

# Growth of *Geobacter sulfurreducens* under nutrient-limiting conditions in continuous culture

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## Summary

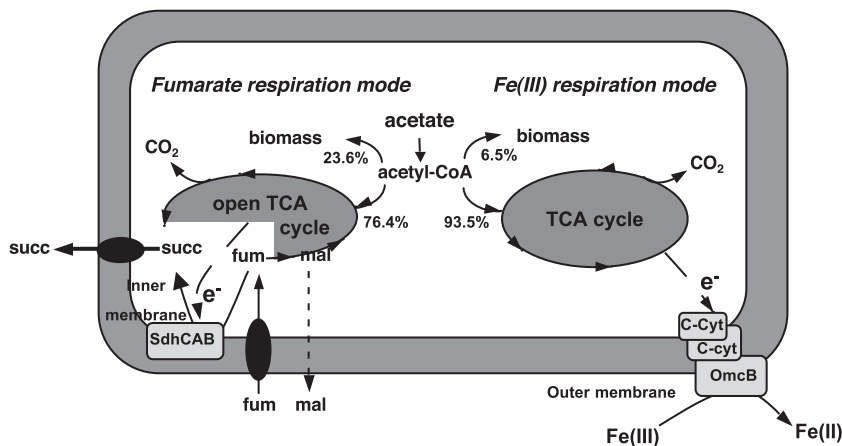
**A system for growing *Geobacter sulfurreducens* under anaerobic conditions in chemostats was developed in order to study the physiology of this organism under conditions that might more closely approximate those found in the subsurface than batch cultures. *Geobacter sulfurreducens* could be cultured under acetate-limiting conditions with fumarate or Fe(III)-citrate as the electron acceptor at growth rates between 0.04 and 0.09 h<sup>-1</sup>. The molar growth yield was threefold higher with fumarate as the electron acceptor than with Fe(III), despite the lower mid-point potential of the fumarate/succinate redox couple. When growth was limited by availability of fumarate, high steady-state concentrations were detected, suggesting that fumarate is unlikely to be an important electron acceptor in sedimentary environments. The half-saturation constant, K<sub>s</sub>, for acetate in Fe(III)-grown cultures (10 μM) suggested that the growth of *Geobacter* species is likely to be acetate limited in most subsurface sediments, but that when millimolar quantities of acetate are added to the subsurface in order to promote the growth of *Geobacter* for bioremediation applications, this should be enough to overcome any acetate limitations. When the availability of electron acceptors, rather than acetate, limited growth, *G. sulfurreducens* was less efficient in incorporating acetate into biomass but had higher respiration rates, a desirable physiological characteristic when adding acetate to stimulate the activity of *Geobacter* species during *in situ* uranium bioremediation. These results demonstrate that the ability to study the growth of *G. sulfurreducens* under steady-state conditions can provide insights into its physiological characteristics that have relevance for its activity in a diversity of sedimentary environments.**

## Introduction

An understanding of the physiology of *Geobacter* species is important because microorganisms closely related to *Geobacter* species available in pure culture are the predominant microorganisms in a diversity of sedimentary environments in which Fe(III) reduction is important either in the natural degradation of organic matter (Stein *et al.*, 2001) or in the bioremediation of organic (Sneyenbos-West *et al.*, 2000; Roling *et al.*, 2003) or metal contaminants (Sneyenbos-West *et al.*, 2000; Holmes *et al.*, 2002; Anderson *et al.*, 2003; Ortiz-Bernad *et al.*, 2004). *Geobacter sulfurreducens* (Caccavo *et al.*, 1994) serves as a model for the study of *Geobacter* physiology (Lovley, 2003) because it is closely related to *Geobacter* species that are predominant in subsurface environments; its complete genome has been sequenced (Methe *et al.*, 2003); and a genetic system is available (Coppi *et al.*, 2001).

