Water-Rock Interactions, Ore Deposits, and Environmental Geochemistry: A Tribute to David A. Crerar
© The Geochemical Society, Special Publication No. 7, 2002
Editors: Roland Hellmann and Scott A. Wood

Microbial acquisition of nutrients from mineral surfaces

CHRISTOPHER J. TADANIER[1,2,3*], JOHN C. LITTLE[1], DUANE F. BERRY[2], AND MICHAEL F. HOCHELLA, JR.[3]

[1]Charles E. Via Department of Civil and Environmental Engineering,
Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA
[2]Department of Crop and Soil Environmental Sciences,
Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA
[3]Department of Geological Sciences,
Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA

* Author to whom correspondence should be addressed:

Christopher J. Tadanier
Department of Geological Sciences
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061 U.S.A.
e-mail: ctadanie@vt.edu

Abstract—Mineral surfaces act as concentrators for both organic and inorganic microbial nutrients and environmental contaminants. The bioavailability of mineral surface-associated nutrients and contaminants is controlled by a complicated set of both biotically- and abioticially-mediated processes which occur at microbe-solution, mineral-solution, and microbe-mineral interfaces. This study was conducted to 1) develop a generalized transport model which can be used to describe nutrient uptake by bacteria, 2) conduct continuous growth experiments using a model system in which the growth-limiting nutrient is adsorbed on a colloidal mineral, and 3) use uptake rate calculations and model-system data to evaluate various physiological responses bacteria may use to obtain nutrients from mineral surfaces. Microorganisms from an undisturbed natural soil were maintained in chemostat (continuous) culture for 45 d, using P_i (orthophosphate) adsorbed on colloidal goethite as a sole phosphorous source. After a short initial acclimation period microbial utilization of adsorbed P_i averaged 82±6 %. The predominant Gram-negative bacterium isolated from chemostat effluent was *Burkholderia cepacia*. Free energy (ΔG) considerations demonstrate that sufficient energy was available for complete bacterial P_i uptake; therefore, trans-membrane P_i transport was not thermodynamically-limited. Rate calculations, which model P_i uptake as a series of discrete transport processes in each cell wall and near-cell compartment, suggest that the microbial community must alter the chemostat environment in order to obtain sufficient P_i for continuous steady-state growth. The nutrient uptake model outlined here, used in conjunction with steady-state chemostat data, may ultimately provide a means to address the broader environmental implications of microbial nutrient acquisition from mineral surfaces on the fate and transport of mineral surface-associated contaminants.

1. INTRODUCTION

Microorganisms display a wide range of nutritional requirements, some being adaptable to varied nutrient conditions, while others are fastidious, occupying only a narrow environmental niche due to strict nutritional needs. However, all microorganisms share the same fundamental biomolecular building blocks — proteins, lipids, nucleic acids, and carbohydrates — which require carbon, oxygen, hydrogen, nitrogen, phosphorous, sulfur, and transition metals in varying amounts. Alkali and alkaline earth metals (typically Na, K, Mg, and Ca) are also required for membrane transport processes, charge balance, structural conformation, signaling, and a variety of other physiological processes. One or more nutrients ultimately limit microbial growth in natural environments, most often carbon, nitrogen, or phosphorous. Oligotrophic (nutrient-limited) conditions generally prevail in natural environments; solution phase nutrients at μM to pM concentrations are commonly encountered due to depletion by microbial growth and sorption onto mineral surfaces.

Mineral surfaces act as concentrators for both organic and inorganic microbial nutrients, with the strength of nutrient-surface association dependent on the physicochemical properties of each. The affinity of microbial nutrients for mineral surfaces, from weakly to strongly associated, is based on one or more fundamental sorptive interactions. Organic molecules may be sorbed to mineral surfaces due to hydrophobic interaction or electrostatic, covalent, or hydrogen bonding mechanisms. Natural organic compounds known to interact with mineral surfaces to varying degrees include biopolymers of plant, animal, and microbial origin such as proteins, lipids, nucleic acids, and polysaccharides; and their associated degradation products such as amino and low molecular-weight fatty acids, nucleosides, and a vari-

ety of phosphorylated and aminated sugars (Kummert and Stumm, 1980; Schwertmann et al., 1986; Stevenson, 1994; Chenu, 1995; Cornell and Schwertmann, 1996). Synthetic organic compounds (SOCs) including solvents, manufacturing by-products, hydrocarbon fuels, and pesticides, may also serve as microbial nutrients and have been widely observed in association with mineral surfaces (Kung and McBride, 1991; Rao et al., 1995; Ristori and Fusi, 1995; Brady and Weil, 1999). Inorganic nutrients may be sorbed to mineral surfaces through surface complexation reactions, or they may be contained in surface precipitates, the bulk mineral, or occluded phases. Surface-complexed inorganics may be strongly associated through covalent bonds formed by ligand exchange (inner-sphere-complexed) or relatively weakly associated due to electrostatic interaction through an intervening water molecule (outer-sphere-complexed), whereas inorganic nutrients contained in a mineral phase are characterized as strongly associated (Brown, 1990). Examples of inorganic nutrients observed in association with mineral surfaces include, but are not limited to, nitrogen (NH_4^+, NO_3^-, NO_2^-), phosphorous ($H_2PO_4^-$, HPO_4^{2-}), sulfur (SO_4^{2-}, HS^-), and transition metals (particularly $Fe^{2+/3+}$, and $Mn^{2+/4+}$) (Cornell and Schwertmann, 1996).

The bioavailability of mineral surface-associated contaminants has received substantial attention from both biotic and abiotic points of view, with the impact of environmental factors such as pH, ionic strength, and redox potential on contaminant phase distribution studied extensively (Stotzky, 1986; Lonergan and Lovley, 1991; Ristori and Fusi, 1995; Stone and Torrents, 1995; Bollag et al., 1995; Andreux et al., 1995). However, the influence of microbially mediated processes on nutrient availability has been infrequently studied at environmentally relevant nutrient concentrations. Furthermore, phenomenological mechanisms responsible for macroscopically observed nutrient bioavailability have invariably been speculative in nature, with little or no supporting experimental data available. Therefore, we feel it is imperative to study nutrient bioavailability in natural environments from a biogeochemical perspective, in which microbially-mediated mechanisms responsible for macroscopically observable nutrient bioavailability are considered in terms of interactions at microbe-solution, mineral-solution, and microbe-mineral interfaces. Figure 1 illustrates the conceptual approach we have developed and adopted for studying biogeochemical processes associated with microbial acquisition of nutrients from mineral surfaces.

Chemostat (continuous flow, steady-state) culture offers many possibilities for studying environmental influences on microbial community structure not available using batch culture techniques (Veldkamp, 1977; Smith and Waltman, 1995). Unlike batch culture experiments, in which continuously changing environmental and progressively depleted nutrient conditions bring about a continuous succession of species distributions and phenotypic traits within a microbial community, maintenance of limited but sufficient nutrient conditions in a chemostat ultimately results in development of a stable or steady-state community. Moreover, the initially high nutrient concentrations required for batch culture confer a competitive advantage to microbial species which rely on high growth rate as a survival strategy (*r* strategists), and preferentially inhibit species which optimize utilization of scarce resources (*K* strategists) (Atlas and Bartha, 1998). Steady-state cultures (growth rate of each species is balanced by dilution rate – see Appendix A.1) can be exposed to environmental changes in a systematic manner, allowing controlled study of the physiological response of microorganisms to nutrient-limited conditions more representative of natural environments.

For the research described herein, sorption of orthophosphate (P_i) on colloidal goethite (α-FeOOH) was used as a model nutrient mineral-surface system. Use of this model system is based on its importance from both microbiological and mineralogical points of view. Phosphorous is a major nutrient for all microorganisms, regardless of physiological and metabolic character or environmental niche occupied. Because of its high affinity for mineral surfaces and lack of volatile forms, phosphorous is often the least available of the biologically essential elements, and in global terms may be the single most important factor limiting the extent of life on Earth (Cox, 1995). Indeed, dissipation of P_i in the environment due to current land use practices threatens to adversely impact the food chain at its foundation (i.e., microbial activity and production) and may ultimately limit food production in the future (Runge-Metzger, 1995; Abelson, 1999). Goethite is the most abundant iron-oxide in the environment, and in soils and sediments it may represent a majority of the total surface area exposed (Cornell and Schwertmann, 1996). Phosphate complexes strongly with goethite surfaces, and although not completely characterized, the sorptive behavior of phosphate on goethite is perhaps the most widely studied nutrient mineral-surface system.

Our ultimate goal is to study the actively and passively mediated processes which microorganisms may use to enhance bioavailability of nutrients sorbed on mineral surfaces. Actively mediated processes are based on physiological responses that either enhance nutrient release at the mineral-solu-

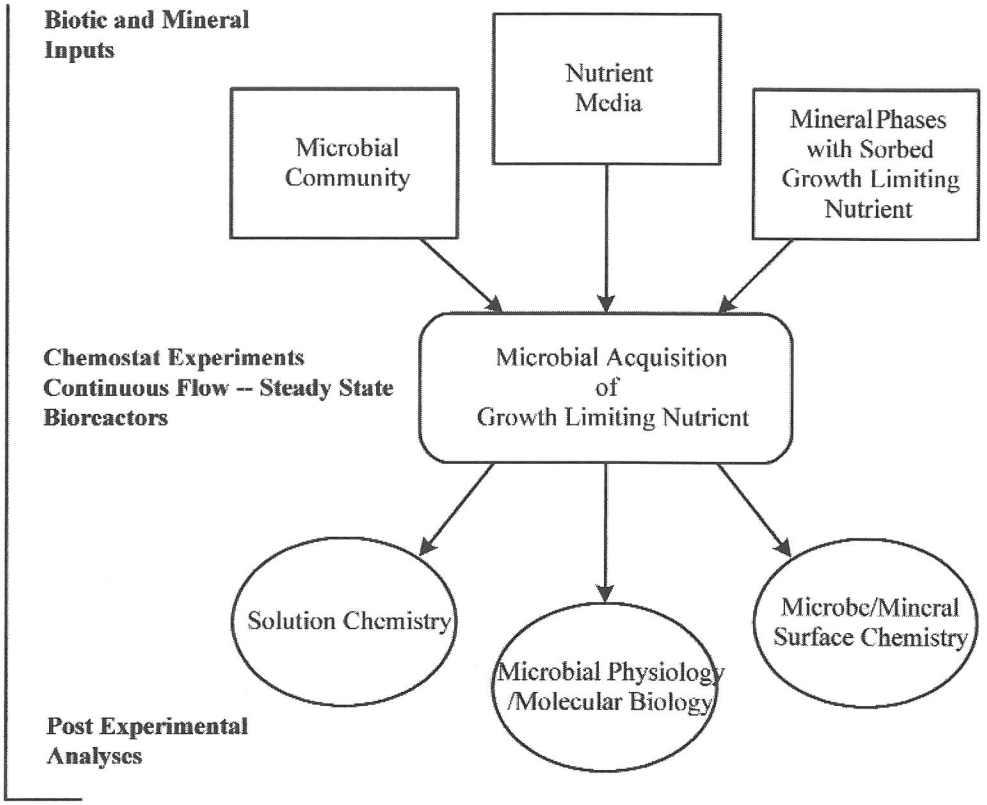

Fig. 1. Conceptual biogeochemical approach for studying microbial acquisition of nutrients from mineral surfaces.

tion interface or minimize resistance to nutrient mass transport in the bulk solution, whereas passively mediated processes involve physiological responses that either enhance nutrient transport across the microbial cell envelope or reduce the cellular nutrient requirement. In this sense, the distinction between actively and passively mediated responses is based on whether or not microbes alter their surrounding environment as a means of increasing sorbed nutrient bioavailability. Specific objectives of the research described here are to 1) develop a generalized nutrient transport/uptake model which allows P_i mass transfer in near-mineral surface, bulk solution, and near-microbe environmental compartments to be described in terms of mineral, solution, and bacterial cell envelop properties, 2) conduct a set of chemostat experiments to obtain basic microbiological, kinetic (growth rate), and solution chemistry data for a natural bacterial community able to effectively utilize P_i sorbed on colloidal goethite as the sole source of phosphorous, and 3) evaluate various active and passive physiological responses which bacteria may use to enhance P_i acquisition from mineral surfaces by comparing chemostat data with previous observations of microbial P_i bioavailability.

2. BIOGEOCHEMISTRY OF P_i-GOETHITE ASSOCIATION

2.1. Phosphate-Goethite as a Model Nutrient-Mineral System

2.1.1. Occurrence of goethite in the environment

Goethite is one of the most widespread minerals in the earth's near-surface environment, and is by far the most abundant iron (hydr)oxide in soils worldwide (Cornell and Schwertmann, 1996; and references therein). It predominates especially under humid and cool to temperate climates. Although the absolute abundance of goethite in a soil is typically only a few

weight percent (tens of g goethite/kg soil), its surfaces may account for the majority of the total surface area of the soil as a whole (Schwertmann and Taylor, 1990). This is in part due to the relatively small size and high specific surface area of natural goethite crystals, typically acicular or needle-like with surface area of 20 to 200 m^2/g (Schwertmann and Cornell, 1988), and in part due to formation of goethite coatings on larger mineral grains in soil. Furthermore, because goethite has a high affinity for sorption of many cations and anions (see below), it is thought to be a major factor in the retention and cycling of geochemically and environmentally important elements and compounds.

