

## Continuation Progress Report

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### Microbial Mineral Transformations at the Fe(II)/Fe(III) Redox Boundary for Solid Phase Capture of Strontium and Other Metal/Radionuclide Contaminants

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Current Amount of Unexpended Funds: \$57,828.00

Note: Project expenditures are on target for the first budget period. After three quarters of work, the amount of unexpended funds comprise 35% of the budget, within 10% of the amount expected to be on hand with one quarter remaining (25%). Thus, the amount of unexpended funds at the end of the first budget period is expected to be less than 10% of the planned fiscal year budget.

## **Project Objectives**

The objectives of the project remain the same as those stated in the original proposal. Specifically, to determine microbiological and geochemical controls on carbonate mineral precipitation reactions that are caused by bacterial reduction of Fe(III)-oxides, and identify contributions of these processes to solid phase capture of strontium and other metal/radionuclide contaminants.

## **Personnel**

In accordance with project plans, there are two postdoctoral research associates working the project. Dr. Lesley Warren is based at the University of Toronto (UT), and has prior experience working with bacterial-iron oxide-metal interactions through the DOE co-contaminant chemistry subprogram of the Subsurface Science Program. Dr. Michael Leonardo, who has been working in the laboratory of Dr. Kenneth Nealson at the the University of Wisconsin in Milwaukee, recently started work on the project at the University of Alabama (UA).

At UT, Ms. Tracey Fluery has started on the project as an M.Sc. graduate student and Ms. Jenny Cox (a 4th year undergraduate) is helping out with ongoing work over the summer. Two M.Sc. graduate students have participated thus far in the UA portion of the work, Ms. Karrie Weber (Department of Biological Sciences) and Mr. Robert Howell (Department of Geology).

## **Research Accomplishments to Date**

Studies at UT and UA have thus far been targeted on a series of related research questions, as stipulated in the original project outline:

- To what extent and how quickly does microbial reduction of Fe(III)-oxides (HFO) change the alkalinity and pH of carbonate buffered aqueous media?

- o Does strontium sorb to bacterial cells or HFO, and does this inhibit microbial HFO oxide reduction?

These research questions are being addressed initially with experiments on the solid phase partitioning of strontium in batch cultures of two related Fe(III)-reducing bacteria. The dissimilatory Fe(III)-reducing bacteria *Shewanella alga* strain BrY and *Geobacter metallireducens* are being used as test organisms. A brief outline of experimental methods follows, along with a description of results obtained to date.

### **Rate and extent of bacterial Fe(III) reduction and alkalinity changes that occur in carbonate buffered aqueous media (UA)**

Two initial experiments were conducted in bicarbonate buffered medium (5-10 mM **NaHCO<sub>3</sub>**) containing synthetic HFO as the electron acceptor and acetate (10 mM) as the electron donor. A third experiment was conducted in bicarbonate buffered medium containing natural amorphous HFO-rich sediment from a freshwater wetland in Alabama (Roden and Wetzel, 1996). In each of these experiments, the growth medium contained 4.4 mM **NH<sub>4</sub>Cl** and 0.44 mM **KH<sub>2</sub>PO<sub>4</sub>** as inorganic nutrients, and 5 mL of separate vitamin and trace mineral solutions (Lovley and Philips, 1988). The concentration of inorganic nutrients represents somewhat of a departure from that used in previous studies (Roden and Lovley, 1993; Roden and Zachara, 1996) which contained 18 mM **NH<sub>4</sub>Cl** and 4.4 mM **KH<sub>2</sub>PO<sub>4</sub>**. Because there is a strong potential for PO<sub>4</sub> to react with Fe(II) generated during HFO reduction (Emerson and Widmer, 1978; Roden and Edmonds, 1997), and since our current research is focused (at least in part) on the interaction of Fe(II) with carbonate rather than phosphate, we felt it would be advantageous to use a medium which provided sufficient, but not excess, PO<sub>4</sub> in our experimental systems.

The choice of 0.44 mM PO<sub>4</sub> (and as a consequence, 4.4 mM **NH<sub>4</sub>Cl**, assuming that a 10:1 N:P ratio would be appropriate for virtually any type of bacterium) was based on a test experiment which compared the growth of acetate-oxidizing *G. metallireducens* in medium which contained either the normal high level of **NH<sub>4</sub>Cl** and P<sub>04</sub>, an intermediate level of N and P (4.4 and 0.44 mM,

respectively), or a low level and N and P (0.44 and 0.044 mM, respectively). The results showed that rate and extent of HFO reduction was maximal in the “medium nutrient” medium (fig. 1). Based on these results, it was decided to use the “medium nutrient” medium for routine experiments. Note, that at least several mM Fe(II) is typically generated in batch HFO reduction experiments, such that with only 0.44 mM PO<sub>4</sub> present, only a small fraction of the Fe(II) could end up becoming associated with PO<sub>4</sub>.

The medium in the initial experiments also contained 10 mM **CaCl<sub>2</sub>** and 1 mM **SrCl<sub>2</sub>**. The calcium and strontium were added in order to make a preliminary examination of the potential for solid phase capture of a contaminant metal (strontium) in medium which contained both abundant Fe(II) and abundant calcium - i.e., an adequate supply of divalent cations capable of undergoing precipitation with carbonate ions, thereby providing for incorporation of strontium into siderite or calcite by solid solution (Ferris et al., 1995). Other experiments showed that neither the strontium or calcium at the above levels had an inhibitory effect on HFO reduction (see below).

The HFO reduction experiments were initiated by adding a 10% (v/v) inoculum of *G. metallireducens*. This organism was chosen for our initial studies as it is likely to be representative of one of the major groups of organic-carbon oxidizing, carbonate alkalinity generating Fe(III) reducing bacteria in anaerobic sediments (Lovley, 1991; Coates et al., 1996). Future studies will employ both *G. metallireducens* and *S. alga* strain BrY, the test organism used in the strontium sorption studies. For the initial growth experiments, however, it was not critical which of the two Fe(III) reducing bacteria was used as the major goal was to detail the pH/Alkalinity shifts which occur during iron reduction in carbonate buffered media.

The total amount of Fe(II) produced over time during the HFO reduction experiments was quantified by extracting a small volume of culture in dilute 0.5N HCl, and measuring the Fe(II) content of the extract using the calorimetric reagent ferrozine, as previously described (Roden and Zachara, 1996). The total dissolved inorganic carbon (DIC) content of the cultures was determined by acidifying 1 mL aliquots of filtered culture medium with 1 mL of 1 M HCl in a 10 mL serum bottle, followed by gas chromatographic determination of the CO<sub>2</sub>.

content of the headspace (Roden and Wetzel, 1996). The pH of the cultures was measured on small subsamples using a semi-micro pH electrode inside a Coy Products anaerobic chamber (Roden and Edmonds, 1997). Concentrations of dissolved iron, strontium, and calcium in the cultures was determined by inductively coupled plasma (ICP) spectroscopy. The MINTEQA2 (Allison et al., 1991) was used to calculate saturation index (log IAP) values for different solids at various time points during the incubations

Significant increases in pH were observed in all three experiments (Figs. 2-4). A major increase in DIC was observed only in HFO exp 2 and the wetland sediment experiment, in which the initial DIC concentrations were somewhat lower (i.e there was a lower DIC background), and in which greater amounts of acetate oxidation occurred relative to the 1 mM acetate oxidized in the first HFO experiment. The higher amounts of acetate oxidation in HFO exp 2 and the wetland sediment experiment occurred as a result of there being a higher concentration of reducible Fe(III) in the medium. It is important to note that typically only 1/3 of the synthetic HFO used to cultivate Fe(III) reducing bacteria is reduced during a batch culture reduction experiment (Roden and Zachara, 1996). In contrast, natural amorphous HFO is essentially completely reduced over time, as shown with *G. metallireducens* in fig. 3, or with *S. alga* in Roden and Edmonds (1997).