The study of the physiology of *Geobacter* species is in its infancy. The hallmark physiological characteristic of *Geobacter* species is their ability to couple the oxidation of acetate to the reduction of Fe(III) (Lovley *et al.*, 1987; Lovley and Phillips, 1988; Lovley, 2000). The ability to oxidize acetate is important because acetate is the central intermediate in the anaerobic degradation of organic matter in sedimentary environments (Lovley and Chapelle, 1995). *Geobacter* species metabolize acetate via the tricarboxylic acid cycle (TCA) cycle (Champine and Goodwin, 1991; Galushko and Schink, 2000). Fe(III) reduction appears to take place in the outer membrane and electron transfer to Fe(III) requires several periplasmic and outer membrane *c*-type cytochromes (Fig. 1) (Leang *et al.*, 2003; Lloyd *et al.*, 2003; J.E. Butler *et al.*, submitted). In addition to Fe(III), other metals, most notably U(VI) (Lovley *et al.*, 1991), Tc(VII) (Lloyd *et al.*, 2000), Co(III) (Caccavo *et al.*, 1994) and V(V) (Ortiz-Bernad *et al.*, 2004), can be reduced. Reduction of soluble U(VI) to insoluble U(IV) removes uranium from contaminated groundwater and stimulating the growth and activity of *Geobacter* species in the subsurface by adding acetate to the groundwater is a promising bioremediation strategy for uranium-contaminated aquifers (Finneran *et al.*, 2002; Anderson *et al.*, 2003). Vanadium may be removed in a similar manner (Ortiz-Bernad *et al.*, 2004). However, because of the relative abundance of

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**Fig. 1.** Potential mechanism for acetate metabolism in *Geobacter sulfurreducens* with either fumarate or Fe(III) serving as the electron acceptor. TCA, tricarboxylic acid cycle; fum, fumarate; succ, succinate; mal, malate; SdhCAB, succinate dehydrogenase; C-cyt, c-type cytochrome; OmcB, outer membrane c-type cytochrome. The percentage of acetate either converted into biomass or mineralized is shown for both modes of respiration when acetate is the limiting substrate.

Fe(III) versus the contaminant metals, most of the growth of *Geobacter* during bioremediation can be attributed to Fe(III) reduction (Finneran *et al.*, 2002). Some *Geobacter* species can oxidize aromatic compounds and oxidation of aromatic contaminants coupled to Fe(III) reduction is an important process for removing aromatic compounds from polluted aquifers (Lovley *et al.*, 1989, 1994; Anderson and Lovley, 1999).

The chemostat provides one of the best opportunities to study microbial physiology under environmentally relevant conditions (Kovarova-Kovar and Egli, 1998). For example, growing cells under electron donor limitation mimics the slow steady release of electron donors from complex organic matter in many sedimentary environments. Furthermore, only by studying cells that are physiologically at steady state is it possible to reproducibly compare the physiological response of cells to different environmental conditions.

The goal of the study reported here was to develop a chemostat system for culturing *G. sulfurreducens* under steady-state conditions. Here we describe steady-state growth of *G. sulfurreducens* under electron donor and electron acceptor-limiting conditions with acetate as the electron donor and either fumarate or Fe(III)-citrate as the electron acceptor. The results provide insights into the growth of *Geobacter* species in subsurface environ-

ments and provide data for a developing *in silico* model of *G. sulfurreducens* metabolism (R. Mahadevan *et al.*, submitted).

## Results

### Acetate-limited growth

When *G. sulfurreducens* was grown under continuous culture with acetate as limiting substrate (5.5 mM) and fumarate (30 mM) as the electron acceptor, biomass concentration remained constant and the biomass productivity was linear at dilution rates (*D*) of 0.04–0.09 h<sup>-1</sup> (Fig. 2), consistent with classical Monod-type growth. Steady-state acetate concentrations were not detectable (detection limit 10 µM) at the lowest dilution rates and steadily increased at higher dilution rates. Up to 5 mM malate accumulated in the medium. When fumarate consumption other than conversion to malate was considered, the stoichiometry of fumarate utilization and succinate accumulation in the extracellular medium were equivalent (Table 1), indicating that fumarate was not an important source of cell carbon. When acetate assimilation into cell mass was considered, electrons recovered via fumarate reduction compared well with the number of electrons provided by acetate oxidation (Table 1).

**Table 1.** Acetate consumption, fumarate reduction and growth of *Geobacter sulfurreducens* at *D* = 0.05 h<sup>-1</sup>.