2.1.2. Chemical forms of phosphorus in the environment

Phosphorus is not an abundant element in the environment, comprising on average less than 0.1 weight percent (1000 mg/kg) of the earth's crust and a negligible percentage of the atmosphere. Total phosphorus content in soils varies from < 20 to 6800 mg P/kg soil (< 0.002 to 0.68 %), with a median value of approximately 500 mg P/kg soil (0.05 %) (Bowen, 1979; Pierzynski et al., 1994; Barber, 1995). Due to strong retention by mineral phase constituents, soil-solution phosphorus concentrations are quite low, with values in the range of 0.002 to 0.03 mg P/kg soil solution ($\leq 10^{-6}$ M) typical in temperate soils (Bohn et al., 1985). Soluble phosphorus concentrations in fresh and ocean waters are also quite low, with average values of 0.02 and 0.07 mg P/kg solution, respectively (Taylor and McLennan, 1985). Phosphorus comprises a relatively small mass fraction in microbiota and plants; 1 to 3 % in bacteria, 0.3 to 0.4 % in marine algae, 0.4 to 1.8 % in marine phytoplankton, 0.5 % in fungi, 0.074 % in lichens, and 0.012 to 1 % in vascular plants (Bowen, 1979). However, these modest phosphorus contents represent bioconcentration of 2 to 4 orders of magnitude due to metabolic activity of living organisms.

Environmental phosphorus, whether in organic or inorganic form, almost invariably occurs as phosphate (PO_4^{3-}, oxidation state +5) (Corbridge, 1990; Frossard et al., 1995). Reduced forms of phosphorus such as orthophosphite (PO_3^{3-}, oxidation state +3), hypophosphite (PO_2^{3-}, oxidation state +1), and phosphine (PH_3, oxidation state -3) occur only rarely in the earth's surface environment, and are generally rapidly oxidized to phosphate (Cox, 1995). Phosphate is incorporated in a wide variety of inorganic and organic forms in nature, which display a broad range of solubilities. Soluble inorganic forms of phosphorus include orthophosphate (P_i) and polyphosphates – linear polymers with 2 or 3 phosphate molecules linked by phosphoanhydride bonds. Both ortho- and polyphosphates undergo protonation, resulting in pH-dependent distributions of partially to fully protonated species. In the range of pH values encountered in all but the most extreme environments (pH 3–11), singly and doubly protonated forms predominate (i.e., HPO_4^{2-} and $H_2PO_4^-$ for P_i; Stumm and Morgan, 1981).

Phosphorus also occurs commonly in relatively insoluble phosphate mineral forms. More than 200 phosphate minerals are known, but those in the apatite group (calcium phosphates – $Ca_{10}(PO_4)_6X_2$, where X represents an anion such as F^-, Cl^-, OH^-, or CO_3^{2-}) are by far the most abundant igneous and sedimentary forms (Corbridge, 1990). A variety of secondary phosphate minerals are formed in soils due to weathering of primary apatite minerals, especially highly insoluble Al- and Fe-phosphate precipitates. Phosphate is also strongly adsorbed on the surfaces of both crystalline and amorphous Al and Fe (hydr)oxide secondary minerals, as discussed in detail in the following sections. The various inorganic and adsorbed mineral forms of phosphate often account for 50 to 70 % of the total phosphorus in soils (Pierzynski et al., 1994).

Phosphorus in organic form occurs in soils and sediments exclusively as phosphate incorporated in biomolecules, and associated degradation products produced by living organisms. Organic forms of phosphorus have been observed to account from as little as 5 % to as much as 95 % of total soil phosphorus content (Stevenson, 1994; Senesi and Loffredo, 1999), with 29 to 65 % being a more representative range (Harrison, 1987; Pierzynski et al., 1994; Paul and Clark, 1996). Less than 50 % of organic phosphorus in most soils can be accounted for in identifiable forms (Senesi and Loffredo, 1999). Chemically identifiable organic phosphorus compounds in soils include inositol phosphates (IP, 2 to 50 %), phospholipids (1 to 5 %), nucleic acids (0.2 to 2.5 %), and trace amounts of phosphoproteins and metabolic phosphates (e.g., ATP and NAD^+) (Stevenson, 1994). The inability to chemically describe the remaining forms of organic phosphorus may be attributed to the occurrence of polymeric phosphate-containing molecules such as teichoic acids and phosphorylated polysaccharides from bacterial cell walls, incorporation of phosphate in soil organic matter (SOM), such as humic and fulvic acids, and formation of insoluble complexes with metal ions or sorption on mineral surfaces (Anderson et al., 1974; Shvets et al., 1991; Stevenson, 1994).

2.1.3. Phosphorus-goethite sorptive interactions

The phosphate-goethite adsorption envelope is typical of oxyanion adsorption on mineral surfaces: fractional phosphate adsorption is maximum at low pH, decreases with increasing pH, and displays maxima or slope changes at the pK_a(s) of the conjugate acid(s) of the adsorbing anion (Hingston et al., 1968; Hingston et al., 1972). Phosphate adsorption on goethite generally exhibits maximum adsorption at about pH 2 (~ pK_1), with discontinuities in the adsorption envelope slope at pH values of approximately 7 (~ pK_2) and 12 (~ pK_3) (Hingston et al., 1968; Hingston et al., 1972; Sigg and Stumm, 1981; Hawke et al., 1989; Geelhoed et al., 1998). The extent of phosphate adsorption on goethite at any pH increases with rising concentration, and often conforms to the Langmuir isotherm (McBride, 1994). However, results of phosphate desorption experiments demonstrate that substantial hysteresis exists between adsorption and desorption isotherms, with residual sorbed phosphate concentrations substantially greater than the equilibrium adsorption isotherm values (Hingston et al., 1974; Barrow, 1983). Explanations for the observed hysteresis in phosphate adsorption-desorption on goethite include kinetic differences (Hingston et al., 1974), slow diffusion out of micropores (Barrow, 1983), and precipitation of a relatively insoluble phosphate mineral phase on the goethite surface (Martin et al., 1988).

Of the common inorganic and organic oxyanions, phosphate is the most strongly adsorbed by goethite surfaces (McBride, 1994). Based on experimental evidence from a variety of direct and indirect methods, a consensus exists supporting inner-sphere complexation of phosphate on goethite by ligand exchange with reactive surface hydroxyl groups (Goldberg and Sposito, 1985; and references therein). Studies of the phosphate-goethite adsorption envelope indicate little influence of ionic strength, a characteristic associated with inner-sphere complexation of metal and oxyanion adsorption on mineral surfaces (Ryden et al., 1977; Barrow et al., 1980; Hayes et al., 1988; Hawke et al., 1989; Davis and Kent, 1990). Several other lines of evidence support inner-sphere complexation, including the adsorption of phosphate on goethite at pH values greater than the point of zero charge (PZC) - i.e., negatively charged surface - (Hingston et al., 1972; Mott, 1981), differences in the kinetics of phosphate adsorption and desorption on goethite (Mott, 1981) and in phosphate adsorption on Fe(III) and Cr(III) oxides (Yates and Healy, 1975), and spectroscopic studies which suggest phosphate (and its structural analog arsenate) adsorption on goethite occurs by ligand exchange with surface hydroxyls (Parfitt et al., 1975; Tejedor-Tejedor and Anderson, 1986; Martin and Smart, 1987; Sun and Doner, 1996; Fendorf et al., 1997).

2.2. Bioavailability of Phosphate in the Environment

The bioavailability of P_i in soil environments is largely determined by its distribution among various organic and inorganic chemical forms and sorption on or presence within mineral and solution phases. About 0.1 % of the estimated 10^{15} kg of P in the earth's crust is subject to at least some turnover due to cycling of P_i between various forms and phases (Paul and Clark, 1996). Phosphate cycling is critical in soils because the uptake of phosphorus by both microorganisms and plants is generally in the form of soluble P_i from the soil solution (Van Veen et al., 1994; Frossard et al., 1995).

Soil solution P_i concentration, and hence bioavailability, is a complex function of pH and soil solution geochemistry, which may vary considerably between soils and with recent environmental conditions and usage for a given soil. Inputs of P_i that impact soil environments include weathering and dissolution of primary and secondary minerals, desorption from mineral surfaces, hydrolysis of phosphate containing biomolecules, and anthropogenic sources such as fertilizers. Conversely, processes which remove P_i from soil solution include incorporation in biomass by microbes and plants, sorption on mineral oxide surfaces, precipitation of secondary minerals, and dilution by infiltration or advective groundwater flow. In general, neither biotically- nor abiotically-mediated reactions involved in phosphorus cycling alter its oxidation state from +5 (Stewart and McKercher, 1982; Tate, 1995).

2.2.1. Influence of P_i distribution on bioavailability

In principle, soil solution P_i concentration may be controlled by the solubility of Ca, Al, and Fe-containing minerals, or sorption on Al and Fe (hydr)oxide, clay, and calcium carbonate mineral surfaces (Lindsay, 1979; Pierzynski et al., 1994; Barber, 1995; Frossard et al., 1995). In acidic to neutral environments phosphate bioavailability is dominated by sorption/desorption to Al- and Fe-(hydr)oxides (Haynes, 1982; Sanyal and De Datta, 1991; Pierzynski et al., 1994; Geelhoed et al., 1998), whereas in alkaline environments solubility of calcium phosphate minerals is thought to control P_i bioavailability (Goldstein, 1994; Lajtha and Harrison, 1995; Iyamuremye and Dick, 1996). The time-dependence

of abiotic P_i transformations in soils also supports the role of sorption reactions in determining P_i bioavailability: the kinetics of P_i chemisorption on mineral surfaces are much faster than for precipitation of P_i-containing secondary minerals (Van Der Zee and Van Riemsdijk, 1991).

2.2.2. Microbial response to environmental stimuli - phosphate uptake by bacteria

Essentially all microorganisms are capable of adapting their physiology/metabolism to some extent in response to environmental changes (Stock et al., 1990). Microbial response to environmental stimuli is accomplished through alternative patterns of gene expression, which result in a wide variety of both membrane-bound and cytoplasmic proteins with functions related to nutrient uptake, respiration, sporulation, virulence, resistance to temperature and osmotic shock, and chemical communication between bacterial cells. When confronted with P_i limitation, expression of as many as 38 phosphate starvation-inducible (*psi*) genes has been observed in *E. coli* and closely related bacteria (Wanner, 1994). The *psi* genes of E. coli are arranged in eight separate transcription units (i.e., operons), collectively known as the Pho regulon (Wanner, 1995). Specific functions of Pho regulon gene products include enhanced P_i uptake (Pst system and associated outer-membrane porin PhoE), uptake of small phosphorylated organic molecules (Ugp system), utilization of phosphonate compounds (Phn system), and extracellular phosphoester hydrolysis (periplasmic phosphatase enzymes) (Wanner, 1996).

Because of the continuous-flow design and use of goethite-sorbed P_i as the sole phosphorous source in this study, a microbial community must adapt to solution P_i limitation to remain viable in the long term. Also, a relatively detailed physical description of the pathways involved in bacterial P_i uptake is required to model transmembrane P_i transport. Therefore, consideration of the physiology and regulation of P_i uptake is particularly relevant to this study. Phosphate transport has been most extensively studied in *E. coli*, which serves as the paradigm for environmentally regulated bacterial P_i uptake (Wanner et al., 1996).

Bacterial P_i uptake occurs by a series of diffusionally-driven and enzymatically-mediated energy dependent processes. Phosphorylated molecules diffuse through the outer membrane of Gram-negative bacteria through protein channels and through the peptidoglycan cell wall of Gram-positive cells to the vicinity of the cytoplasmic membrane. With few exceptions, bacteria preferentially transport P_i across the cytoplasmic membrane. P_i is typically released from small phosphorylated organic molecules by enzymatic hydrolysis prior to trans-membrane transport (Torriani-Gorini, 1994). Transmembrane P_i transport is energy-dependent, as it must be transported into the cell against a very unfavorable concentration gradient (cytoplasmic P_i is typically on the order of 10 mM (Shulman et al., 1979; Wanner, 1994), whereas extracellular P_i in natural environments is typically at $\leq$ μM levels).

When P_i is present at ~ μM or greater concentrations, *E. coli* take up P_i through the constitutively (continuously) expressed non-specific phosphate inorganic transport (Pit – low affinity) system, whereas at lower extracellular concentrations uptake is primarily through the inducible P_i specific transport system (Pst – high affinity) of the Pho regulon (Rosenberg, 1987). The Pit system utilizes symport (simultaneous transport) of a divalent metal-phosphate complex ($MeHPO_4$ – Me may be Mg^{2+}, Ca^{2+}, Mn^{2+}, or Co^{2+}) and a proton across the cytoplasmic membrane, and is therefore energetically driven by a transmembrane H^+ gradient (Van Veen et al., 1994). The Pst system relies on direct ATP hydrolysis by a membrane-bound transport enzyme to provide the energy required to overcome the energetically unfavorable P_i concentration gradient (Webb and Cox, 1994).

Microbial uptake of P_i from the environment is a highly coordinated activity, subject to a high level of genetic regulation. The Pho regulon is influenced by multiple controls, but its principle regulator is extracellular P_i concentration (Wanner, 1994). P_i control of the Pho regulon involves a form of transmembrane signal transduction in which a cell surface receptor protein (PhoR) modulates phosphorylation of a cytoplasmic transcription regulator protein (PhoB) (Wanner, 1995). When extracellular P_i levels become limiting, PhoR activates PhoB by phosphorylation, thus triggering transcription of Pho regulon *psi* proteins.