The measured increases in pH and DIC were sufficient to cause the aqueous phase of the medium to become supersaturated with respect to all of the potential metal-carbonate phases, i.e. **FeCO<sub>3</sub>**, SrCO<sub>3</sub>, and calcite/aragonite (**CaCO<sub>3</sub>**) (figs. 5-7; note that the dissolved calcium and strontium data are not yet available for the wetland sediment experiment). The absence of major declines in DIC suggest that very limited carbonate mineral formation occurred in these preliminary experiments, although strontium and calcium levels were clearly decreased by 40 to 50 % relative to their initial concentrations. A wet-chemical approach was subsequently developed in attempt to directly detect the presence of solid-phase carbonates in the cultures. Solid-phase carbonate content was estimated by comparing the amount of inorganic carbon liberated by acidification of whole medium (including the bacteria) versus the filtered aqueous phase alone. The results showed that solid phase carbonate precipitation had not yet occurred, in agreement with the DIC data. In this context, it is likely that the

extent of carbonate development in the HFO reduction experiments is sensitive to kinetic constraints imposed by saturation state of the medium (Stumm and Morgan, 1981; Stumm, 1992). Thus, until the onset of carbonate precipitation, sorption reactions must dominate the solid phase partitioning and aqueous concentrations of strontium and calcium. This is consistent with sorption data (see below) which indicates that bacteria (Ferris et al., 1988; 1989; 1995), as well as HFO (Dzombak and Morel, 1990), interact strongly with strontium by sorbing the metal ion from solution.

Analysis of one of numerous ongoing transfers of *G. metallireducens* growing in HFO culture medium with no added strontium or calcium showed evidence of solid-phase carbonate formation: the whole medium contained  $25.6 \pm 0.1$  mM DIC versus  $16.2 \pm 0.2$  mM for the aqueous phase alone, corresponding to approximately 9 mM of solid phase carbonate and 30 % of the total Fe(II) content of the cultures. A similar analyses performed on a set of triplicate cultures (once again in medium without strontium or calcium) from an unrelated experiment indicated formation of  $7.3 \pm 0.3$  mM of solid phase carbonate over a 30 day incubation, which again accounted for ca. 30 % of the total Fe(II) content of the cultures. This confirms that carbonate formation may be coupled to HFO reduction, as suggested by Coleman and others (1993).

Thus far one experiment has been conducted to examine how the relatively high concentrations of strontium and calcium (compared to typical uncontaminated groundwater aquifer environments) in our initial experiments may have affected the ability of *G. metallireducens* to reduce synthetic and natural amorphous HFO. Duplicate tubes of acetate HFO medium were amended with either 1 mM strontium or 10 mM calcium prior to inoculation; duplicate control tubes to which no additions were made were also inoculated. The presence of 1 mM strontium (probably about half of which was in solution, based on the results of the pH/alkalinity change experiments, figs. 2 and 3) had no discernable negative impact on the rate or extent of Fe(III) reduction (fig. 8). The presence of 10 mM calcium, however, did seem to slightly inhibit Fe(III) reduction during the first week of incubation by ca. 25 %, and the total amount of Fe(III) reduced by ca. 15 %. These results indicate that there was no gross toxicological effect of these metals on HFO reduction by *G. metallireducens*. These experiments will soon be repeated with *S. alga*, and in this case the effect of preexposure of the

cells to strontium will also be assessed to examine the extent to which the presence of abundant HFO may alter the sensitivity of the bacteria to strontium.

### **Strontium sorption by Fe(III)-reducing bacteria and Fe(III)-oxides under non-growth conditions (UT)**

A series of strontium sorption studies have been conducted with *S. alga* at low ionic strength under non-growth conditions. Sorption studies have also been done with the synthetic HFO used in culture media (see growth studies above). *G. metallireducens* has not been examined, but is Gram-negative like *S. alga* (i.e., their cell walls are comprised of a thin layer of peptidoglycan that underlies an exterior outer membrane) and should have very similar strontium sorption characteristics (Ferris and Beveridge, 1985; McLean et al., 1996). A great advantage in using *S. alga* is that this bacterium, unlike *G. metallireducens*, is a facultative anaerobe and can be grown to very high culture densities under aerobic conditions. In this way, large amounts of bacteria biomass can be rapidly generated for experimental work. Typically, *S. alga* cultures were grown overnight in tryptic soy broth (TSB) at **30°C**. Then cells were recovered and washed twice by centrifugation in ultrapure water (UPW) for the strontium sorption studies.

To avoid metal contamination in the sorption studies, we use only polypropylene plasticware that is leached overnight in 7N **HNO<sub>3</sub>** and rinsed with UPW. Bacterial cells are resuspended and adjusted to a constant optical density **OD<sub>600 nm</sub> = 0.2** (standard curves relating the optical density of the bacterial suspensions to number of cells and their dry weight have been constructed) in UPW containing 87 to 8700 ppb (**10<sup>-6</sup> to 10<sup>-4</sup> M**) strontium, added as **SrCl<sub>2</sub>**. A concentration of **10<sup>-4</sup> M** total Fe was used for HFO in the sorption experiments. Two separate sets of sorption experiments have been run at pH values of 6.0 and 7.0 to span the initial pH range of the HFO growth medium used in the growth studies. Test strontium concentrations bracket the 500 to 1200 ppb amounts measured in waste management areas at Oak Ridge National Laboratory (Saunders and Toran, 1995). The suspensions are typically allowed to equilibrate for two hours, although sorption time course studies conducted for periods of up to 48 hours show that equilibrium is reached in a matter of minutes. Separation of solids (bacterial cells and HFO) from the suspensions is done by filtration

through 0.22 mm membrane filters. The equilibrium dissolved strontium concentrations of the  $\text{HNO}_3$  acidified filtrates is measured by-flame atomic adsorption spectroscopy (AAS) using an acetylene-nitrous oxide flame. The extent of solid phase adsorption is, in turn, calculated from the difference between initial and equilibrium dissolved strontium concentrations.

Standard curves relating the optical density of *S. alga* suspensions to their dry weight is shown in fig. 8. These curves allow us to normalize strontium sorption data, measured using a constant cell density dry weight of bacteria in suspension. At an  $\text{OD}_{600 \text{ nm}} = 0.2$ , suspensions of *S. alga* contain approximately 0.22 mg dry weight bacteria/ml, and  $10^8$  cells/mL as enumerated using DAPI staining and epifluorescence microscopy. A growth curve for *S. alga* in trypticase soy broth is also shown (fig. 9). This nutrient medium accommodates rapid bacterial growth, and yields more than sufficient biomass to conduct strontium sorption studies. It would take considerably longer to generate equivalent amounts of biomass with the obligate anaerobe, *G. metallireducens*.