TEA	Acetate consumed (mM)	Fumarate consumed (mM)	Malate secreted (mM)	Acetate dissimilated (mM) <sup>a</sup>	Succinate (mM)	Fe(II) (mM)	Cell mass (mg dw/l)	Electron recovery (%) <sup>b</sup>
Fumarate	5.5	21.0	5.0	4.2	16.0	–	60.6	95
Fe(III)-citrate	5.5	–	–	5.1	–	43.0	18.6	105

**a.** The difference between acetate consumed and acetate used for cell synthesis was calculated from the assimilation equation given in *Experimental procedures*.

**b.** Electrons found in reduced electron acceptor compared with amount of electrons derived from acetate dissimilation. TEA, terminal electron acceptor; dw, dry weight.

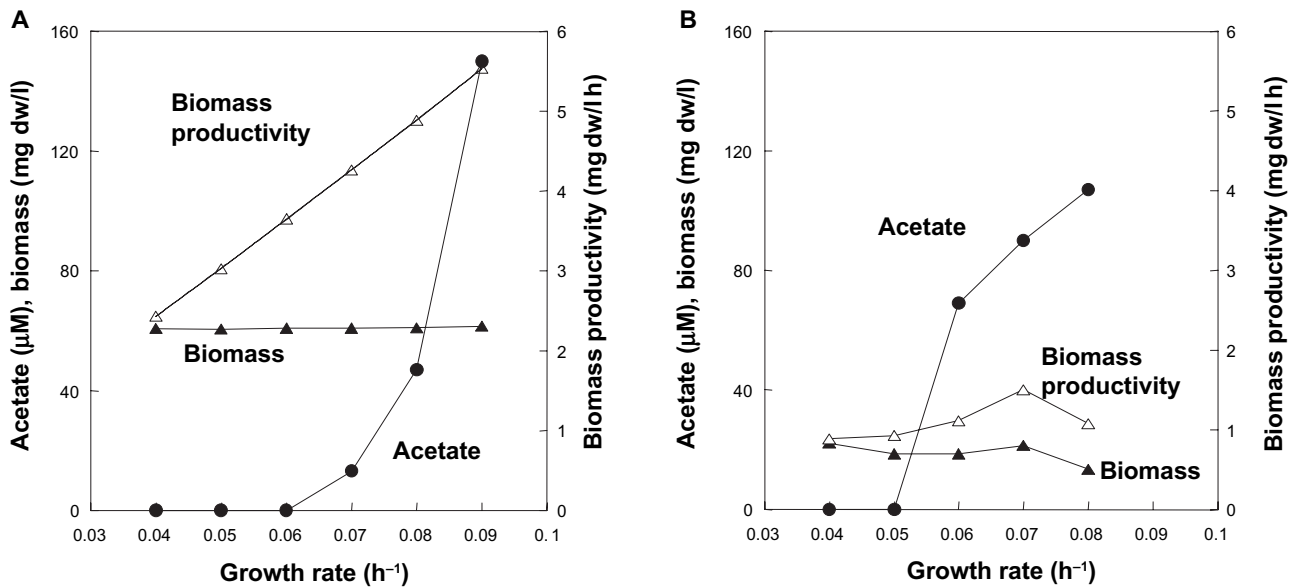


Fig. 2. Influence of the growth rate on steady-state values of residual acetate concentration (closed circle), biomass (closed triangles), and volumetric biomass productivity (open triangles) in acetate-limited chemostat under fumarate-respiring conditions (A) and Fe(III)-respiring conditions (B). dw, dry weight.

The steady-state biomass with Fe(III) (60 mM) as the electron acceptor, was threefold lower than with fumarate (Fig. 2). The steady-state, biomass and biomass productivity declined at the highest dilution rate evaluated (0.08 h<sup>-1</sup>), presumably because this dilution rate was approaching the maximal dilution rate (see below).

As in fumarate-grown cultures, acetate was not detectable at the lowest dilution rates and steadily increased at

higher dilution rates. For each mol of acetate consumed 7.9 mol of Fe(III) were reduced (Table 1), which is consistent with the expected stoichiometry of acetate oxidation with Fe(III) serving as the sole electron acceptor.

The maximum specific growth rate ( $\mu_{max}$ ) calculated from acetate-grown batch cultures was  $0.15 \pm 0.01$  h<sup>-1</sup> with fumarate as the electron acceptor and  $0.1 \pm 0.01$  h<sup>-1</sup> with Fe(III). Using this value and the Lineweaver-Burk linearization method, the half-saturation constant,  $K_s$ , for acetate was calculated as  $0.030 \pm 0.002$  mM with fumarate as the electron acceptor and  $0.010 \pm 0.001$  mM with Fe(III).