Although P_i uptake has been more extensively characterized for *E. coli* than other bacteria, several significant aspects of P_i uptake by *E. coli* appear common among diverse bacterial species. First, the *E. coli* PhoB and PhoR proteins belong to a superfamily of environmentally responsive, two-component regulatory systems which are prevalent in bacteria (Ronson et al., 1987; Parkinson and Kofoid, 1992). Also, both low- and high-affinity P_i transport systems have been observed in a number of both Gram-positive and Gram-negative bacteria, including *Salmonella typhimurium* (Foster and Spector, 1986), *Pseudomonas aeroginosa* (Poole and Hancock, 1984), *Bacillus cereus* (Guddal et al., 1989) and *Bacillus subtilis* (Liu and Hulett, 1997; Qi et al., 1997), *Synechococcus* sp WH7803 (Scanlan et al.,

1997), *Rhizobium meliloti* (Voegele et al., 1997), *Acinetobacter johnsonii* (Van Veen et al., 1993), and *Enterobacter cloacae* and *Klebsiella pneumoniae* (Van Der Ley et al., 1987).

3. NUTRIENT TRANSPORT AND UPTAKE MODELING

Based on the previous discussion of P_i biogeochemistry, it is clear that the soluble P_i concentration has a central role in the interfacial processes which occur between mineral surfaces and bulk solution, and bulk solution and cell envelope. Therefore, those aspects of P_i bioavailability related to transport may be framed in terms of a generalized nutrient transport/uptake model which considers the impact of resistance to mass transfer in the near-mineral surface, bulk fluid, and near-cell membrane environments. However, in a well-mixed chemostat with two-phase mass transport and rapid equilibrium of sorbed components at the mineral-solution interface, the rate of bacterial P_i uptake is likely to be limited by resistance to P_i transport across the cell envelope. Therefore, expressing the steady-state bacterial P_i uptake rate in terms of cell envelope physiology and properties provides a means of evaluating the potential contributions of active and passive microbial responses to P_i limitation.

3.1. Generalized Nutrient Transport/Uptake Model

The acquisition of mineral surface-associated nutrients by microorganisms can be viewed as a sequence of discrete mass transfer steps in which a nutrient molecule must sequentially traverse each of the environmental compartments illustrated in Fig. 2. Each environmental compartment exerts a resistance to nutrient transport, the relative magnitude of which

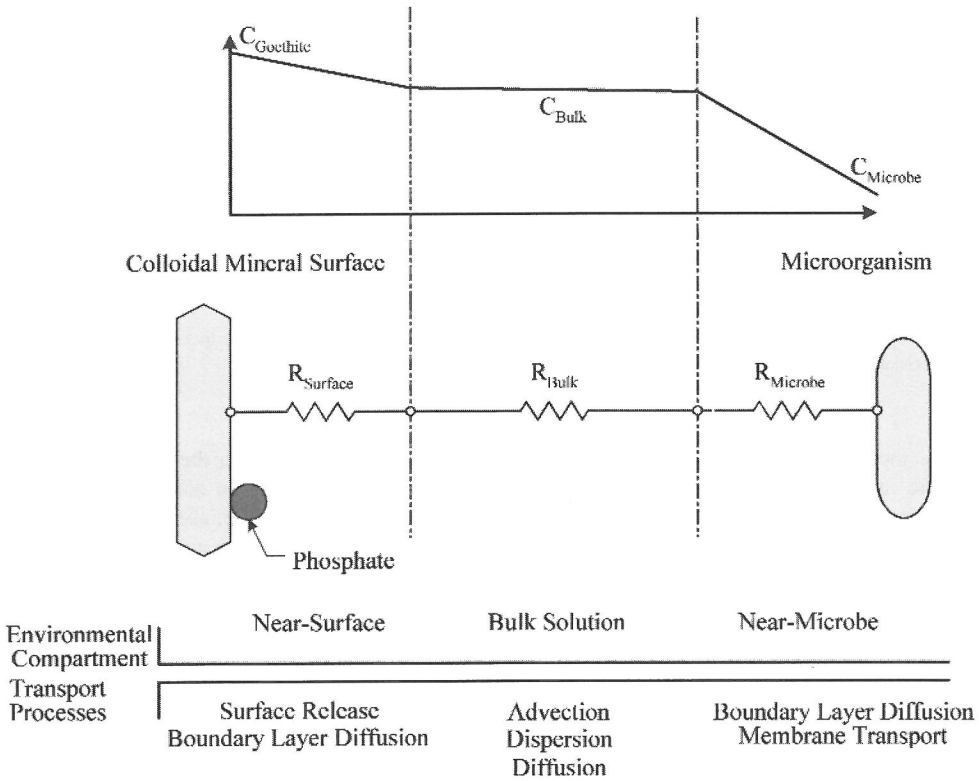

Fig. 2. Graphical representation of the Resistances in Series Model for mass transport in a well-mixed chemostat. Mass transfer in each environmental compartment may be composed of several transport processes acting in series and/or parallel, which combine to yield a composite resistance to mass transfer.

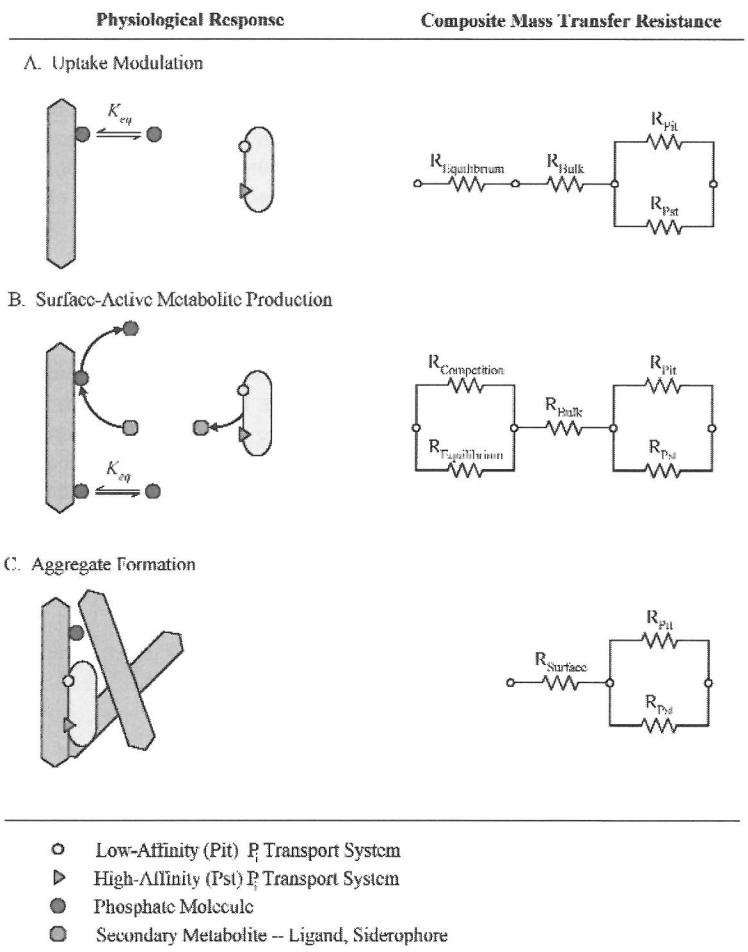

Fig. 3. Conceptual model of several physiological responses microbes may use to lower the overall resistance to phosphate transport or increase its bioavailability.

depends on the biophysicochemical characteristics of the specific mass transfer processes involved. The overall resistance to nutrient transport is given by the sum of resistances of compartments traversed, or in the nomenclature of Fig. 2:

$$R_{Total} = R_{Surface} + R_{Bulk} + R_{Microbe} \quad (1)$$

Furthermore, nutrient transport through each environmental compartment may be accomplished by several processes acting in parallel, resulting in a decreased resistance to mass transfer. For two transport processes acting in parallel the composite resistance to mass transfer is given by:

$$\frac{1}{R_{Composite}} = \frac{1}{R_1} + \frac{1}{R_2} = \frac{R_1 R_2}{R_1 + R_2} \quad (2)$$

Mass transport through the various environments defined in Fig. 2 may be accomplished by several mechanisms. In the near mineral surface environment solute molecules diffuse to and from the mineral surface, where rapid sorption-desorption reactions occur. Transport in the bulk environment may occur by molecular diffusion, dispersion, or advection, with the dominant mechanism being determined by the characteristic dimension or scale of interest. Nutrients diffuse to the microbial surface though a fluid boundary layer after which one or more of several possible diffusive or energy dependent membrane transport processes occur.

Transport of P_i to or across the microbial membrane may in principle limit growth. However, the extent to which microorganisms physiologically respond to imposed environmental conditions,

either as single species or in consortia, to lower the overall resistance to P_i transport is not well known. Three possibilities are immediately apparent: (1) microorganisms may alter their membrane character to enhance uptake rate from solution (passive response), (2) microorganisms may excrete surface-active metabolites which alter the equilibrium position of P_i-goethite surface association by competitive exchange or reductive dissolution (active response), or (3) microorganisms may increase their proximity to phosphate-covered mineral surfaces by chemotaxis or inducing microbe-aggregate formation (active response). Each of these responses is conceptually illustrated in Fig. 3. In complex natural environments these responses may occur singly or in combination, with the operative response(s) at any point in time dependent on imposed environmental conditions.

3.2. Modeling P_i Transport Across the Bacterial Cell Envelope

Phosphate transport in the near-microbe environment generally involves a series of three discrete steps: 1. diffusion through the cell boundary layer (i.e., from bulk solution to cell envelope outer surface), 2. diffusion through the outer membrane and periplasm (Gram-negative cells) or S-layer and cell wall (Gram-positive cells), 3. energy-dependent transport across the cytoplasmic membrane (Fig. 4). A composite expression for P_i transport in the near-microbe environment in terms of component mass transfer resistances can be developed by mathematically expressing each mass transfer process as diffusive in nature.

The P_i mass transfer rate J_i through each compartment of the near-microbe environment can be expressed in terms of the resistance to mass transport R_i through the compartment and the concentration difference ΔC_i across the compartment. The general expression for mass transfer rate through any compartment is written as:

$$J_i = \frac{\Delta C_i}{R_i} \tag{3}$$

Physicochemical properties of outer-membrane transport channel proteins and kinetic parameters for energy-dependent cytoplasmic membrane P_i

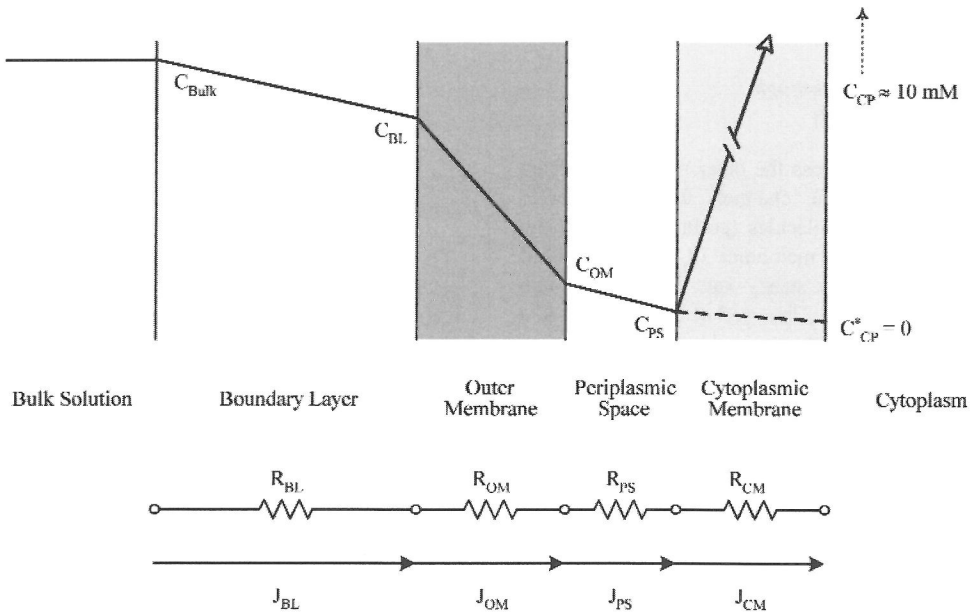

Fig. 4. Phosphate uptake by Gram-negative bacteria, with representative cell envelope P_i concentration profile. P_i transport is by diffusion through the aqueous boundary layer (BL), outer membrane (OM), and periplasmic space (PS), followed by an energy-dependent enzymatically-mediated process in the cytoplasmic membrane (CM). At steady state the rates of P_i transport through all near-cell environmental compartments are equal. Note that the actual cytoplasmic P_i concentration C_{CP} is approximately 10 mM, whereas the apparent P_i concentration C^*_{CP} with respect to the energy-dependent enzymatically-mediated trans-membrane transport process is set to 0.

transport proteins are listed in Table A.2.1 and Table A.2.2, respectively. In the individual near-microbe P_i transport rate expression derivations which follow, parameters in parentheses are either physical constants or representative values used in subsequent numerical calculations.