According to surface complexation theory, sorption to particulate solids like HFO or bacterial cells can be approached in the same way as the formation of soluble complexes in solution (Dzombak and Morel, 1990; Stumm, 1992). Thus, a general expression for strontium sorption to HFO and bacteria can be formulated as follows:



where S-OH represents reactive amphoteric surface hydroxo groups, and S-OSr<sup>+</sup> is the solid phase complexed form of strontium. The corresponding mass law and apparent surface complexation constant  $K_s$  is:

$$K_s = \frac{\{\text{S-OSr}^+\}\{\text{H}^+\}}{\{\text{S-OH}\}\{\text{Sr}^{2+}\}}$$

At the low ionic strength of our experiments, the activity  $\{\}$  and concentration  $[\ ]$  of the various components can be taken to be equal; e.g.,  $\{\text{Sr}^{2+}\} = [\text{Sr}^{2+}]$ . Mass balance considerations dictate further that the total available solid phase sorption sites ( $\Gamma_{\text{max}}$ ) is equivalent to the sum of the occupied sites  $[\text{S-OSr}^+](\Gamma)$  and unoccupied sites  $[\text{S-OH}]$ . Incorporation of the mass balance into the mass law expression yields a Langmuir sorption equation:

$$\Gamma = \Gamma_{\max} K_s ([\text{Sr}^{2+}]/[\text{H}^+]) / (1 + K_s ([\text{Sr}^{2+}]/[\text{H}^+]))$$

When plotted in a double reciprocal form, the Langmuir equation permits estimation of the surface complexation constant  $K_s$  and maximum binding capacity  $\Gamma_{\max}$  of the solid phase sorbent. The incorporation of the  $[\text{Sr}^{2+}]/[\text{H}^+]$  ratio into the equation is particularly useful as this permits direct comparison of sorption data compiled at different pH values. This is because the proton condition has a great influence on sorption affinity according to:

$$K_{\text{pH}} = K_s / [\text{H}^+]$$

where  $K_{\text{pH}}$  is a conditional sorption constant that varies according to pH (Stumm, 1992).

A double reciprocal plot for strontium sorption to HFO is shown in fig. 10. With a total HFO concentration of  $10^{-4}$  M, very little strontium sorption was actually measured over the concentration and pH range of used in the experiments completed to date. This corresponds to a strontium  $K_s$  value of 0.19 (dimensionless) and sorption capacity around  $0.13 \text{ mmole.g}^{-1}$  (Table 1). These experiments are being repeated at higher HFO concentrations. Regression analysis, however, of the available sorption data reveals a fairly robust relationship which suggests that 94 % of the variance in the measured strontium uptake by HFO is explained by the equilibrium  $[\text{Sr}^{2+}]/[\text{H}^+]$  ratio, as anticipated by the Langmuir equation.

**Table 1: Sorption characteristics for hydrous ferric oxide and *S. alga* determined from double reciprocal Langmuir plots**

	$r^2$	$K_s$	$\Gamma_{\max}$ (M)	$\Gamma_{\max}$ (mmol.g <sup>-1</sup> )
HFO	0.94	0.19	$10^{-6.2}$	0.13
<i>S. alga</i> (low)	0.99	0.13	$10^{-4.3}$	0.23
<i>S. alga</i> (high)	0.98	0.40	$10^{-5.3}$	0.02

The sorption of strontium by *S. alga* follows a much more complex pattern than that observed with the HFO. In double logarithm isotherm plots, sorption data from pH 6.0 and 7.0 are not fully conformable as might be expected from the mass action relationships outlined above (fig. 11). Instead, two separate curves emerge from the data implying that there are two separate sorption sites on the surface of *S. alga* with quite different sorption characteristics (Stumm, 1992). The lower curve obtained at pH 6.0 must reflect the present of high affinity sorption sites that become saturated at low  $[\text{Sr}^{2+}]/[\text{H}^+]$  values; however, apparent saturation (normally evidenced by a leveling of sorption curves in double logarithm plots) is never reached because at higher  $[\text{Sr}^{2+}]/[\text{H}^+]$  values strontium continues to be sorbed by weaker affinity sites. At pH 7.0, the high affinity sites are filled almost immediately, then sorption continues with the low affinity sites as increasing amounts of strontium are complexed by the cells.

Construction of double reciprocal plots for the two curves shown in fig. 11 more clearly reveals differences between the two types of sorption sites on *S. alga* (figs. 12 and 13). The high affinity sites have a  $K_s$  value of 0.41, close to 3 times greater than the  $K_s$  value of 0.13 determined for the low affinity sites (and about 2 times greater than the HFO  $K_s$ ) (Table 1). The high affinity sorption capacity amounts to 0.02  $\text{mmole.g}^{-1}$  dry wt of cells, while the low affinity sorption capacity is ten times higher at 0.23  $\text{mmole.g}^{-1}$  dry wt of cells. We suspect that the high affinity sites correspond to hydroxo groups associated with surface carboxylates ( $\text{pK}_a$ 's around 3 to 4) on the bacterial cells. Conversely, the low affinity sites are probably hydroxo groups associated with phosphorylated polymers (the  $\text{pK}_a2$  of  $\text{P}_i$  is around 7.2). On a dry weight basis, the combined strontium sorption capacity of *S. alga* is comparable to that of HFO (Table 1).

Based on our sorption data, and the concentration of HFO used in the growth experiments, one would expect that 20 to 50 % of the total strontium in the starting culture medium would be complexed. This agrees well with the analytical aqueous chemistry data for strontium in the growth experiments (shown in figs. 2 and 3). On the other hand, the sorption studies conducted with BrY were done with a cell density of about  $10^8$  cells / mL, approximately the starting cell density in culture medium after inoculation. As such, the bacterial cells would account for less than 5 % of the solid phase partitioning of strontium in the culture medium.

## Summary of Major Research Accomplishments to Date

Our project on microbial mineral transformations at the Fe(II)/Fe(III) redox boundary for the solid phase capture of strontium is progressing well. Thus far, we have been able to demonstrate that:

- pH and DIC concentrations increase during microbial reduction of HFO in batch culture experiments with *G. metallireducens* lasting 30 days
- high concentrations of strontium (1.0 mM) and calcium (10 mM) do not inhibit microbial HFO reduction
- the extent of change in pH and DIC concentrations brings about supersaturation with respect to carbonate minerals including siderite (**FeCO<sub>3</sub>**), strontianite (**SrCO<sub>3</sub>**), and calcite/aragonite (CaCO<sub>3</sub>); in addition, precipitation of siderite has been documented in cultures of HFO reducing bacteria
- significant amounts of strontium and calcium (40 to 50 % of the total initial concentration) sorb to particulate solids (i.e., HFO and bacteria cells)-in batch culture experiments
- sorption of strontium to HFO conforms with Langmuir single site sorption models derived from corresponding mass action and mass balance relationships anticipated from thermodynamic equilibrium considerations
- the sorption behavior of strontium with *S. alga* is more complex and seems to involve two sets of reactive surface sites on the bacterial cells; a high affinity site of low total sorption capacity, and a low affinity site with high sorption capacity
- the total strontium sorption capacities of *S. alga* and HFO are comparable
- the observed solid phase partitioning of strontium in the culture experiments is in excellent agreement with sorption characteristics measured with HFO and *S. alga*

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## Figure Legends

Figure 1. Synthetic HFO reduction by *G. metallireducens* in “high nutrient”, “medium nutrient”, and “low nutrient” growth medium (see text for further details). Data represent the mean  $\pm$  SD of 3 replicate cultures.

Figure 2. Time course results of the first synthetic HFO reduction experiment with *G. metallireducens* . Results of duplicate cultures are shown.

Figure 3. Time course results of the second synthetic HFO reduction experiment with *G. metallireducens* . Results of duplicate cultures are shown.

Figure 4. Time course results of the natural wetland sediment amorphous Fe(III) oxide reduction experiment with *G. metallireducens* . Results of duplicate cultures are shown.