The specific respiration rates ( $q_e$ ) and the acetate consumption rates ( $q_{acetate}$ ) increased linearly with growth rate with both electron acceptors (Fig. 3). These rates were always higher for Fe(III)-grown cells than for fumarate-grown cells (Table 2). The maximal yield values for electron transfer ( $Y_e^{max}$ ) were ninefold higher in fumarate medium than in Fe(III)-citrate medium, further emphasizing that fumarate reduction is a more effective respiratory mechanism. Furthermore, the calculated metabolism required for maintenance was higher when Fe(III) was the electron acceptor (Table 2).

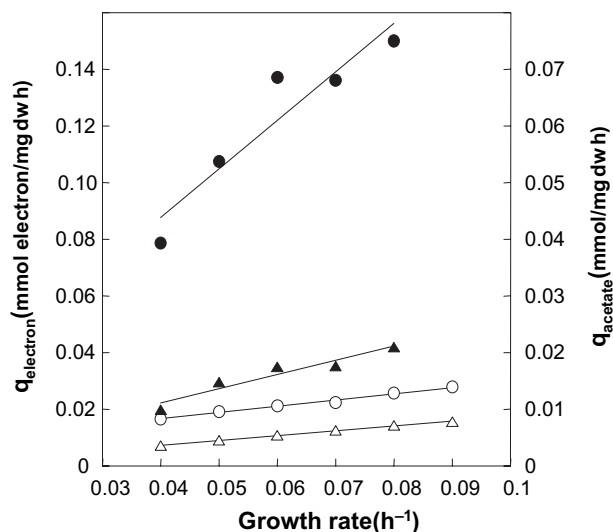


Fig. 3. Influence of the growth rate on steady-state values of specific acetate consumption (triangles) and specific respiration rates (circles). Cells were cultured under acetate-limited conditions with either fumarate as electron acceptor (open symbols) or Fe(III) (closed symbols). The values are the mean of three replicates. dw, dry weight.

#### Electron acceptor-limited growth

Chemostats growth with acetate (10 mM) in excess and fumarate (10 mM) as the growth-limiting electron acceptor did not follow typical Monod-type growth kinetics over the range of dilution rates examined (Fig. 4). The steady-state biomass was lower at lower dilution rates. This was associated with apparent stress to the cells as evidenced by

**Table 2.** Calculated metabolic rates for maintenance purposes and maximal growth yield under acetate-limited conditions.

TEA in culture	Metabolic rates for maintenance, $m^a$		Theoretical maximal yield, $^a Y^{\max}$	
	Respiration rate (mmol electron/g dw h)	Acetate consumption rate (mmol acetate/g dw h)	$Y_e^{\max}$ (mg dw/mol electron)	$Y_{\text{acetate}}^{\max}$ (mg dw/mol acetate)
Fumarate	7.9	0.3	4.5	11.8
Fe-citrate	19.0	1.2	0.6	3.8

a. Metabolic rates for maintenance purposes ( $m$ ) and maximal yield ( $Y^{\max}$ ) values were calculated from the plot showed in Fig. 3.  $m$ -values correspond with the intercepts, and  $Y^{\max}$  with the inverse of the slopes. TEA, terminal electron acceptor; dw, dry weight.

significant aggregation of cells at the lower dilution rates. The ratio of fumarate consumed to succinate produced was *c.* 1, with 20-fold less malate (*c.* 0.15 mM) excreted than when acetate was limiting and fumarate was in excess.