3.2.1. Diffusion rate through microbial boundary layer (BL)

The P_i diffusion rate through the bacterial cell fluid boundary layer (J_{BL}) is calculated for steady-state diffusion of a solute from solution to the surface of an absorbing sphere, with the concentration difference across the boundary layer as shown in Fig. 4. Based on the cell morphology of bacteria observed in this study (discussed later), a spherical cell shape with diameter of 1.6 μm was used. The diffusion rate J_{BL} and mass transport resistance R_{BL} are expressed as:

$$J_{BL} = 4\pi aD\left(C_{Bulk} - C_{OM}\right); \quad \frac{1}{R_{BL}} = 4\pi aD \qquad (4)$$

where

a = cell radius (800 nm)

D = diffusivity of P_i in water (0.8×10^{-5} cm^2 sec^{-1} – as tabulated in CRC, 1992).

3.2.2. Diffusion rate through outer membrane (OM)

Diffusion of P_i across the outer membrane occurs through anion-specific channels defined by outer membrane protein molecules (porins). The diffusion rate across the outer membrane (J_{OM}) is calculated from the equation for steady-state diffusion through pores with total area A_P in a wall of thickness x, and a concentration difference across the wall, as shown in Fig. 4. A trans-membrane pore diameter of 1.0 nm is used, based on representative values for Gram negative bacteria listed in Table A.2.1. The diffusion rate J_{OM} and mass transport resistance R_{OM} are expressed as:

$$J_{OM} = \frac{A_p f D}{x}\left(C_{OM} - C_{PS}\right); \quad \frac{1}{R_{OM}} = \frac{A_p f D}{x} \qquad (5)$$

where

A_p = area for diffusion through outer membrane porin channels (3.0×10^4 P_i transport porins/cell (Hancock et al., 1982; Nikaido, 1996), porin channel diameter of 1.0 nm; $A_p = 2.36\times10^4$ nm^2)

f = Renkin correction factor (Koch, 1990) for porin channel inlet geometry and Stokes drag for diffusion through the channel $f = [1\text{-}b/r_p]^2\,[1 - 2.104(b/r_p) + 2.09(b/r_p)^3 - 0.95(b/r_p)^5] = 2.21\times10^{-2}$ (HPO_4^{2-} ionic radius b = 0.29 nm (Hancock et al., 1987) and porin channel radius r_p = 0.5 nm)

D = diffusivity of P_i in water (0.8×10^{-5} cm^2 sec^{-1} – as tabulated in CRC, 1992)

x = thickness of outer membrane (7.5 nm, Koch and Wang, 1982).

3.2.3. Diffusion rate through periplasmic space (PS)

The P_i diffusion rate through the periplasmic space between the outer and cytoplasmic membranes (J_{PS}) is calculated from the equation for steady-state diffusion through a spherical shell of thickness y and radius a (Crank, 1975; Middleman, 1998), with a concentration difference as shown in Fig. 4. Thus, the diffusion rate J_{PS} and mass transport resistance R_{PS} are expressed as:

$$J_{PS} = \frac{4\pi a^2 D}{y}\left(C_{PS} - C_{CM}\right); \quad \frac{1}{R_{PS}} = \frac{4\pi a^2 D}{y} \qquad (6)$$

where

y = thickness of periplasmic space (7.5 nm, Koch and Wang, 1982)

D = diffusivity of P_i in periplasmic space is conservatively assumed to be the same as in water

a = cell radius (800 nm), as in section *3.2.1* because outer membrane and periplasmic space thicknesses x and y, respectively, are << than cell radius a.

3.2.4. Enzymatically mediated uptake rate through cytoplasmic membrane (CM)

The energy-dependent P_i transport across the cytoplasmic membrane is accomplished by the Pit and Pst enzymatic systems, as discussed in section *2.2.2*. Because P_i transport across the cytoplasmic membrane is an enzymatic process it is subject to substrate saturation, and Michaelis-Menton enzyme kinetics can therefore be used to describe the transport rate across the cell membrane. In terms of Michaelis-Menton enzyme kinetics, the P_i transport rate across the cell membrane (J_{CM}) is a function of the substrate concentration at the surface of the cytoplasmic membrane (C_{CM}):

$$J_{CM} = V = \frac{V_{\max}\left[C_{CM}\right]}{K_M + \left[C_{CM}\right]} \qquad (7)$$

where

V_{max} = maximum transport rate when enzyme is saturated by P_i (30 nmol P_i/mg DW·min – Pit system, 20 nmol P_i/mg DW·min – Pst system), 2.8×10^{-13} g DW/cell)

K_M = Michaelis-Menton enzyme half-saturation con-

stant (25×10^{-6} mol P_i/L – Pit system, 0.2×10^{-6} mol P_i/L – Pst system).

Because it is difficult to directly measure the concentration of cytoplasmic membrane-bound enzymes, the Michaelis-Menton constants V_{max} and K_M are expressed on a dry weight of constituents per cell basis (DW/cell).

The energy-dependent enzymatically-mediated P_i transport process described by Eqn. 7, although not physically diffusive in nature, can be modeled using the same mathematical form as a purely diffusive process. Because $[C_{CM}] << K_M$ for the chemostat conditions encountered in this work (see section 4.7), the denominator of Eqn. 7 reduces to K_M. Furthermore, an *apparent* diffusive concentration difference ($C_{CM} - C^*_{CP}$) can be defined across the cytoplasmic membrane as shown in Fig. 4, where C^*_{CP} is set to zero to reflect the independence of the *actual* physical transport process on cytoplasmic P_i concentration; note: the actual C_{CP} is ≈ 10 mM. With these thoughts in mind, Eqn. 7 simplifies to these expressions for J_{CM}, and mass transport resistance R_{CM}:

$$J_{CM} = \frac{V_{\max}}{K_M}\left(C_{CM} - C^*_{CP}\right) \; ; \; \frac{1}{R_{CM}} = \frac{V_{\max}}{K_M} \qquad (8)$$

As depicted in Fig. 3, the Pit and Pst transport systems operate in parallel across the cytoplasmic membrane. However, the resistance to P_i transport of the Pit system is much greater than that of the Pst system ($R_{Pit} \approx 20$ to $200R_{Pst}$), as determined using representative kinetic properties for the two transport systems in several bacterial species (Table A.2.2) and Eqn. 8. Then by Eqn. 2, $R_{composite} \approx R_{Pst}$ for parallel transport, and the Pst system controls P_i transport across the cytoplasmic membrane under P_i -limiting conditions. Therefore, P_i transport by the Pit system will not be considered further.

3.2.5. Microbial cell envelope composite P_i mass transfer resistance

Because at steady state the P_i transport rate through all near-microbe environmental compartments is equal, the transport rate J through the composite near-microbe environment can be found by rearranging Eqns. 4, 5, 6, and 8, respectively, as follows:

$$J_{BL}R_{BL} = \left(C_{Bulk} - C_{OM}\right) \qquad (9)$$

$$J_{OM}R_{OM} = \left(C_{OM} - C_{PS}\right) \qquad (10)$$

$$J_{PS}R_{PS} = \left(C_{PS} - C_{CM}\right) \qquad (11)$$

$$J_{CM}R_{CM} = \left(C_{CM} - C^*_{CP}\right) \qquad (12)$$

Summing Eqns. 9–12 yields the following expression:

$$J\left(R_{BL} + R_{OM} + R_{PS} + R_{CM}\right) = \left(C_{Bulk} - C^*_{CP}\right) \qquad (13)$$

Rearranging Eqn. 13 and substituting $C^*_{CP} = 0$ yields:

$$J = \frac{C_{Bulk}}{R_{BL} + R_{OM} + R_{PS} + R_{CM}} \qquad (14)$$

where J (mol/s) is the transport rate of P_i into the cell, C_{Bulk} (mol/L) is bulk solution P_i concentration, and R_{BL}, R_{OM}, R_{PS}, and R_{CM} (s/L) are the resistances to P_i transport through the cell boundary layer, outer membrane, periplasmic space, and cytoplasmic membrane, respectively.

4. CHEMOSTAT EXPERIMENTS

A set of chemostat experiments was conducted using a natural soil microbial community and P_i sorbed on goethite as the sole phosphorous source. Basic microbiological, kinetic, and solution chemistry data were collected to facilitate evaluation of various active and passive responses bacteria may use to enhance bioavailability of mineral surface associated P_i.

4.1. Goethite Synthesis and Phosphate Adsorption Characterization

Colloidal goethite was synthesized by precipitation from $FeSO_4$ in an aerated solution buffered to pH 7 with 0.1 M $NaHCO_3$ (Schwertmann and Cornell, 1991). The identity and purity of the synthetic goethite sample was verified by powder X-ray diffraction analysis. No other crystalline mineral phases were detected. Broad weak features, indicative of amorphous material, were also absent in the diffraction pattern. Scanning electron microscopy (SEM) indicated that the size and shape of goethite crystals in our synthetic sample were very similar to those found in natural environments (Schwertmann and Cornell, 1991); colloids were relatively uniform euhedral, acicular crystals, approximately 700 nm long by 70 nm wide. Specific surface area was 85.4 m^2/g as measured by N_2 BET Analysis. The PZC of the prepared goethite was estimated to be 7.9 using the intersection of pH titration curves in 0.001, 0.01, and 0.1 M KCl.

The sorptive behavior of P_i on the goethite synthesized here was characterized by generating adsorption edges at several surface coverages and adsorption isotherms at several pH values. Standard wet-chemical techniques were used to generate these adsorption data, and both adsorption edge and isotherm data were

typical of strongly adsorbed oxyanions (Fig. 5). Desorption of phosphate by repeated rinsing with 0.01 N KCl showed strong hysteresis. Phosphate adsorption on goethite was also examined with X-ray photoelectron spectroscopy (XPS) (Fig. 5). The phosphorus signal measured directly from the surface of dried samples was well correlated with the isotherm developed from wet chemical experiments.

4.2. Chemostat Design and Operation

A chemostat was fabricated from a 2.2-L cylindrical borosilicate glass environmental sample bottle. Openings through the teflon-lined screw top lid were provided for glucose-mineral salts (GMS) microbial nutrient media and goethite suspension, pH probe, acid and base for pH control, and low-pressure (0.03 MPa, 5 psig) filtered air. Outflow from the chemostat was by gravity through a horizontal 0.64-cm (0.25-in) diameter teflon tube which penetrated the chemostat wall at a level corresponding to a volume of 2 L. Circumferential and vertical mixing were achieved using a floating teflon stir-bar pressure fit to the chemostat wall and an air-driven lift pump mounted atop the floating stir-bar assembly.

GMS media and phosphate loaded goethite suspension were admitted to the chemostat at equal volumetric rates from separate reservoirs using a dual channel cassette pump. Both GMS media and goethite suspension were prepared as 2X solutions, which resulted in a 1X concentration for all components in the chemostat. Both chemostat influent flow paths were exchanged with autoclaved components at each replenishment of GMS media and goethite suspension. Constant pH was maintained using a pH/ORP controller monitoring an epoxy body pH Triode. Two single-channel peristaltic tubing pumps, actuated by high or low pH setpoint solenoids in the controller, periodically added small volumes of 0.1 N HCl or 0.1 N NaOH to the chemostat in response to minor pH fluctuations. The mathematics of chemostat operation are described in Appendix A.1.

4.3. Glucose-Mineral-Salts Media

Glucose was selected as a carbon and energy source because it can be utilized by most aerobic microorganisms. In addition, glucose does not interact with mineral surfaces to an appreciable extent, and therefore does not compete with P_i for surface adsorption sites. A pH of 4.5 was selected to approximate natural soil pH (~4.2), and to inhibit use of NH_4^+ as an energy source through nitrification (inhibited at pH < 5.5). The mineral salts composition of GMS media is listed in Table 1. In order to replace the natural organic and inorganic nutrient components of an actual soil matrix, vitamin and SL-8 trace-mineral solutions (Atlas, 1995) were added to support growth of fastidious microbial species.

4.4. Analytical Methods

Orthophosphate was measured spectrophotometrically using the molybdate-blue method (APHA et al., 1995). Microbial P_i utilization was determined as the difference between influent P_i concentration and acid hydrolyzable P_i in chemostat effluent. Goethite in the chemostat effluent was reductively dissolved by mild heating at acidic pH with added ascorbic acid prior to determination of acid hydrolyzable P_i. XPS analysis of phosphate loaded goethite surfaces was performed using a Perkin-Elmer 5400 X-ray photoelectron spectrophotometer. Glucose concentration was measured using a commercially available Glucose HK (Hexokinase) enzymatic assay kit (Sigma® Kit GAHK-20). Dissolved oxygen concentration was measured and found to be at saturation in the bulk chemostat effluent. Soluble iron was measured using both graphite furnace atomic emission spectrometry (AES) and ferrozine spectrophotometry (Gibbs, 1978). The concentrations of inorganic cations (Na^+, NH_4^+,K^+, Mg^{2+}, Ca^{2+}) and anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) were determined by high-pressure ion chromatography (HPIC). The concentrations of low molecular-weight organic acids were measured using high-pressure ion exclusion chromatography (HPIEC). Microbe-mineral aggregates in chemostat effluent were imaged using scanning laser confocal microscopy (SLCM) with acridine orange as a fluorescent biological probe.