Figure 5. Carbonate mineral saturation index (reported as log IAP) calculations (MINTEQA2) for the first synthetic HFO reduction experiment. The data represent calculations performed on averages from duplicate cultures.

Figure 6. Carbonate mineral saturation index (reported as log IAP) calculations (MINTEQA2) for the second synthetic HFO reduction experiment. The data represent calculations performed on averages from duplicate cultures.

Figure 7. Carbonate mineral saturation index (reported as log IAP) calculations (MINTEQA2) for the second synthetic HFO reduction experiment. The data represent calculations performed on averages from duplicate cultures.

Figure 8. Standard curve relating the optical density (at 600 nm) of *S. alga* suspensions to the dry weight of cells

Figure 9. A growth curve for *S. alga* at 30°C in trypticase soy broth showing very strong exponential growth.

Figure 10. A double reciprocal Langmuir plot for strontium sorption on HFO.

Regression analysis of the sorption data (Table 1) indicated that 94 % of the variance in the measured strontium uptake by HFO is explained by the equilibrium  $[Sr^{2+}]/[H^+]$  ratio, as anticipated by equilibrium thermodynamic considerations.

Figure 11. Double logarithmic isotherm plots for strontium sorption on *S. alga* at pH 6.0 and pH 7.0. Note, the curves for the two pH values are not fully conformable as anticipated from equilibrium thermodynamic considerations for single site sorption. The implication is that two separate sorption sites exist on the surface of *S. alga*.

Figure 12. A double reciprocal Langmuir plot for apparent high affinity strontium sorption on *S. alga* at pH 6.0. Corresponding  $K_s$  and  $\Gamma_{max}$  values are given in Table 1.

Figure 13. A double reciprocal Langmuir plot for apparent low affinity strontium sorption on *S. alga* at pH 7.0. Corresponding  $K_s$  and  $\Gamma_{max}$  values are given in Table 1.