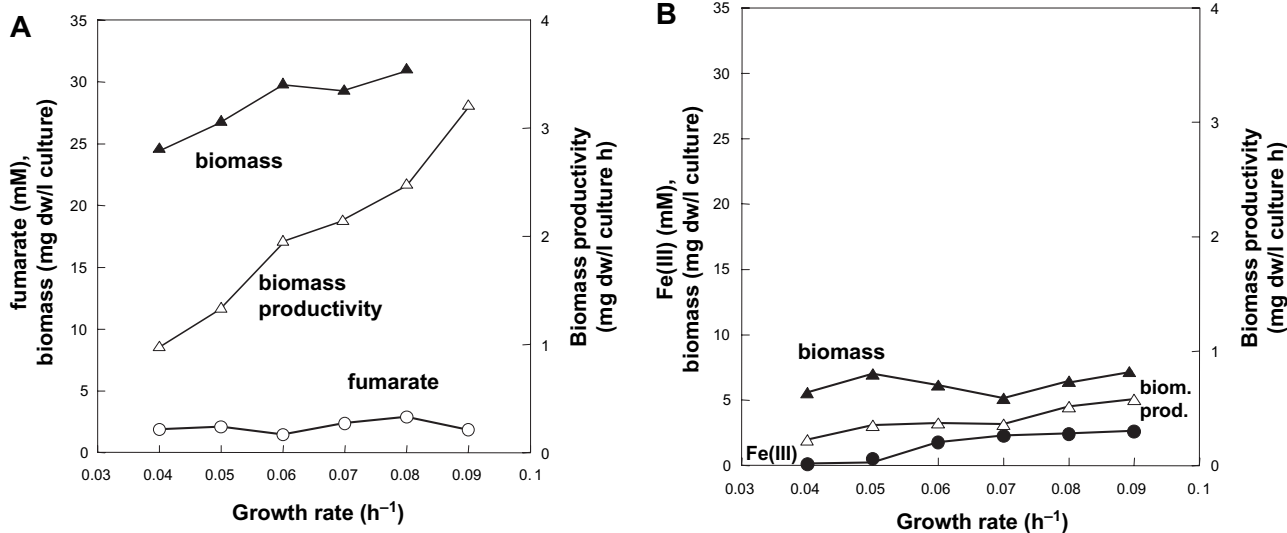
There was no cell aggregation with Fe(III) as the limiting electron acceptor and biomass remained constant as expected with Monod-type growth kinetics. The half-saturation constant  $K_s$ , based on Lineweaver-Burk linerasitation method, for Fe(III) was 1.0 mM. Although the amounts of fumarate or Fe(III) provided were equivalent in terms of their electron-accepting capacity, steady-state concentrations of fumarate were much higher than those for Fe(III) at low growth rates (Fig. 4).

As was observed under acetate-limiting conditions, the growth yield on fumarate was substantially higher (four-fold) than during growth on Fe(III) (Table 3). The efficiency of acetate metabolism was lower under electron acceptor-limited conditions than when acetate availability limited growth (Table 3). In addition, the specific respiration rates ( $q_e$ ) were 10% and 15% higher for fumarate and Fe(III)-grown cells, respectively, and the metabolic rates for ace-

tate consumption ( $q_{\text{acetate}}$ ) increased over 40% for both electron acceptors. Correspondingly, the molar growth yield for acetate ( $Y_{\text{acetate}}$ ) were reduced as less carbon was incorporated into cells (Table 3).

## Discussion

The results demonstrate that it is possible to grow *G. sulfurreducens* at steady state, under anaerobic conditions in chemostats. This is significant because although microorganisms closely related to available pure cultures of *Geobacter* are the predominant microorganisms in a variety of subsurface environments in which Fe(III) reduction is important (Sneyenbos *et al.*, 2000; Holmes *et al.*, 2002; Anderson *et al.*, 2003), little is known about the physiology of these organisms. Growth in chemostats is likely to be a better representation of life in subsurface environments than typical batch growth and cells grown under steady-state conditions provide more reproducible and interpretable studies on gene expression and cell content than cells grown in batch. As detailed below, the



**Fig. 4.** Influence of the growth rate on steady-state values of residual electron acceptor concentration (circles), biomass (closed triangles) and volumetric biomass productivity (open triangles). Cells were cultured under electron acceptor-limited conditions with either fumarate (A) or Fe(III) (B), and acetate as sole electron donor. The values are the mean of three replicates. dw, dry weight.

**Table 3.** Influence of electron acceptor availability on the growth of *Geobacter sulfurreducens* under continuous culture at  $D = 0.05 \text{ h}^{-1}$ .