4.5. Chemostat Inputs, Operation, and Monitoring

The inoculum used to seed the chemostat consisted of the microbial community present in an undisturbed acidic mineral soil. A dilute slurry was prepared by placing 1 g of well dispersed soil in 100 mL of mineral salts media and gently agitating the mixture for 1 h. Ten mL of the dilute slurry were added to the chemostat, and the selective pressure of the nutrient and physical environments imposed by chemostat operation applied for 45 d. GMS media and goethite slurry were added at equal rates to give a 4-d hydraulic retention time. Growth in chemostat culture under these conditions was expected to result in selective enrichment of those microbial species that were most competitive under the low-pH, low-carbon, mineral-sorbed P_i conditions characteristic of the acidic mineral soil used to prepare the initial

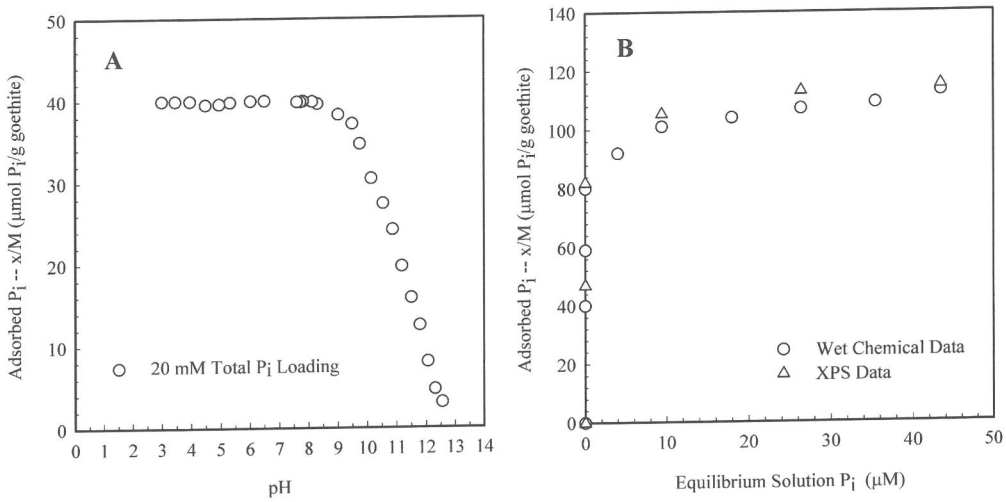

Fig. 5. Equilibrium adsorption of P_i on goethite described by adsorption edge and adsorption isotherm behavior (0-100 µM total P_i, 0.5 g/L goethite, and 0.01 M KCl as background electrolyte). Panel A: Typical adsorption edge for 20 µM total P_i concentration. Sorbed P_i (x/M – µM P_i/g goethite) as a function of pH was determined by both indirect wet-chemical and direct XPS techniques. Indirect wet-chemical measurement of sorbed P_i was made by difference between the total initial P_i concentration of the goethite suspension and the residual P_i concentrations of filtered sub-samples collected at several pH values. XPS peak areas measured from dried goethite suspensions of varying initial pH were directly correlated to sorbed P_i (data not shown). The effect of slight variations in source-sample-detector geometry on measured P_i peak areas was accounted for by using the Fe_{3S} peak area of each sample as an instrumental correction factor. Panel B: Adsorption isotherm data generated from adsorption edge data, pH 6.3. Sorbed P_i (x/M) plotted as a function of equilibrium solution P_i (residual concentration in filtered sample) from both wet-chemical and XPS-based measurements.

inoculum. Phosphorus loading was 40 µmol P_i/g goethite (~ 6 to 12 % surface area coverage for monodentate and bidentate complexes, respectively) and influent glucose concentration was 0.56 mM. After operation for 14 d, influent glucose concentration was increased to 2.78 mM. Effluent samples were collected daily and analyzed for glucose, residual P_i, anions and cations, soluble iron (< 0.2 µm), and organic ligand production.

4.6. Chemostat Effluent Parameters

After a short acclimation period (~ 2 d) in which residual sorbed P_i dropped rapidly, microbial utilization of goethite sorbed P_i averaged 82 ± 6 % throughout the remainder of the experiment. Measurements of inorganic ion concentrations showed that flow rates from the GMS media and goethite reservoirs were balanced and mixing in the chemostat was adequate to preclude hydraulic short-circuiting. A low molecular-weight organic acid (LMWOA – tentatively identified by HPIEC retention time as either citric or gluconic acid) was detected in filtered chemostat effluent. LMWOA production appeared related to carbon availability, increasing from ~ 4 µM at an influent glucose concentration of 0.56 mM to ~ 100 µM at an influent glucose concentration of 2.78 mM. Soluble iron was detected for the duration of the experiment, ranging between 1 and 3 times the solubility limit of amorphous $Fe(OH)_3$ (0.11 µM at pH 4.5).

Formation of colloidal microbe-goethite aggregates was observed, and also appeared related to carbon availability. Aggregates predominated in the chemostat at the low influent glucose level, while at the high-glucose level microbes and goethite were characteristically well dispersed. Soluble polysaccharide levels as high as 20 mg/L were noted under aggregate-forming conditions. The aggregates appeared loosely associated, as brief sonication (30 s) dispersed the microbes and goethite colloids. However, re-association of microbes and goethite was observed within minutes following sonification. A typical microbe-goethite aggregate is illustrated in Fig. 6.

Microscopic examination and Gram staining of chemostat effluent showed that bacteria with motile Gram-negative rod-shaped morphology predominat-

Table 1. GMS media mineral salts composition (1X).

Compound	Concentration (M)
NaCl	4.28×10^{-3}
KCl	4.02×10^{-3}
NH_4Cl	1.08×10^{-3}
$MgSO_4{\cdot}7H_2O$	6.49×10^{-5}
$CaCl_2{\cdot}2H_2O$	6.80×10^{-5}

ed, although small numbers of bacteria with other morphologies were observed. Fatty acid profile and 16S rDNA analyses (Midi Labs, Newark, DE) both indicated that the dominant Gram-negative species was *Burkholderia cepacia*. No yeast cells or filamentous fungi were observed. Evidence of approach to steady-state chemostat operation was seen at both influent glucose concentrations used in this study. At the initial influent glucose level of 0.56 mM the culturable cell population was $6.0 \pm 0.8\times10^5$ CFU/mL, LMWOA concentration was 4 µM, and effluent glucose concentration was 1.5 µM; whereas at the subsequent influent glucose level of 2.78 mM, these effluent parameters were $1.2 \pm 0.2\times10^8$ CFU/mL, 105 µM, and 140 µM, respectively.

4.7. Thermodynamics of Microbial P_i Uptake

In accordance with Le Châtelier's principle, P_i uptake from solution by microbes results in desorption from the mineral surface until a new equilibrium condition is established. In a chemostat operating at steady state, and assuming rapid exchange kinetics at the surface-solution interface, the net effect of microbial phosphate uptake is to shift the position of surface complexation equilibrium toward the solution phase. The extent to which phosphate desorbs from goethite surfaces in response to microbial uptake from solution may ultimately depend on the energetics of transmembrane phosphate transport against a very unfavorable concentration gradient or the availability of phosphate at the membrane surface. Simply stated, either the thermodynamics or kinetics of membrane transport may control the influence of solution phase uptake on P_i-mineral surface equilibrium.

Due to the very high equilibrium constant for P_i complexation on goethite surfaces ($\log K_{int} \geq 22$, Sigg and Stumm, 1981), when a goethite suspension is loaded with phosphate at less than the total surface sorptive capacity, the solution phase phosphate concentration is extremely low. For example, at the phosphate loading of 40 µmol P_i/g goethite used in our bioavailability study, the observed solution phase P_i concentration was below the 0.1 µM limit of spectrophotometric detection. In fact, the equilibrium solution concentration for the total P_i loading used in chemostat experiments (40 µmol P_i/g goethite in GMS media at pH 4.5) is predicted to be approximately 0.1 to 1 nM, based on numerical simulation using MINTEQA2 (Allison et al., 1991) and K_{int} values available in the literature (Sigg and Stumm, 1981). Direct measurement of P_i concentrations ≤ 1 nM are problematic, even using sensitive radiolabeled ^{32}P methods. Therefore, we must at present rely on predictions made using surface complexation modeling of sorption data to estimate solution phase P_i.

Microorganisms must expend energy to take up P_i against the unfavorable concentration gradient across the cytoplasmic membrane typical of most natural environments (see section *2.2.2*). Energy is also required to transport negatively charged P_i molecules into microbial cells against a negative electrical potential difference ($\Delta\Psi = \Psi_{intercellular} - \Psi_{extracellular}$) across the cytoplasmic membrane. The membrane potential $\Delta\Psi$ varies between about −60 mV and −200 mV depending on the bacterial species and growth conditions (Kashket, 1985), with an average value of about −100 mV for slowly growing or resting cells (Futai and Tsuchiya, 1987). The total electrochemical energy, expressed in terms of free energy ΔG (kJ/mol), required to transport P_i into bacterial cells is:

$$\Delta G = RT \ln \frac{C_{inter}}{C_{extra}} + ZF\Delta\Psi \qquad (15)$$

where C_{inter} and C_{extra} are intercellular and extracellular P_i concentrations, Z is anion charge, F is Faraday's constant, and $\Delta\Psi$ is membrane potential. Under conditions imposed in our bioavailability experiments (T = 298 K, $C_{inter} \approx 10$ mM, $C_{extra} \approx 1$ nM, $Z = -1$ for $H_2PO_4^-$, and $\Delta\Psi \approx -0.1$ V), from Eqn. 15 the ΔG required for P_i uptake is 49.6 kJ/mol, or 9.9×10^{-4} kJ/L for complete uptake of 20 µM P_i. Because of uncertainty in the chemical composition and ionization state of solutes in the cytoplasm, calculation of ionic strength and hence use of activities rather than concentrations in Eqn. 15 is impractical. However, due to the logarithmic nature of the concentration dependence of Eqn. 15 and the large difference in P_i concentration across the cytoplasmic membrane typical of natural systems, the error induced in ΔG calculated using concentrations is minor. For example, assuming cytoplasmic and external ionic strengths of 0.3 and 0.01 M, and using the other values in Eqn. 15 as above, changes the value of ΔG by less than 1 %.

Chemoorganotrophic microorganisms obtain energy for cell maintenance and growth by oxida-

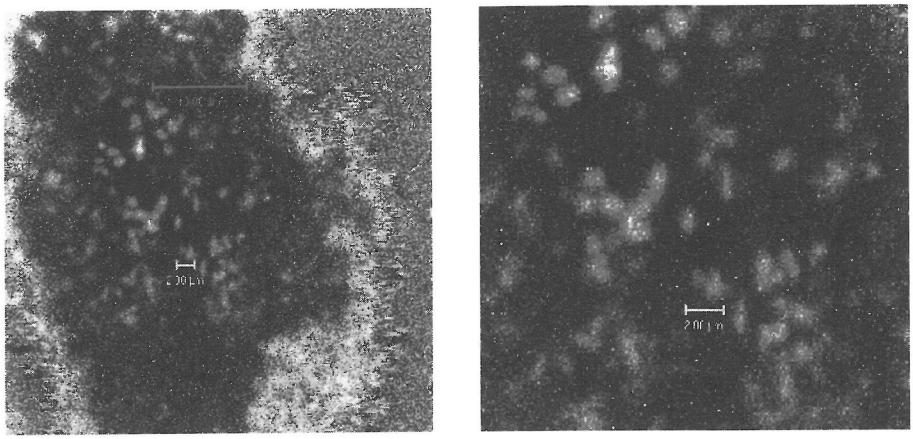

Fig. 6. Scanning laser confocal microscopy (SLCM) images of bacteria-goethite aggregates from chemostat effluent (large scale bar = 10 μm, small scale bar = 2 μm). Bacteria stained with acridine orange dye (lightly contrasted ~ 1-2 μm rod-shaped objects) can be seen in close proximity to colloidal goethite particles (dark regions) within aggregates. These images were collected using a Zeiss LSM-510 SLCM with a 40X, 1.2 n.a. water immersion lens.

tion of organic carbon compounds coupled to formation of high energy phosphoanhydride bonds in ATP. For aerobically respiring bacteria, complete oxidation of 1 mole of glucose to CO_2 and H_2O through the glycolytic and Krebs cycle pathways yields 38 moles of ATP. Given that hydrolysis of 1 mole of phosphoanhydride bonds in ATP gives 31 kJ of energy, the total free energy available due to microbial glucose oxidation in our experiments (0.56 to 2.78 mM glucose) is 0.65 to 3.3 kJ/L. Thus, if 9.9×10^{-4} kJ/L of free energy is thermodynamically required for complete P_i uptake as calculated above, ATP-dependent enzymatically-mediated P_i uptake by the Pst system would require less than 1 % of the energy potentially available by glucose oxidation. Because microorganisms typically spend a large fraction of their energy resources to support ATP-driven solute transport (Voet and Voet, 1995) (30 to 50 % is not uncommon), it appears that transmembrane P_i transport was not thermodynamically-limited in our experiments.

5. MICROBIAL RESPONSES TO P_i LIMITATION

Microbes respond to environmental stimuli such as P_i limitation through alternative patterns of gene expression, which regulate cellular physiology and metabolism. These physiological responses may be grouped into two fundamental categories: passive responses in which the effects of modulated cellular physiology responsible for adaptation to low solution P_i concentrations are confined to the cell itself, or active responses in which cell surface physiology or an exported metabolic product physicochemically alters the surrounding environment to enhance bioavailability of adsorbed P_i. A number of potential passive and active microbial responses to P_i limitation are described in the following sections, and evaluated with respect to both the chemostat experiments presented here and previous observations in the literature.

5.1. Passive Responses

5.1.1. Alteration of cell envelope to enhance P_i uptake

Under P_i-limiting conditions, i.e., several μM or less depending on the species, an enhanced P_i uptake system is commonly induced. In Gram-negative bacteria, these enhanced transport systems are composed of a porin trimer protein channel that spans the outer membrane and a P_i specific transporter protein complex that spans the cytoplasmic membrane. The physicochemical and kinetic properties of P_i transport-related porins and cytoplasmic membrane protein channels for several bacterial species are listed in Tables A.2.1 and A.2.2.