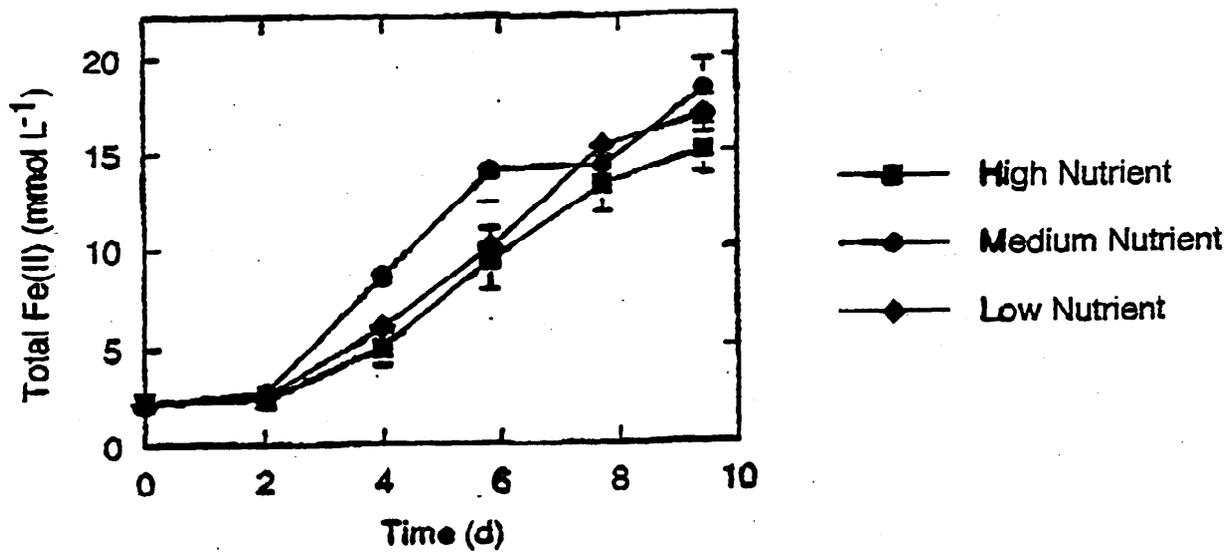


Fig. 1

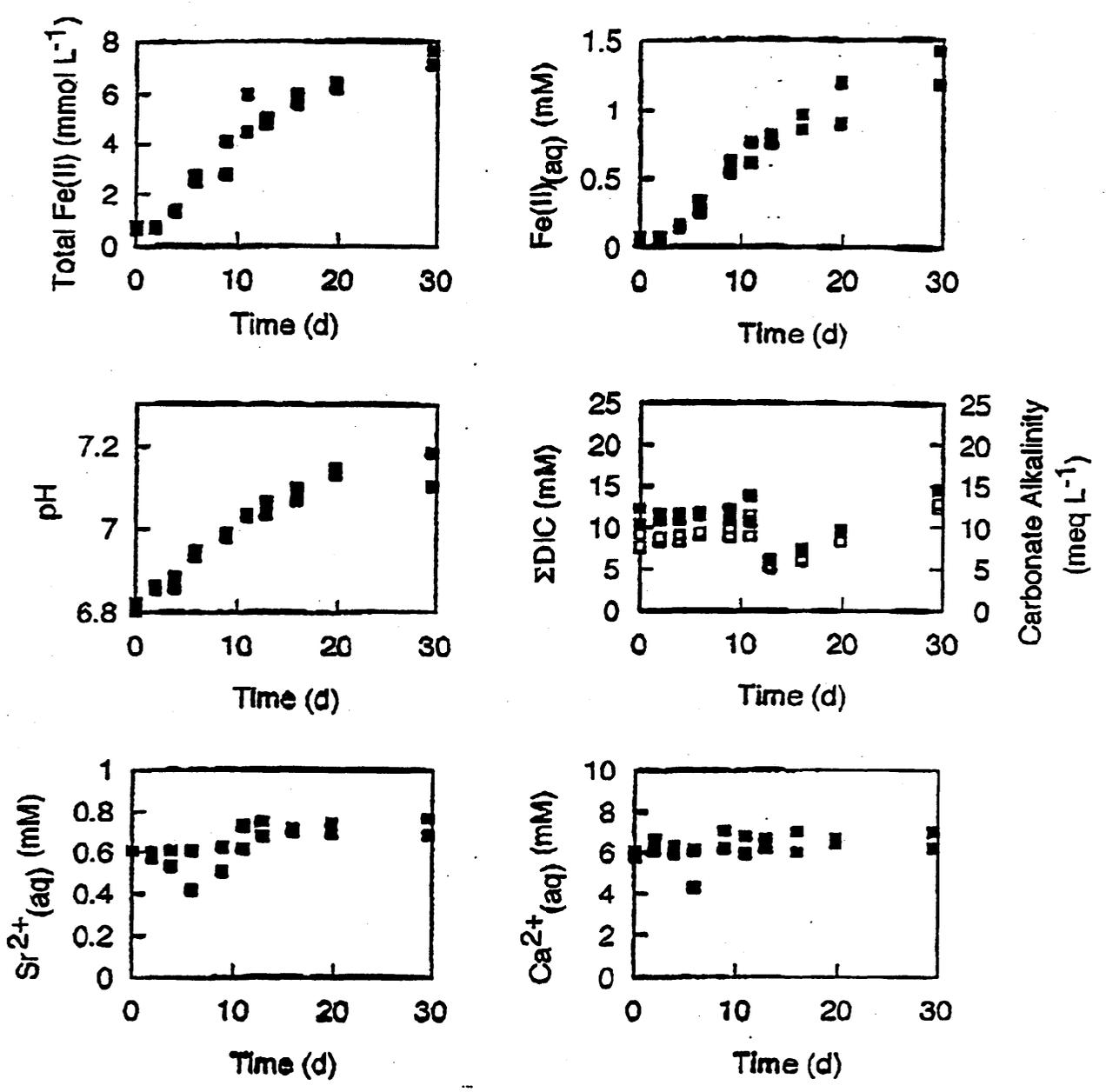


Fig. 2

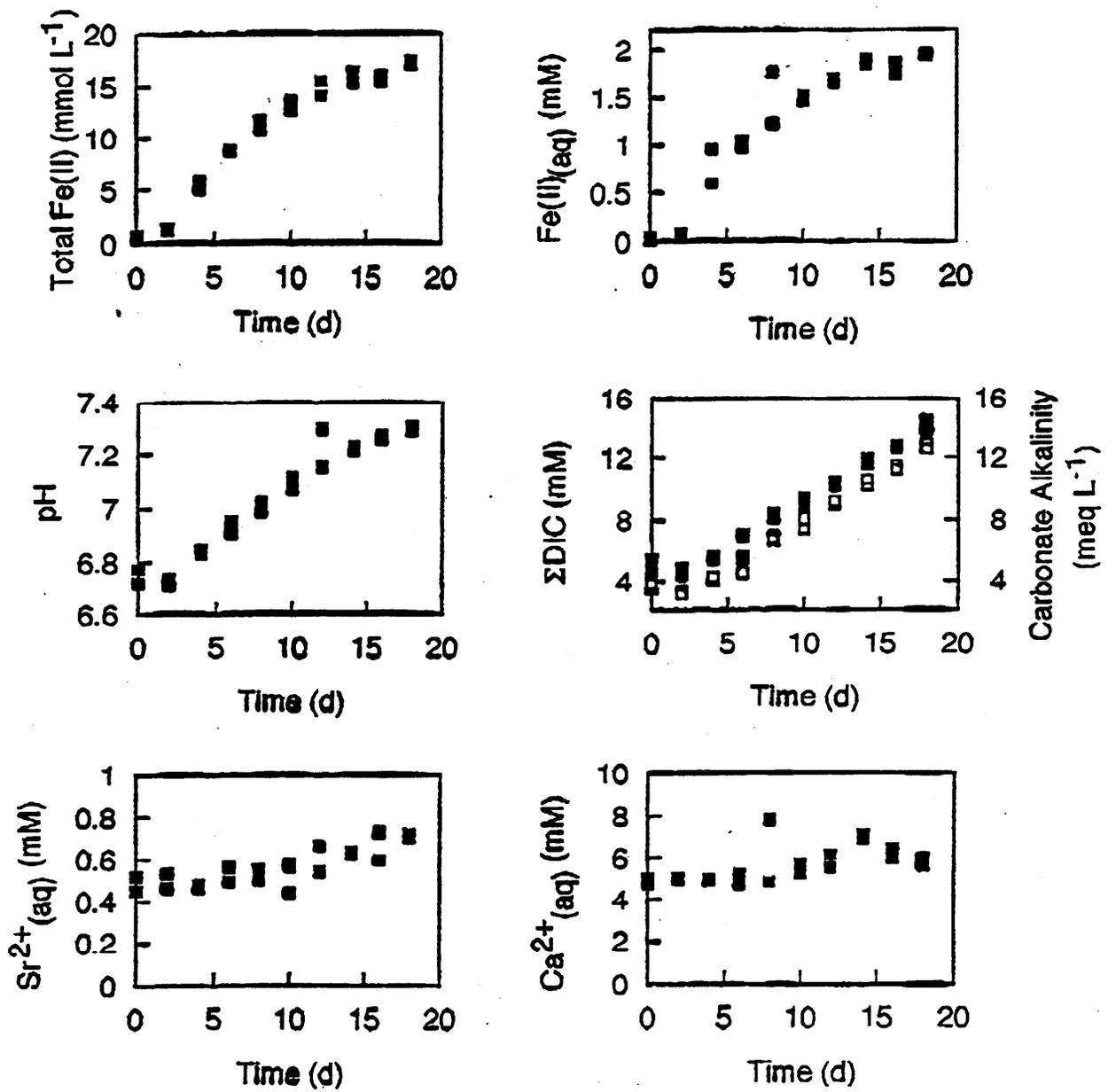


Fig. 3

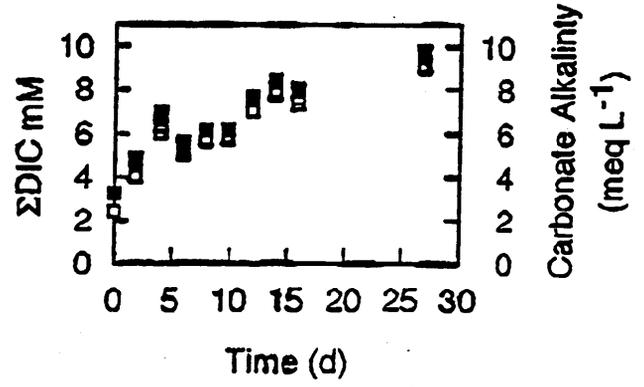
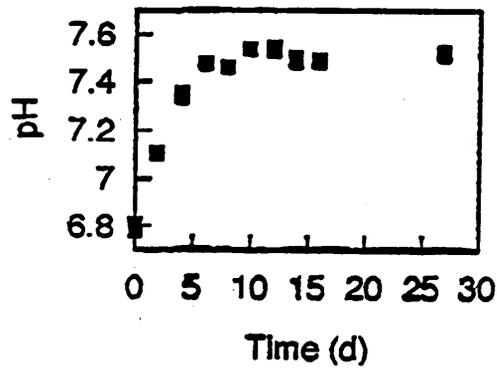
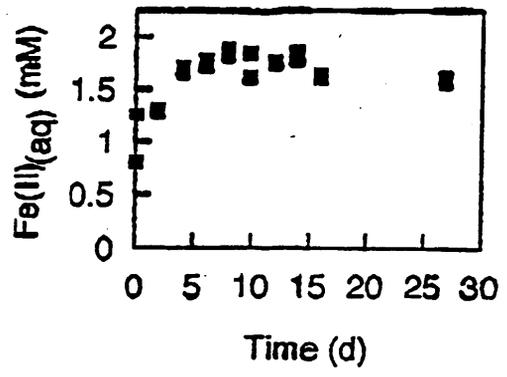
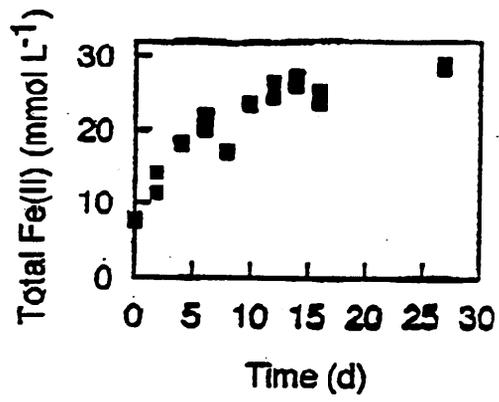