Growth parameters <sup>a</sup>	TEA in culture			
	Fumarate		Fe(III)-citrate	
	Acetate-limited TEA-excess	Acetate-excess TEA-limited	Acetate-limited TEA-excess	Acetate-excess TEA-limited
$q_{\text{acetate}}$ (mmol/g dw h)	4.50	6.75	14.8	28.12
$q_{\text{electron}}$ (mmol electron/g dw h)	19.21	21.30	107.61	126.90
$Y_{\text{acetate}}$ (mg dw/mmol)	11.01	7.40	3.30	1.18
$Y_{\text{electron}}$ (mg dw/mol electron)	2.60	2.30	0.46	0.39
CCE (%)	23.6	16.4	6.5	3.6

a. The results are the mean of three replicates.

TEA, terminal electron acceptor; dw, dry weight;  $q_{\text{acetate}}$ , acetate consumption rate;  $q_{\text{respiration}}$ , respiration rate based on electron transferred to the TEA;  $Y_{\text{acetate}}$ , growth yield based on acetate consumption;  $Y_{\text{respiration}}$ , growth yield based on electron transferred to the TEA; CCE, carbon conversion efficiency (per cent of carbon from acetate that is converted into biomass); dw, dry weight.

physiological data obtained in these first studies provide some important insights, perhaps most surprisingly that it is not possible to predict relative growth yields of *G. sulfurreducens* based solely on the potential energy theoretically available from the reduction of different electron acceptors.

#### *Disparity between growth yield and energy theoretically available from electron acceptor reduction*

A common observation is that the relative growth yield of microorganisms is directly related to the amount of energy theoretically available from reduction of different electron acceptors (John and Gunsalus, 1987; Gunsalus, 1992; Uden, 1998). For example, growth yields on oxygen are typically higher than those on nitrate (Koike and Hattori, 1975; Yamamoto and Ishimoto, 1977) and microorganisms that use sulfate as an electron acceptor have higher growth yields than methane-producing microorganisms (Lovley and Klug, 1986). Therefore, given the fact that the Fe(III)-citrate/Fe(II)-citrate mid-point potential (+372 mV) (Thamdrup, 2000) is more positive than the fumarate/succinate mid-point potential (+35 mV), it was expected that growth yields on Fe(III) might be higher than on fumarate. However, growth yields with fumarate as the electron acceptor were substantially higher than those with Fe(III) under both electron donor- and electron acceptor-limiting conditions.

The higher growth yields on fumarate can not be attributed to fumarate providing an additional source of cell carbon. Fumarate not reduced to succinate could be accounted for as malate accumulating in the medium and the stoichiometry of acetate oxidation and fumarate reduction was consistent with acetate serving as the sole electron donor for fumarate reduction. However, in the presence of both Fe(III) and fumarate, *G. sulfurreducens* preferentially reduces Fe(III) and switches off the fumarate respiration (Esteve-Núñez *et al.*, 2004). Under these spe-

cific conditions, small amounts of fumarate can be used as electron donor or carbon source in a pathway in which fumarate is converted to acetyl-CoA via malate and pyruvate (Esteve-Núñez *et al.*, 2004). One factor potentially leading to higher yields when fumarate serves as the electron acceptor is that *G. sulfurreducens* can use an open TCA cycle which alleviates the need for succinate oxidation to fumarate, an energy-requiring reaction (Galushko and Schink, 2000; J.E. Butler *et al.*, submitted) (Fig. 1).

Furthermore, reduction of an extracellular electron acceptor, such as Fe(III), poses unique problems for proton balance within the cell (R. Mahadevan *et al.*, submitted). Oxidation of acetate at physiological pH results in the production of eight protons, which remain in the cytosol while eight electrons are transferred to the inner membrane, and eventually to the outer matrix to reduce extracellular Fe(III). Thus, Fe(III) reduction results in net proton production inside the cell, which has to be compensated for by proton translocation that is energetically expensive. In contrast, the reduction of fumarate inside the cell consumes both protons and electrons, and thus all protons produced by acetate oxidation are balanced by consumption at fumarate reductase (R. Mahadevan *et al.*, submitted). This phenomenon, of cytosolic proton production having a negative effect of adenosine triphosphate (ATP) production, is unique to electron acceptors that are reduced outside the cell, such as Fe(III) and  $\text{S}^{\circ}$ .