The feasibility of transport system modulation (induction of a high-affinity Pst-like P_i transport system – Fig. 3a) as the sole strategy for enhancing microbial uptake of P_i adsorbed on colloidal goethite

surfaces was evaluated by comparing the cell generation time g_{Pi} (h) estimated for P_i uptake limited by cell envelope transport with the chemostat MCRT: if g_{Pi} is greater than MCRT (96 h) then modulated uptake is not sufficient to support steady-state chemostat culture. The resistance to P_i transport through each cell envelope compartment was calculated using representative cell envelope data for Gram-negative bacteria (Tables A.2.1 and A.2.2, and Sections *3.2.1* through *3.2.4*), and values for the individual compartment and total cell envelope P_i transport resistances given in Table 2. Assuming a bulk solution phase P_i concentration of 1 nM, from Eqn. 14 the growth-limiting P_i uptake rate J is 4.9×10^{-23} mol/cell·sec. The amount of P_i required by a single representative Gram-negative cell, estimated using an average dry weight of 2.8×10^{-13} g/cell (Neidhardt et al., 1990) and an average phosphorus content of 2 percent (w/w) (Bowen, 1979), is 1.8×10^{-16} mol/cell. Dividing the phosphate content per cell by the limiting P_i uptake rate J gives a cell generation time g_{Pi} of 1020 h.

Although representative cell envelope parameters were selected in a conservative manner, i.e., to lower the calculated P_i uptake-limited cell generation time g_{Pi} , the time required for steady-state cell growth based on P_i uptake rate limitation exceeded the available chemostat residence time by an order of magnitude ($g_{Pi} \cong 10\times$MCRT). Thus, based on the kinetics of P_i uptake, it appears likely that in addition to inducing a high-affinity Pst-like transport system the microbial culture in this study utilized other as yet unspecified strategies to obtain P_i at a rate sufficient to maintain steady-state chemostat growth. Also, even though the resistance to P_i transport across the outer membrane dominates the overall cell envelope resistance (Table 2), the resistance to P_i transport across the cytoplasmic membrane is not negligible, as has been previously assumed (Koch and Wang, 1982; Koch, 1990).

5.1.2. Reduction in P_i required

In addition to the passively mediated transport strategy outlined in the previous section, which addresses P_i limitation by altering cell envelope structure to enhance P_i uptake through the cell envelope, microorganisms may also reduce their requirement for P_i by substituting non-phosphorylated molecules where possible. For instance, some bacterial species may substitute glycolipids for phospholipids in membranes when exposed to P_i limited environments (Tempest and Neijssel, 1978; Brown and Gilbert, 1993). However, the majority of cellular macromolecules require P_i as an integral component, for which there is no suitable alternative. Using *E. coli* B/r cells grown aerobically on glucose minimal media as an example, complete substitution of non-phosphorylated membrane lipids would only decrease the total cellular P_i requirement by about 12% (Table 3).

5.1.3. Reduction in cell size

Reduction in cell size has also been widely postulated as a general physiological survival response of microbes exposed to nutrient limitation in oligotrophic natural environments. It has been proposed and widely accepted that, in addition to reducing the requirement for limiting nutrients associated with less cell mass, smaller size increases the "efficiency" of microbial nutrient transport. However, we contend that smaller cell size, in and of itself, is not an effective strategy for dealing with nutrient limitation, and use P_i uptake by bacteria to illustrate this point.

Considering only the effect of size on membrane transport by bacteria, smaller size actually decreases P_i uptake rate J. Examination of Eqns. 5-8 shows that cell size (as measured by cell radius a) explicitly appears only in the expressions for diffusion to the outer-membrane surface and diffusion through the periplasmic space. Decreasing a increases the resistance to diffusive mass transport in both the cell boundary layer and periplasmic space (R_{BL} and R_{PS} terms), which by Eqn. 14 actually decreases rather than increases the overall P_i uptake rate J. However, as demonstrated in Table 2, the collective resistance to mass transport in the cell boundary layer and periplasmic space represents < 1% of the total resistance to P_i uptake, and any decrease in overall uptake rate J due to decreased cell size a should be minimal. Conservatively assuming that decreasing cell size does not affect transport across either cell membrane (R_{OM} and R_{CM} terms), the influence of cell size on P_i uptake rate only becomes noticeable at extremely small cell sizes, on the order of 0.1 μm, as illustrated in Fig. 7.

When bacteria encounter nutrient limitation, growth rates slow, and smaller cell sizes are commonly observed. However, reduction in cell size is not a direct physiological response to limitation of any specific nutrient, but rather, a coordinated global response to less favorable growth conditions. Nutritional conditions control the growth rate of bacteria, which in turn dictates the macromolecular composition (amounts of DNA, RNA, ribosomes, etc.) required to support that growth rate (Neidhardt et al., 1990). In fact, cellular components, including total cell mass, protein, DNA, and RNA, are individually varying exponential functions of growth rate, which

Table 2. Representative resistance to P_i transport in cell envelope compartments of Gram-negative bacteria.[a]

Near Microbe Compartment	Resistance to P_i Transport (s/nm^3)	Proportion of Total Resistance (%)
Cell Boundary Layer (R_{BL})	1.24×10^{-13}	0.6
Outer Membrane (R_{OM})	1.80×10^{-11}	88.8
Periplasmic Space (R_{PS})	1.17×10^{-15}	0.006
Cytoplasmic Membrane (R_{CM})	2.14×10^{-12}	10.6
Cell Envelope Total (R_T)	2.03×10^{-11}	100

[a]Values for Resistance to P_i transport in near microbe compartments calculated using Eqns. 4, 5, 6, and 8, respectively, and representative cell envelope data for Gram-negative bacteria listed in Tables A.2.1 and A.2.2.

forms the fundamental relationship between nutrition-imposed growth rate and cellular composition (Schaecter et al., 1958). Obviously, cell size varies in accordance with the amount of cellular machinery required to support any given growth rate. The relationship between cell mass (size) and growth rate is related to the initiation of chromosomal replication: replication is initiated when cell mass reaches a specific value M_i per chromosomal replication origin (*oriC*) (Donachie, 1968).

Reduction in growth rate, and hence cell mass and size, may reduce the requirement for P_i. For the example of *E. coli* B/r cells given in Table 3, 68% of cellular P_i is contained in nucleic acids, the quantities of which are strictly regulated with respect to growth rate. However, there are physical, chemical, and biological constraints which limit the extent to which a cell can down-regulate critical cellular components. In order to remain viable a cell must maintain some minimum set of synthetic machinery, including a complete copy of its genome and some basal level of macromolecules for protein synthesis. The physical size of a bacterial chromosome places a minimum dimension on cell size: the supercoiled nucleoid configuration of bacterial chromosomes has a species-dependent characteristic dimension of hundreds to thousands of nm. Small size also has implications with respect to solution chemistry in the cell cytoplasm (Nealson and Stahl, 1997). For example, at a cytoplasmic concentration typical of many enzymatically mediated reactions (1 μM), a spherical cell of 0.1 μm radius would contain only about 2.5 reactant molecules, whereas a cell of 0.8 mm radius would contain about 1290. The low number of solute molecules which results from small cell size presents a potential impediment to synthesis of macromolecular components and cell growth.

The cells observed in the chemostat experiments described here were characteristically small (~1 μm), but were of a size consistent with the slow growth rate imposed by the oligotrophic nutritional environment and low dilution rate of chemostat operation. The distribution of P_i among cellular components was not examined in this study, and the extent to which substitution of non-phosphorylated lipids were utilized in cell membranes is not known. However, based on the apparent inability of the high affinity Pst uptake system to transport P_i at a rate sufficient to support chemostat growth under the environment applied in our experiments, the example cellular P_i distribution of Table 3, and the insensitivity of P_i uptake rate with cell size shown in Fig. 7, we believe that passive microbial responses to P_i limitation do not adequately explain the high level of P_i bioavailability observed in this study. This suggests that the presence of bacteria altered the physicochemical environment in the chemostat resulting in enhanced P_i release from goethite surfaces.

5.2. Active Microbial Responses to P_i Limitation

5.2.1. Production of surface active biomolecules

Overproduction of several LMWOA metabolites has been observed in chemostat cultures exposed to P_i-limitation (Tempest and Neijssel, 1992). Organic acids may compete directly for sorption sites on mineral (hydr)oxides or inhibit the formation of mineral phase precipitates (Earl et al., 1979; Sibanda and Young, 1986; Schwertmann et al., 1986; Hue, 1991; Violante and Gianfreda, 1993; Geelhoed et al., 1998). Microbial excretion of LMWOA may also accelerate dissolution of (hydr)oxides by lowering pH or by chelating Al or Fe on the mineral surface (Furrer and Stumm, 1986; Zinder et al., 1986; Robert and Berthelin, 1986; Stevenson, 1994; Hersman et al., 1995; Hersman et al., 1996; Holmén and Casey, 1996; Stone, 1997; Barker et al., 1997), thereby releasing P_i from the mineral (Dalton et al., 1952; Banik and Dey, 1983; Bolan et al., 1987; Jayachandran et al., 1989). Reductive dissolution of Fe (hydr)oxides, known to occur in anaerobic environments (Willett, 1982; 1985; 1989; Suter et al., 1991; Ehrlich, 1996), may also occur in aerobic environments when chelating or reducing metabolites are present (Iyamuremye and Dick, 1996). Exopolysaccharides retain water and contain reactive functional groups which may participate in dissolution reactions (Barker et al., 1998). Extracellular enzymes such as Fe(III)-chelate reductase may enhance dissolution of Fe-oxide surfaces (Robinson et al., 1999).

Microbial utilization of P_i and P_i-containing organic compounds sorbed on non-crystalline alu-

Table 3. Distribution of P_i among cellular components of *E. coli*[a] B/r.

Cellular Component	Molecules/Cell	P_i/Molecule	Molecules P_i	% of Total
Cell Envelope				
Phospholipid[b]	2.2×10^7	1	2.2×10^7	12.1
Lipopolysaccharide[c]	1.4×10^6	6	8.6×10^6	4.7
Cytoplasm[d]	1.3×10^7	n.a.	1.3×10^7	7.1
Nucleic Acids				
DNA[e]	2.13 (4.7×10^6 bp)	2/bp	2.0×10^7	11.0
RNA[f]				
rRNA	18700	4460 (1/nucleotide)	8.3×10^7	46.0
tRNA	198000	80 (1/nucleotide)	1.6×10^7	9.1
mRNA	1380	2000 (1/nucleotide)	2.8×10^6	1.5
Miscellaneous[g]			1.6×10^7	8.7

[a] In balanced growth at 37°C in aerobic glucose minimal medium with a doubling time *g* of 40 min (Bremmer and Dennis, 1996; Neidhardt and Umbarger, 1996). Cell mass is 4.33×10^{-13} g/cell.

[b] One P_i molecule per phospholipid molecule based on 76% phosphatidylethanolamine, 20% phosphatidylglycerol, and small amounts of phosphatidylserine and cardiolipin (Neidhardt et al., 1990).

[c] Six P_i molecules per lipopolysacharride molecule (Neidhardt et al., 1990).

[d] Cytoplasmic P_i concentration of 10 mM (Shulman et al., 1979; Wanner, 1994).

[e] Considers chromosomal DNA only, not plasmid DNA.

[f] RNA nomenclature: rRNA - ribsomal RNA, tRNA - transfer RNA, mRNA - messenger RNA.

[g] Miscellaneous includes phosphorylated proteins, coenzymes, free nucleotides (ATP, GTP, etc.), and polyphosphate storage polymers.

minum-oxide precipitates, goethite, kaolinite, and montmorillonite has previously been noted in batch bioavailability experiments (Shang et al., 1996; He and Zhu, 1998). Microbial acquisition of P_i from phosphate-containing minerals has also been observed in several batch bioavailability studies. Several Gram-negative bacterial spp. have been observed to mediate the dissolution of calcium-phosphate minerals through excretion of glycolic or gluconic acid (Goldstein, 1986; 1987; Illmer and Schinner, 1992; Goldstein, 1994). Microbially-mediated release of P_i from feldspar minerals has also been noted (Rogers et al., 1998). However, P_i release from these minerals is often poorly correlated with organic acid production, and the presence of other unidentified mechanisms has been noted (Illmer and Schinner, 1992).

The presence of a LMWOA, exopolysaccharides, and soluble iron substantially in excess of pH-solubility limits in the chemostat effluent of the present study is consistent with excretion of surface active metabolites as a means of enhancing the bioavailability of P_i adsorbed on mineral surfaces. *Burkholderia cepacia* (formerly classified *Pseudomonas cepacia*) was the dominant microbe isolated and identified in the chemostat effluent. *B. cepacia* is closely related to the *Pseudomonads*, many species of which are known to produce secondary metabolites in response to various environmental stimuli (Wubolts and Witholt, 1998; Meyer and Stintzi, 1998). When exposed to P_i limitation, a number of Gram-negative bacteria, including *Klebsiella pneumoniae*, *Erwinia herbicola*, and *Pseudomonas cepacia,* are known to produce gluconic acid by direct oxidation of glucose in the periplasmic space (Tempest and Neijssel, 1992; Babu-Khan et al., 1995). However, the gene which encodes the periplasmic glucose dehydrogenase enzyme responsible for gluconic acid production is not part of the Pho regulon, and cross-regulation between the Pho regulon and gluconic acid formation has not been demonstrated. Exopolysaccharide production is also common among *Pseudomonads*, and has been observed in *P. cepacia* cultures (Sutherland, 1991; Allison and Goldsbrough, 1994).