Fig. 4

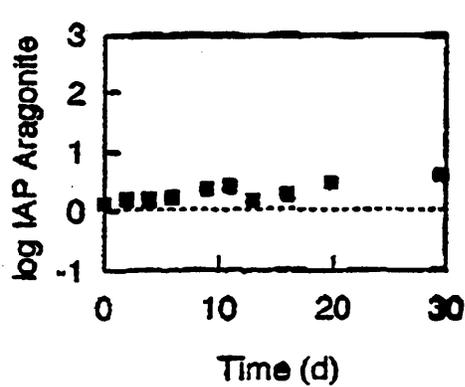
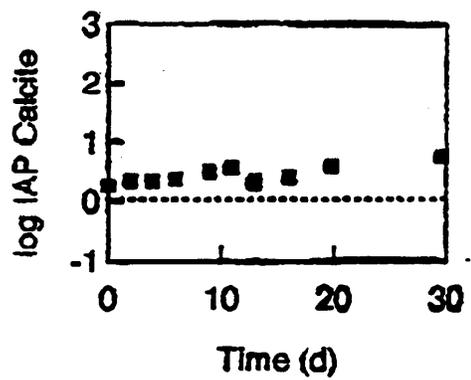
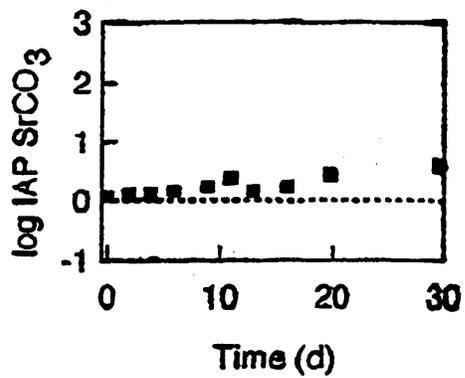
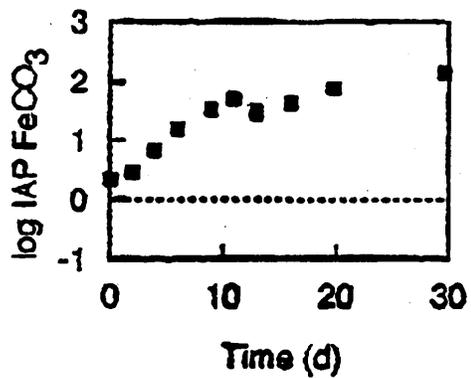


Fig. 5

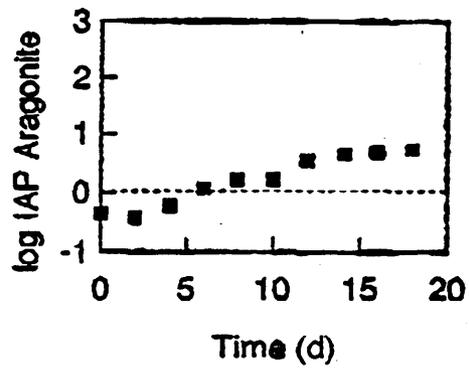
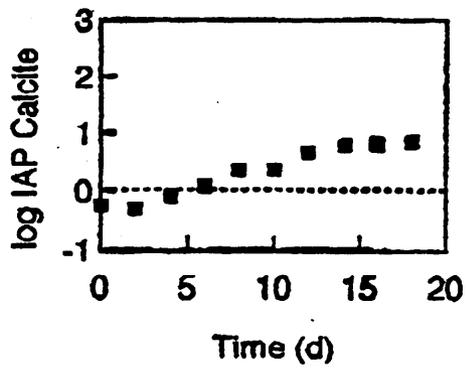
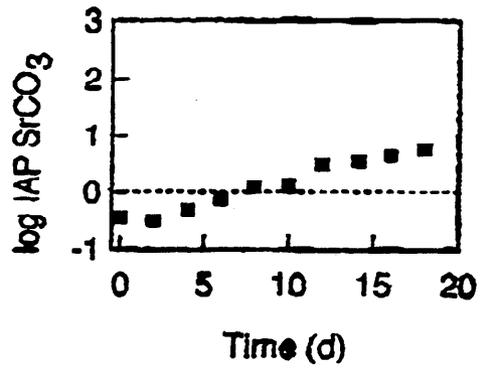
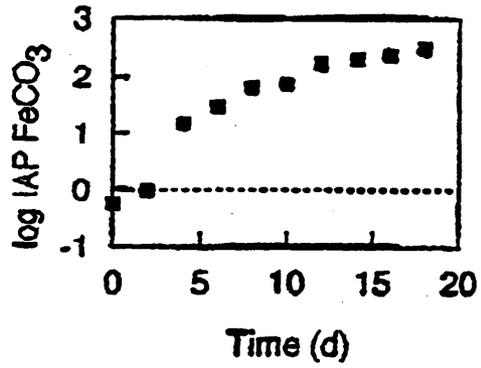


Fig. 6

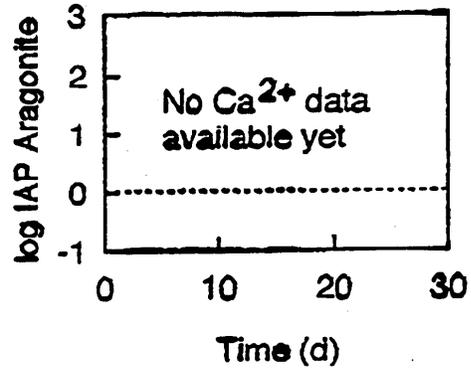
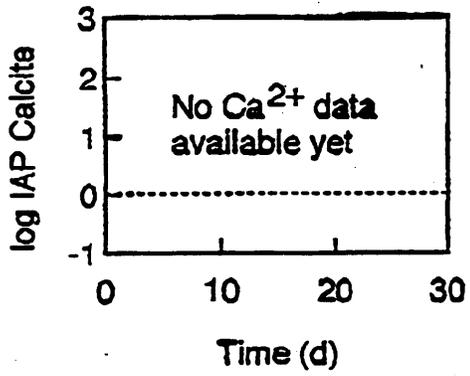
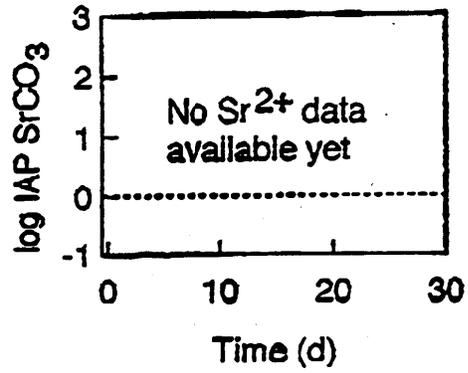
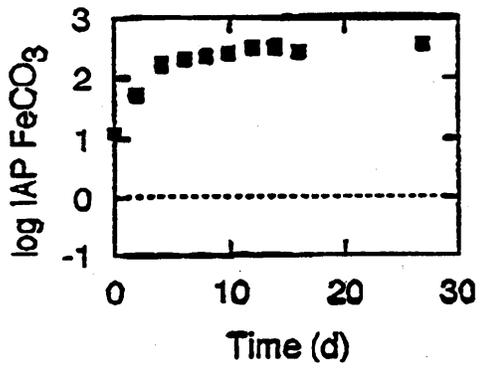