#### *Affinity for acetate and electron acceptors*

Despite the lower growth yield on Fe(III), Fe(III)-grown cells had a higher affinity for acetate, as evidenced by a lower  $K_s$ , than fumarate-grown cells. The mechanisms responsible for this are not known, but the *G. sulfurreducens* genome suggests that *G. sulfurreducens* has multiple options for acetate uptake and the initial steps in acetate metabolism (Methe *et al.*, 2003; R. Mahadevan

*et al.*, submitted). Global analysis of gene expression with currently available whole genome microarrays for *G. sulfurreducens* may provide further insight into this phenomenon.

Acetate concentrations in sedimentary environments in which Fe(III) reduction is the predominant terminal electron-accepting process can be expected to be below 1  $\mu\text{M}$  (Chapelle and Lovley, 1990, 1992). This is substantially below the  $K_s$  for acetate during growth on Fe(III) for *G. sulfurreducens* and is consistent with the concept that anaerobic respiration rates in many sedimentary environments are typically kinetically limited by electron donor availability (Lovley and Phillips, 1987a; Lovley and Goodwin, 1988; Lovley and Chapelle, 1995).

However, electron donor availability may not be the factor limiting growth in some bioremediation strategies. For example, in order to promote reductive precipitation of uranium in uranium-contaminated aquifers, acetate is added to stimulate the growth of *Geobacter* species and dissimilatory metal reduction (Anderson *et al.*, 2003). A target groundwater acetate concentration of 1–2 mM was empirically chosen in those studies. The  $K_s$  for acetate for *G. sulfurreducens* reported here suggests that acetate at this level should ensure that acetate availability is not limiting the growth rate of *Geobacter* species during uranium bioremediation.

The  $K_s$  value for Fe(III)-citrate in *G. sulfurreducens* of 1.0 mM is 29-fold lower than the  $K_s$  (29 mM) reported for *Shewanella putrefaciens* (Liu *et al.*, 2001), but still substantially higher than bulk concentrations of dissolved Fe(III) typically found in sedimentary environments which range from 4  $\mu\text{M}$  to 50  $\mu\text{M}$  (Ratering and Schnell, 2001; Nevin and Lovley, 2002). This suggests that *Geobacter* species may need to primarily rely on insoluble Fe(III) oxide as their primary electron acceptor in most sedimentary environments.

It was not possible to reliably estimate the  $K_s$  for fumarate in *G. sulfurreducens* because the apparent stress conditions during growth at lower dilution rates precluded analysing the data with Monod kinetics. However, it was apparent that *G. sulfurreducens* does not have a high affinity for fumarate as an electron acceptor, as steady-state fumarate concentrations were over 1 mM at all growth rates evaluated when fumarate was the limiting substrate. This is a much higher concentration than is expected to be found in most sedimentary environments as fumarate is typically undetectable with commonly employed techniques and thus likely to be available at less than 10  $\mu\text{M}$ . Therefore, growth with fumarate as an electron acceptor may largely be a laboratory phenomenon with little environmental relevance. We have recently reported that fumarate respiration in *G. sulfurreducens* is catalysed by the TCA cycle enzyme, succinate dehydrogenase, which is able to

catalyse the reverse reaction when no other electron acceptor but fumarate is present in the culture medium (Esteve-Núñez *et al.*, 2004; J.E. Butler *et al.*, submitted). It is likely that the succinate dehydrogenase requires a high cytoplasmic concentration of fumarate to force the reaction in the non-physiological direction.

#### *Implications of growth under electron acceptor-limited conditions for bioremediation*

In bioremediation strategies in which acetate is added to the subsurface, the availability of electron donor may not be the factor limiting dissimilatory metal reduction. Therefore, it is important to understand potential differences in growth and physiology under electron acceptor-limited as well as electron donor-limited conditions. The results demonstrate that under electron acceptor limitation *G. sulfurreducens* is less efficient in converting acetate to cell carbon and has a higher respiration rate. This increase in the electron transfer activity is correlated with the upregulation of respiratory genes (Chin *et al.*, 2004). This is the first time that this phenomena has been shown in bacteria grown under Fe(III)-respiring conditions, but the physiological response of *G. sulfurreducens* is in accordance with previous observations in aerobic bacteria and yeast in which oxygen availability was the factor limiting growth (James and Keevil, 1999; Nobre *et al.*, 2002). This type of response was previously interpreted (Russell and Cook, 1995) as a mechanism to charge the cell with ATP, making it possible to rapidly accelerate growth when electron donor again becomes limiting. The ability of *G. sulfurreducens* to quickly respond to increased acetate availability with a higher respiration rate may be a factor in *Geobacter* species predominance in subsurface environments in which dissimilatory metal reduction has been stimulated with electron donor additions (Sneyenbos-West *et al.*, 2000; Holmes *et al.*, 2002; Anderson *et al.*, 2003; Ortiz-Bernad *et al.*, 2004).