5.2.2. Proximity to mineral surfaces

The pronounced tendency of microbe-mineral aggregate formation observed in this bioavailability study at low glucose levels suggests a possible microbial response to P_i-limitation. In the absence of excess carbon required to produce extracellular sur-

face active metabolites, alteration of cell surface composition or structure in order to induce aggregation with the P_i loaded goethite surfaces may be the least energetically expensive means of enhancing P_i bioavailability. Based on Guoy-Chapman double layer theory, it can be shown that the pH in the electrostatic double layer surrounding a colloidal mineral particle may be as much as several pH units less than that of the surrounding bulk solution (Stumm and Morgan, 1981). The higher $[H^+]$ near the colloidal surface may aid in generation/maintenance of the transmembrane proton gradient which cells use to provide energy for solute transport and ATP formation. Because both the nonspecific (Pit) and specific (Pst) membrane P_i transport systems are endergonic processes which either directly or indirectly depend on a membrane proton gradient, proximity to a colloidal mineral surface with adsorbed P_i not only reduces the mass transfer resistance to the cell surface, but also potentially reduces the energetic expense of transport across the cell envelope.

Aggregation of mineral colloids may also increase the bioavailability of surface-associated nutrients by shifting the position of the solution-surface nutrient equilibrium towards the solution phase in accordance with Le Châtelier's principle. Increased local mineral surface concentration may increase the solution phase P_i concentration within an aggregate by several orders of magnitude. For instance, formation of a goethite aggregate with a characteristic dimension of 50 μm and 90 % free internal volume (solution phase) results in an increase in local goethite surface concentration of approximately 3 orders of magnitude over that in the bulk chemostat solution of this bioavailability study. Furthermore, P_i diffusion within aggregate pore spaces is retarded due to strong association with goethite surfaces, and any increase in solution phase P_i concentration within the aggregate is therefore dissipated extremely slowly by diffusion into the surrounding bulk solution. Analytical solutions for transient mass transport by retarded diffusion

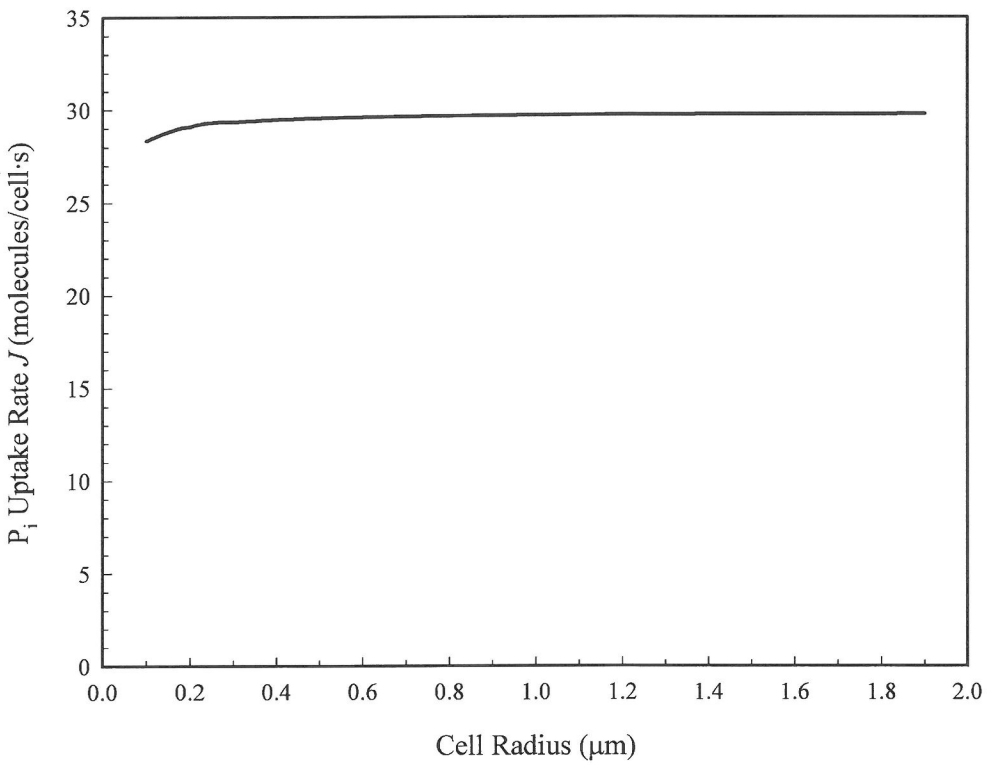

Fig. 7. Effect of cell size on P_i uptake rate by Gram-negative bacteria. P_i uptake rate J calculated using Eqn. 14 and representative P_i transport resistance values for cell envelope compartments listed in Table 2. Note: the numbers of outer membrane Pit system and cytoplasmic membrane Pst system transport protein molecules were conservatively assumed to be constant with cell size; therefore, R_{OM} and R_{CM} terms in Eqn. 14 do not vary with size.

within porous media are found in the literature (Crank, 1975; Middleman, 1998).

Several distinct microbial responses which may be involved in P_i acquisition have been observed in association with microbial adherence/attachment to surfaces, including regulation of extracellular glycoprotein, polysaccharide, and enzyme production (Deretic et al., 1989; Wrangstadh et al., 1990; Dagostino et al., 1991; Giwercman et al., 1991; Davies et al., 1993; Goodman et al., 1993). It has been speculated that microbe-mineral aggregation observed in batch P_i - amorphous Al-oxide bioavailability studies may be involved in release of sorbed P_i (Shang et al., 1996). However, the role and possible mechanisms of exopolysaccharides or other biopolymers in adherence/attachment and subsequent aggregate formation in relation to utilization of mineral-sorbed P_i remain to be elucidated.

6. CONCLUSIONS

Despite the exceptionally strong partitioning of P_i to goethite surfaces, an average of 82 % was bioavailable to a natural soil consortium during the 4-day residence time in chemostat bioreactor studies. Based on extensive experimental and mathematical characterization of the P_i-goethite surface-solution equilibrium in abiotic environments, the release of P_i in our steady-state chemostat experiments appeared clearly related to the presence of a microbial community. This result is also supported by thermodynamic calculations that suggest that sufficient energy was available to these microbes to drive transmembrane P_i transport (i.e., from oxidation of glucose to fuel ATP formation). Further, kinetic calculations of P_i transport from the bulk solution through the cell envelope suggest that the microbes in our chemostat system must actively alter their environment in order to obtain enough P_i to maintain steady chemostat growth. Although, as discussed above, there are a number of possible mechanisms by which this may be accomplished, we have observed formation of microbe-mineral aggregates at low influent glucose concentrations and the production of a competitive ligand (LMWOA) that can enhance P_i desorption at higher influent glucose levels. However, other possible means of microbially-mediated P_i release cannot be excluded based on this study, and the specific roles and interrelationships of these mechanisms remain to be elucidated.

The goethite surface-solution P_i equilibrium which exists in an abiotic setting is disturbed by microbial P_i uptake, and in accordance with Le Châtelier's principle, a different equilibrium condition is established during steady-state chemostat operation. Given the central role of solution concentration in determining the bioavailability of P_i in the two-phase environment of this study, a generalized transport model based on series resistances of the fundamental interfacial and bulk solution transport process can be used to frame microbial acquisition of surface associated nutrients. In combination with steady-state chemostat data, the resistances-in-series model provides a useful tool for examining the presence of and interrelationships among various microbially-mediated nutrient release mechanisms. Ultimately, the generalized resistances in series model may also provide a means to address the broader environmental implications of microbial nutrient acquisition from mineral surfaces on the fate and transport of other mineral surface associated constituents such as heavy metals, agrochemicals, and industrial contaminants.

Acknowledgments—The authors thank Larry Hersman, Jeremy Fein, and an anonymous reviewer for their constructive comments and suggestions.

Editorial handling: R. Hellmann

REFERENCES

Abelson P. H. (1999) A potential phosphate crisis. *Science* **283**, 2015.

Allison D. G. and Goldsbrough M. J. (1994) Polysaccharide production in *Pseudomonas cepacia*. *J. Basic Microbiol.* **34**, 3-10.

Allison J. D., Brown D. S., and Novo-Gradac K. D. (1991) MINTEQA2/PRODEFA2: A geochemical assessment model for environmental systems [computer program], v. 3.0. DOS. Environmental Research Laboratory, ORD, US EPA, Athens, GA.

Anderson G., Williams E. G., and Moir J. O. (1974) A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. *J. Soil Sci.* **25**, 51-62.

Andreux F., Schiavon M., Munier-Lamy C., Mansour M., and Scheunert I. (1995) Factors affecting the movements, reactions, and biotransformations of xenobiotics. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 383-408.

APHA, AWWA, and WEF. (1995) Phosphorus. In *Standard Methods for the Examination of Water and Wastewater*, 19 ed. (eds. A.D. Eaton, L.S. Clesceri, and A.E. Greenberg). American Public Health Association, Washington, D.C. pp. 4-106-4-115.

Atlas R. M. (1995) *Handbook of Media for Environmental Microbiology.* CRC Press, Boca Raton.

Atlas R. M. and Bartha R. (1998) *Microbial Ecology*, 4 ed. Benjamin/Cummings, Menlo Park.

Babu-Khan S., Yeo T. C., Martin W. L., Duron M. R., Rogers R. D., and Goldstein A. H. (1995) Cloning a mineral phosphate-solublizing gene from *Pseudomonas cepacia*. *Appl. Environ. Microbiol.* **61**, 972-978.

Banik S. and Dey B. K. (1983) Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminum phosphate as a sole phosphate source. *Zbl. Mikrobiol.* **139**, 17-23.

Barber S. A. (1995) *Soil Nutrient Bioavailability: A Mechanistic Approach*, 2 ed. John Wiley & Sons, New York.

Barker W. W., Welch S. A., and Banfield J. F. (1997) Biogeochemical weathering of silicate minerals. In *Geomicrobiology: Interactions Between Microbes and Minerals* (eds. J.F. Banfield and K.H. Nealson). Reviews in Mineralogy, vol. 35, Mineralogical Society of America, Washington, D.C. pp. 391-428.

Barker W. W., Welch S. A., Chu S., and Banfield J. F. (1998) Experimental observations of the effects of bacteria on aluminosilicate weathering. *Am. Mineralogist* **83**, 1551-1563.

Barrow N. J. (1983) On the reversibility of phosphate sorption by soils. *J. Soil Sci.* **34**, 751-758.

Barrow N. J., Bowden J. W., Posner A. M., and Quirk J. P. (1980) An objective method for fitting models of anion adsorption on variable charge mineral surfaces. *Austral. J. Soil Res.* **18**, 37-47.

Benz R. (1984) Structure and selectivity of porin channels. In *Current Topics in Membranes and Transport Volume 21. Ion Channels: Molecular and Physiological Aspects* (ed. F. Bronner). Academic Press, Orlando. pp. 199-219.

Benz R. and Hancock R. E. W. (1987) Mechanism of ion transport through the anion-selective channel of the *Pseudomonas aeruginosa* outer membrane. *J. Gen. Physiol.* **89**, 275-295.

Benz R., Egli C., and Hancock R. E. W. (1993) Anion transport through the phosphate-specific opr-channel of the *Pseudomonas aeruginosa* outer membrane: effects of phosphate, di- and tribasic anions and of negatively-charged lipids. *Biochim. Biophys. Acta* **1149**, 224-230.

Bohn H. L., McNeal B. L., and O'Connor G. A. (1985) *Soil Chemistry*, 2 ed. Wiley-Interscience, New York.

Bolan N. S., Robson A. D., Barrow N. J., and Aylmore L. A. G. (1987) Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plants and Soil* **99**, 401-410.

Bollag J.-M., Myers C., Pal S., and Huang P. M. (1995) The role of abiotic and biotic catalysts in the transformation of phenolic compounds. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 299-335.

Bowen H. J. M. (1979) *Environmental Chemistry of the Elements.* Academic Press, London.

Brady N. C. and Weil R. R. (1999) Soils and chemical pollution. In *The Nature and Properties of Soils*, 12 ed. Prentice Hall, Upper Saddle River. pp. 723-758.

Bremmer H. and Dennis P. P. (1996) Modulation of chemical composition and other parameters of the cell by growth rate. In *Eschericia coli and Salmonella: Cellular and Molecular Biology*, 2 ed. (eds. F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, C.C.L. Edmund, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger). ASM Press, Washington, D.C. pp. 1553-1569.

Brown G. E. Jr. (1990) Spectroscopic studies of chemisorption reaction mechanisms at oxide-water interfaces. In *Mineral-Water Interface Geochemistry* (eds. M.F. Hochella Jr. and A.F. White). Reviews in Mineralogy, vol. 23, Minerological Society of America, Washington, D.C. pp. 309-363.

Brown M. R. W. and Gilbert P. (1993) Sensitivity of biofilms to antimicrobial agents. In *Microbial Cell Envelopes: Interactions and Biofilms* (eds. L.B. Quesnel, P. Gilbert, and P.S. Handley). Blackwell Scientific, Oxford. pp. 87-97.

Chenu C. (1995) Extracellular polysaccharides: an interface between microorganisms and soil constituents. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 217-233.