Fig. 7

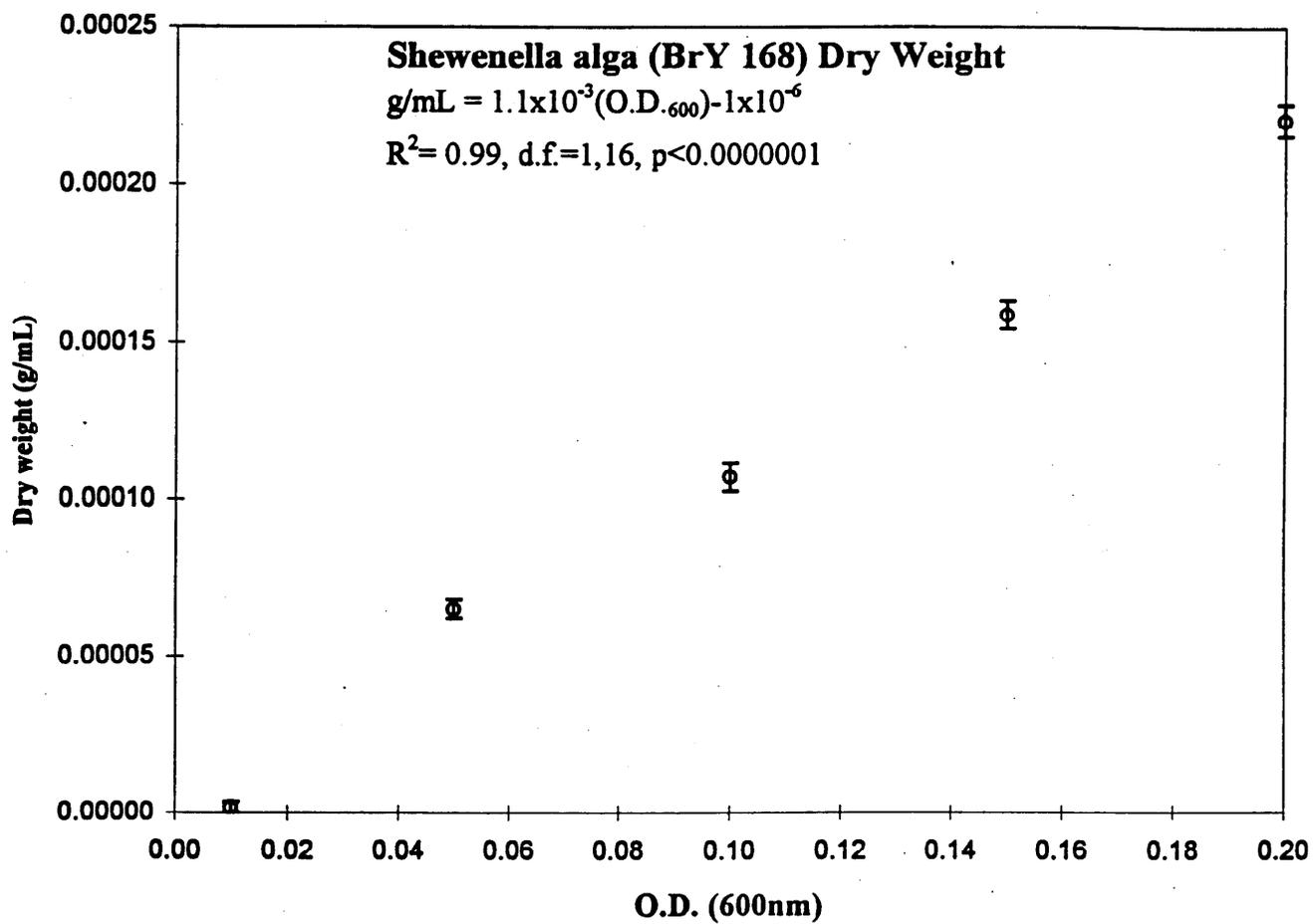


Fig. 8

### Shewenella alga (BrY 168) Growth Curve

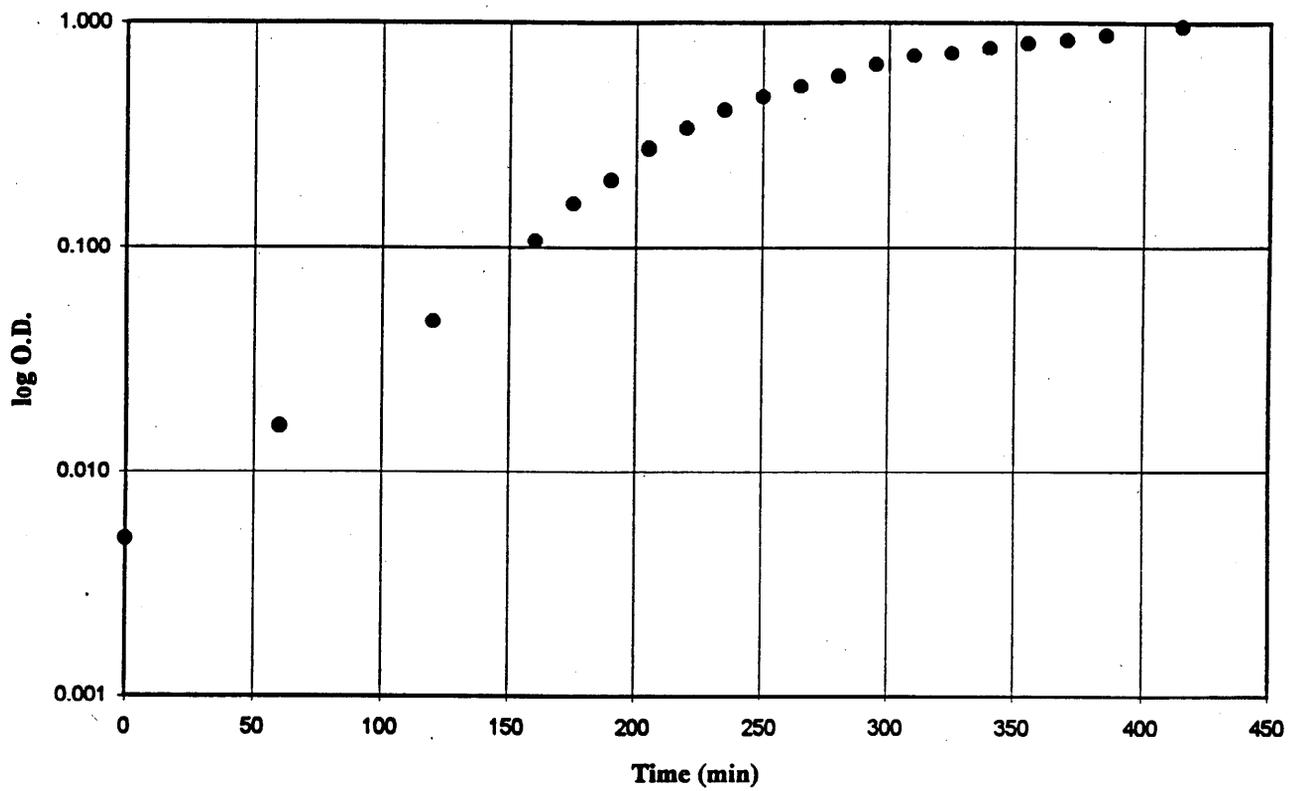


Fig. 9

### Strontium Sorption to HFO

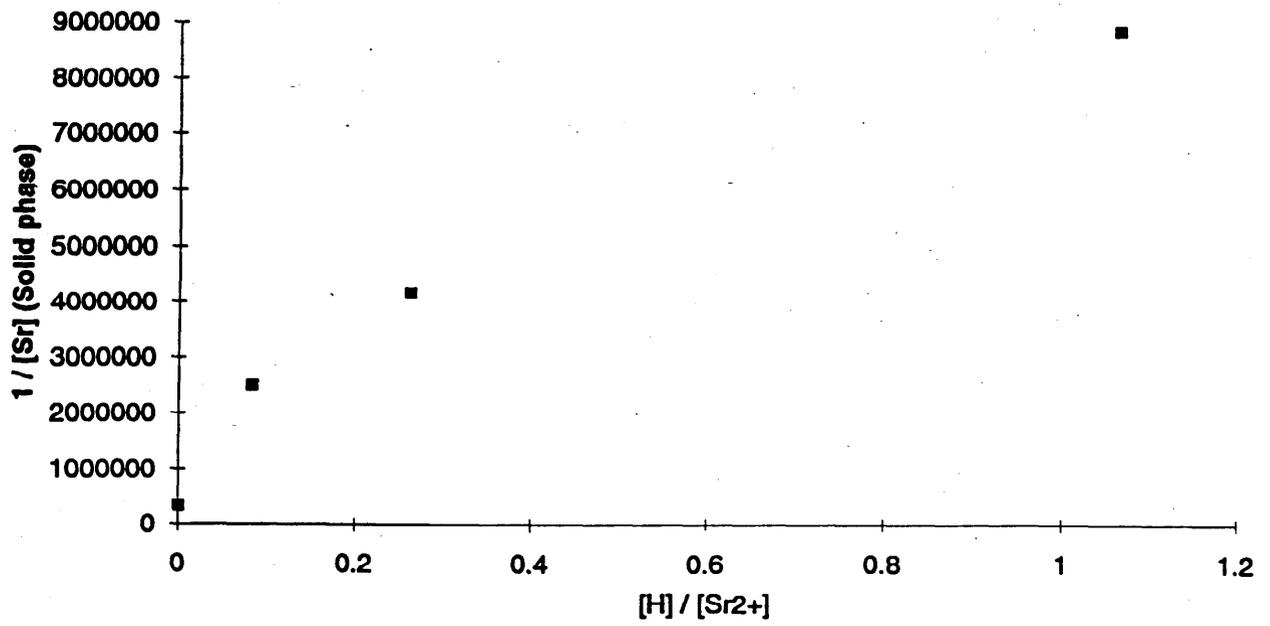


Fig. 10