The lower efficiency of converting acetate to biomass when acetate is not the factor limiting growth is also beneficial in the uranium bioremediation process. Production of less biomass lowers the possibility of accumulated biomass interfering with aquifer porosity while more of the added acetate is directed to metal reduction, which is the desired reaction.

In summary, the development of a chemostat system for culturing *G. sulfurreducens* is likely to greatly accelerate the study of the physiology of this organism which serves as pure culture model for the *Geobacter* species which are predominant in a diversity of Fe(III)-reducing subsurface environments. As shown here, such physiological studies can provide unexpected insights into the functioning of *Geobacter* species in the subsurface under naturally occurring or engineered conditions.

## Experimental procedures

### Culture of the microorganism

*Geobacter sulfurreducens* was grown at 30°C in continuous culture in custom-made glass vessels with a 200 ml working volume, within an outer water jacket that was connected to a water bath for temperature control. The culture vessel and all associated tubing were sterilized by autoclaving. The connections between tubes were made with stainless Luer fittings (Cole Palmer) and the culture was sampled through a steel canula connected to a stainless steel lock.

The growth medium contained the following mineral salts: NaHCO<sub>3</sub> 2.5 g l<sup>-1</sup>; NH<sub>4</sub>Cl 0.25 g l<sup>-1</sup>; NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 0.06 g l<sup>-1</sup>; KCl 0.1 g l<sup>-1</sup>; Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O 0.04 g l<sup>-1</sup>, and was supplemented with a trace mineral and vitamin solutions (Balch and Wolfe, 1979). Sodium acetate was used as electron donor, and either sodium fumarate or ferric-citrate as electron acceptor. The medium was introduced into the culture vessel at a steady rate with a variable-speed dispensing pump (ISMATEC) and calibrated tubing (PharMed; 1.30 mm internal diameter).

The culture media were constantly gassed (50 ml min<sup>-1</sup>) with a certified mixture of N<sub>2</sub>-CO<sub>2</sub> (80:20) passed through heated copper filings to remove any traces of oxygen. The culture exited vertically through a stainless steel canula, pushed by the gas overpressure in the headspace and is collected in the effluent reservoir.

The culture was stirred at a constant speed of 600 r.p.m. with a magnetic bar. Steady-state cell growth was obtained after 5 volume refills and was confirmed by a constant cell density and concentrations of fumarate and succinate or Fe(II).

### Analytical methods

Protein was determined as previously described (Provenzano *et al.*, 1985). Fe(II) was determined with ferrozine as previously described (Lovley and Phillips, 1987b). The content of organic acids in the culture supernatant was measured with HPLC with a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) and a mobile phase of 8 mM H<sub>2</sub>SO<sub>4</sub>.

Acetate consumption for cell mass synthesis was calculated according to the equation (Galushko and Schink, 2000):



### Determination of the kinetic parameters

In order to estimate the maximum specific growth rate ( $\mu_{\text{max}}$ ), batch cultures were grown with 1–10 mM acetate and 30 mM fumarate or 60 mM Fe(III) as electron acceptor and  $\mu_{\text{max}}$  and K<sub>s</sub> were calculated with the Lineweaver-Burk linearization method.

The metabolic rates for maintenance purposes ( $m$ ) and maximal yield ( $Y^{\text{max}}$ ) were calculated from acetate-limiting chemostats with the Tempest and Neijssel equation (Tempest and Neijssel, 1984):

$$q = m + (1/Y^{\text{max}})\mu$$

where  $q$  is the metabolic rate;  $m$  is the coefficient for maintenance purposes; and  $Y^{\text{max}}$  is the theoretical maximum growth yield.

The respiration rate ( $q_e$ ) and the growth yield per electron transferred ( $Y_e$ ) were calculated based on the reduction of the electron acceptor [two electrons for fumarate and one electron for Fe(III)].

## Acknowledgements

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