. A greater yield of the organism was obtained in the presence of Fe(III). This is believed to be the first demonstration of energy conservation associated with H_2 transport to Fe(III) which has been measured in terms of biomass rather than increased viable counts (Balashova & Zavarzin, 1979). The importance of such processes in the reduction of Fe(III) in the lake hypolimnion is not yet known. Although little difficulty has been experienced in the isolation of bacteria which use Fe(III) as a hydrogen sink, lithotrophs and organotrophs which may use the process to conserve energy deserve further attention.

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