### Strontium Sorption to *S. alga* strain BrY

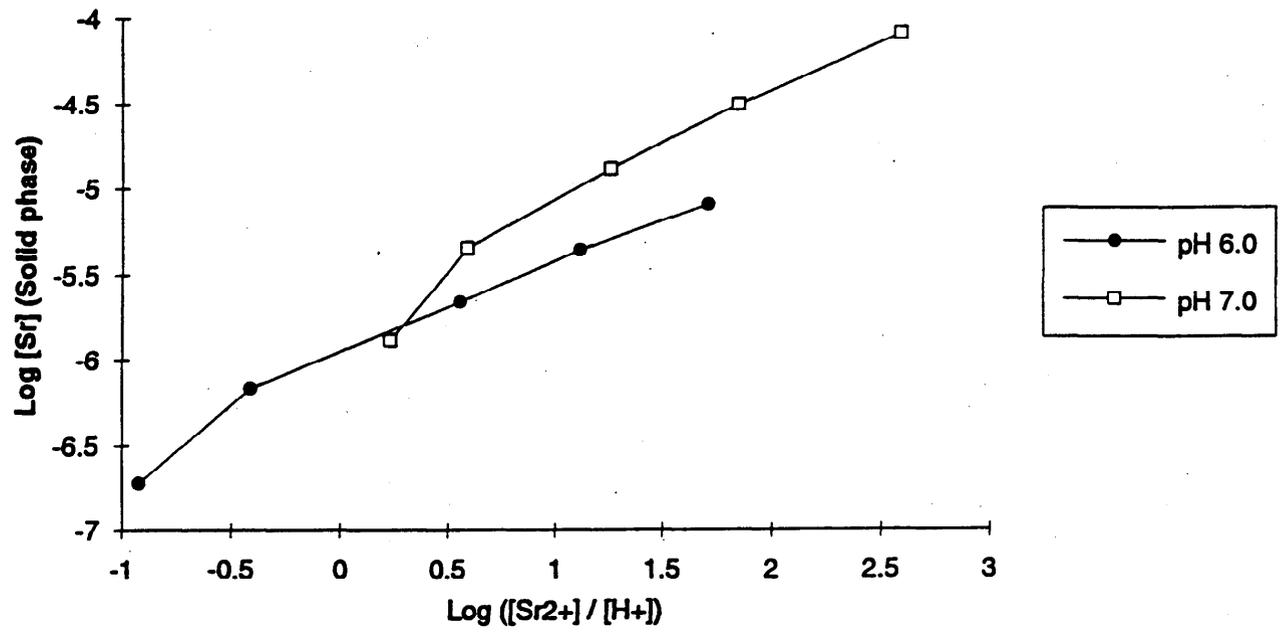
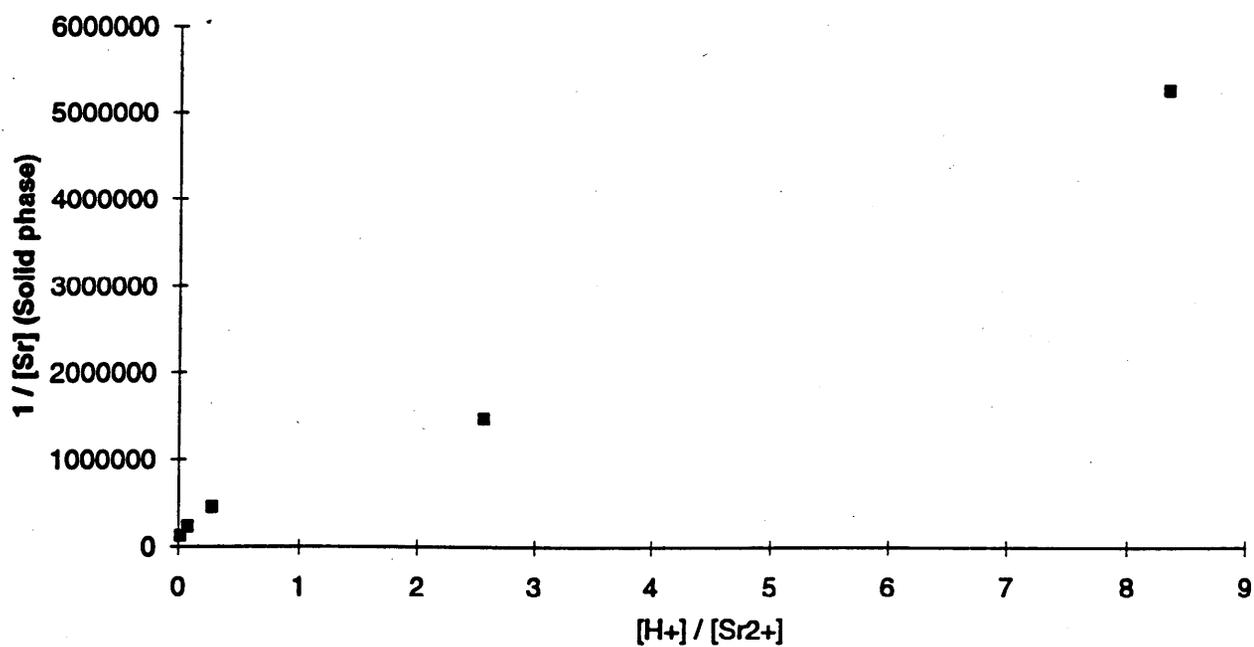


Fig. 11

Strontium Sorption of *S. alga* strain BrY at pH 6



Strontium Sorption of *S. alga* strain BrY at pH 7