Corbridge D. E. C. (1990) *Phosphorous: An Outline of Its Chemistry, Biochemistry, and Technology*, 4 ed. Elsevier Science Publs., Amsterdam.

Cornell R. M. and Schwertmann U. (1996) *The Iron Oxides: Structure, Properties, Reactions, Occurence and Uses.* VCH, Weinheim.

Cox P. A. (1995) *The Elements on Earth: Inorganic Chemistry in the Environment.* Oxford University Press, Oxford.

Crank J. (1975) *The Mathematics of Diffusion*, 2 ed. Clarendon Press, Oxford.

CRC (1992) *Properties of Aqueous Solutions of Electrolytes* (eds. I.D. Zaytsev and G.G. Aseyev). CRC Press, Boca Raton.

Dagostino L., Goodman A. E., and Marshall K. C. (1991) Physiological responses induced in bacteria adhering to surfaces. *Biofouling* **4**, 113-119.

Dalton J. D., Russel G. C., and Sieling D. H. (1952) Effect of organic matter on phosphate availability. *Soil Sci.* **73**, 173-181.

Davies D. G., Chakrabarty A. M., and Geesey G. G. (1993) Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **59**, 1181-1186.

Davis J. A. and Kent D. B. (1990) Surface complexation modeling in aqueous geochemistry. In *Mineral-Water Interface Geochemistry* (eds. M.F. Hochella Jr. and A.F. White). Reviews in Mineralogy, vol. 23, Minerological Society of America, Washington, D.C. pp. 177-260.

Deretic V., Dikshit R., Konyecsni W. M., Chakrabarty A. M., and Misra T. K. (1989) The *alg*R gene, which regulates mucoidy in *Pseudomonas aeruginosa*, belongs to a class of environmentally responsive genes. *J. Bacteriol.* **171**, 1278-1283.

Donachie W. D. (1968) Relationship between cell size and time of initiation of DNA replication. *Nature* **219**, 1077-1079.

Earl K. D., Syers J. K., and McLaughlin J. R. (1979) Origin of the effects of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. *Soil Sci. Soc. Am. J.* **43**, 674-678.

Ehrlich H. L. (1996) *Geomicrobiology*, 3rd ed. Marcel Dekker, New York.

Fendorf S., Eick M. J., Grossl P., and Sparks D. L. (1997) Arsenate and chromate retention mechanisms on goethite. 1. Surface structure. *Environ. Sci. Technol.* **331**, 315-320.

Foster J. W. and Spector M. P. (1986) Phosphate starvation regulon of *Salmonella typhimurium*. *J. Bacteriol.* **166**, 666-669.

Friedberg I. (1977) Phosphate transport in *Micrococcus lysodeikticus*. *Biochim. Biophys. Acta* **466**, 451-460.

Frossard E., Brossard M., Hedley M. J., and Metherell A. (1995) Reactions controlling the cycling of P in soils. In *Phosphorous in the Global Environment: Transfers,*

Cycles, and Management (ed. H. Tiessen). John Wiley & Sons, Chichester. pp. 107-137.

Furrer G. and Stumm W. (1986) The coordination chemistry of weathering: I. Dissolution kinetics of δ-Al_2O_3 and BeO. *Geochim. Cosmochim. Acta* **50**, 1847-1860.

Futai M. and Tsuchiya T. (1987) Proton transport and proton-motive force in prokaryotic cells. In *Ion Transport in Prokaryotes* (eds. B.P. Rosen and S. Silver). Academic Press, San Diego. pp. 3-83.

Geelhoed J. S., Hiemstra T., and Van Riemsdijk W. H. (1998) Competitive interaction between phosphate and citrate on goethite. *Environ. Sci. Technol.* **32**, 2119-2123.

Gibbs M. M. (1978) A simple method for the rapid determination of iron in natural waters. *Wat. Res.* **13**, 295-297.

Giwercman B., Jensen E. T., Hoiby N., Kharazmi A., and Costerton J. W. (1991) Induction of β-lactamase production in *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* **35**, 1008-1010.

Goldberg S. and Sposito G. (1985) On the mechanism of specific phosphate adsorption by hydroxylated mineral surfaces: A review. *Commun. In Soil Sci. Plant Anal.* **16**, 801-821.

Goldstein A. H. (1986) Bacterial solublization of mineral phosphates: historical perspectives and future prospects. *Am. J. Altern. Ag.* **1**, 51-57.

Goldstein A. H. (1987) Molecular cloning and regulation of a mineral phosphate solublizing gene from *Erwina herbicola*. *Biotechnology* **5**, 72-74.

Goldstein A. H. (1994) Involvement of the quinoprotein glucose dehydrogenase in the solublization of exogenous mineral phosphates by Gram-negative bacteria. In *Phosphate in Microorganisms: Cellular and Molecular Biology* (eds. A. Torriani-Gorini, E. Yagil, and S. Silver). ASM Press, Washington, D.C. pp. 197-203.

Goodman A. E., Angles M. L., and Marshall K. C. (1993) Genetic response of bacteria in biofilms. In *Trends in Microbial Ecology* (eds. R. Guerrero and C. Pedrós-Alío). Spanish Society for Microbiology, Barcelona. pp. 119-122.

Guddal P. H., Johanson T., Schulstad K., and Little C. (1989) Apparant phosphate retrieval system in *Bacillus cereus*. *J. Bacteriol.* **171**, 5702-5706.

Hancock R. E. W., Poole K., and Benz R. (1982) Outer membrane protein P of *Pseudomonas aeruginosa*: regulation by phosphate deficiency and formation of small anion-specific channels in lipid bilayer membranes. *J. Bacteriol.* **150**, 730-738.

Hancock R. E., Worobec E. A., Poole K., and Benz R. (1987) Phosphate-binding site of *Pseudomonas aeruginosa* outer membrane protein P. In *Phosphate Metabolism and Cellular Regulation in Microorganisms* (eds. A. Torriani-Gorini, F.G. Rothman, S. Silver, A. Wright, and E. Yagil). American Society for Microbiology, Washington,D.C. pp. 186-190.

Harrison A. F. (1987) *Soil Organic Phosphorous. A Review of World Literature.* CAB International, Oxon.

Hawke D., Carpenter P. D., and Hunter K. A. (1989) Competitive adsorption of phosphate on goethite in marine electrolytes. *Environ. Sci. Technol.* **23**, 187-191.

Hayes K. F., Papelis C., and Leckie J. O. (1988) Modeling ionic strength effects on anion adsorption at hydrous oxide/solution interfaces. *J. Colloid Interface Sci.* **125**, 717-726.

Haynes R. J. (1982) Effects of liming on phosphate availability in acid soils. *Plant and Soil* **68**, 289-308.

He Z. and Zhu J. (1998) Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biol. Biochem.* **30**, 917-923.

Hersman L. E., Lloyd T., and Sposito G. (1995) Siderophore-promoted dissolution of hematite. *Geochim. Cosmochim. Acta* **59**, 3327-3330.

Hersman L. E., Maurice P. A., and Sposito G. (1996) Iron acquisition from hydrous Fe(III)-oxides by an aerobic *Pseudomonas* sp. *Chem. Geol.* **132**, 25-31.

Hingston F. J., Atkinson R. J., Posner A. M., and Quirk J. P. (1968) Specific adsorption of anions on goethite. *Trans. 9th. Int. Cong. Soil Sci.* **1**, 669-678.

Hingston F. J., Posner A. M., and Quirk J. P. (1972) Anion adsorption by goethite and gibbsite: I. The role of the proton in determining adsorption envelopes. *J. Soil Sci.* **23**, 177-192.

Hingston F. J., Posner A. M., and Quirk J. P. (1974) Anion adsorption by goethite and gibbsite II. Desorption of anions from hydrous oxide surfaces. *J. Soil Sci.* **25**, 16-26.

Holmén B. A. and Casey W. H. (1996) Hydroxamate ligands, surface chemistry, and the mechanism of ligand-promoted dissolution of goethite [α-FeOOH(s)]. *Geochim. Cosmochim. Acta* **60**, 4403-4416.

Hue N. V. (1991) Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Sci.* **152**, 463-471.

Illmer P. and Schinner F. (1992) Solublization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol. Biochem.* **24**, 389-395.

Iyamuremye F. and Dick R. P. (1996) Organic amendments and phosphorous sorption by soils. In *Advances in Agronomy* (ed. D.L. Sparks). Academic Press, San Diego. pp. 139-185.

Jap B. K. and Walian P. J. (1990) Biophysics of the structure and function of porins. *Quart. Rev. Biophys.* **23**, 367-403.

Jayachandran K., Schwab A. P., and Hetrick B. A. D. (1989) Mycorrhizal mediation of phosphorous availability: synthetic iron chelate effects on phosphorous solubilization. *Soil Sci. Soc. Am. J.* **53**, 1701-1706.

Kashket E. R. (1985) The proton motive force in bacteria: a critical assessment of methods. *Ann. Rev. Microbiol.* **39**, 219-242.

Koch A. L. (1990) Diffusion. The crucial process in many aspects of the biology of bacteria. In *Advances in Microbial Ecology. Vol. 11* (ed. K.C. Marshall). Plenum Press, New York. pp. 37-70.

Koch A. L. and Wang C. H. (1982) How close to the theoretical diffusion limit do bacterial uptake systems function? *Arch. Microbiol.* **131**, 35-42.

Kreusch A., Neubüser A., Schlitz E., Weckesser J., and Schulz G. E. (1994) Structure of the membrane channel porin from *Rhodopseudomonas blastica* at 2 Å resolution. *Protein Sci.* **3**, 58-63.

Krueger R. D., Harper S. H., Campbell J. W., and Fahrney D. E. (1986) Kinetics of phosphate uptake, growth, and accumulation of cyclic diphosphoglycerate in a phosphate-limited continuous culture of *Methanobacterium thermoautotrophicum*. *J. Bacteriol.* **167**, 49-56.

Kummert R. and Stumm W. (1980) The surface complexation of organic acids on hydrous γ-Al_2O_3. *J. Colloid Interface Sci.* **75**, 373-385.

Kung K. S. and McBride M. B. (1991) Bonding of chlorophenols on iron and aluminum oxides. *Environ. Sci. Technol.* **25**, 702-709.

Lacoste A.-M., Cassaigne A., and Neuzil E. (1981) Transport of inorganic phosphate in *Pseudomonas Aeruginosa*. *Curr. Microbiol.* **6**, 115-120.

Lajtha K. and Harrison A. F. (1995) Strategies of phosphorous acquisition and conservation by plant species and

communities. In *Phosphorous in the Global Environment: Transfers, Cycles, and Management* (ed. H. Tiessen). John Wiley & Sons, Chichester. pp. 139-147.

Lindsay W. L. (1979) Phosphates. In *Chemical Equilibria in Soils.* John Wiley & Sons, New York. pp. 162-209.

Liu W. and Hulett M. (1997) *Bacillus subtilis* PhoP binds to the *phoB* tandem promoter exclusively within the phosphate starvation-inducible promoter. *J. Bacteriol.* **179**, 6302-6310.

Lonergan D. J. and Lovley D. R. (1991) Microbial oxidation of natural and anthropogenic aromatic compounds coupled to Fe(III) reduction. In *Organic Substances and Sediments in Water: Humics and Soil* (ed. R.A. Baker). Lewis Publishers, Inc., Chelsea. pp. 327-338.

Martin R. R. and Smart R. St. C. (1987) X-ray photoelectron studies of anion adsorption on goethite. *Soil Sci. Soc. Am. J.* **51**, 54-56.

Martin R. R., Smart R. St. C., and Tazaki K. (1988) Direct observation of phosphate precipitation in the goethite/phosphate system. *Soil Sci. Soc. Am. J.* **52**, 1492-1500.

McBride M. B. (1994) Chemisorption and precipitation of inorganic ions. In *Environmental Chemistry of Soils.* Oxford University Press, New York. pp. 121-168.

Meyer J.-M. and Stintzi A. (1998) Iron metabolism and siderophores in *Pseudomonas* and related species. In *Pseudomonas* (ed. T.C. Montie). Plenum Press, New York. pp. 201-243.

Middleman S. (1998) *An Introduction to Heat and Mass Transfer: Principles of Analysis and Design.* John Wiley & Sons, New York.

Mott C. J. B. (1981) Anion and ligand exchange. In *Chemistry of Soil Processes* (eds. D.J. Greenland and M.H.B. Hayes). John Wiley & Sons, Inc., New York. pp. 179-219.

Nealson K. H. and Stahl D. A. (1997) Microorganisms and biogeochemical cycles: what can we learn from layered microbial communities. In *Geomicrobiology: Interactions Between Microbes and Minerals* (eds. J.F. Banfield and K.H. Nealson). Reviews in Mineralogy, vol. 35, Mineralogical Society of America, Washington, D.C. pp. 5-34.

Neidhardt F. C., Ingraham J. L., and Schaechter M. (1990) *Physiology of the Bacterial Cell: A Molecular Approach.* Sinauer Associates, Sunderland.

Neidhardt F. C. and Umbarger H. E. (1996) Chemical composition of *Eschericia coli.* In *Eschericia coli and Salmonella: Cellular and Molecular Biology*, 2 ed. (eds. F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, C.C.L. Edmund, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger). ASM Press, Washington, D.C. pp. 13-16.

Nikaido H. (1996) Outer membrane. In *Eschericia coli and Salmonella: Cellular and Molecular Biology*, 2 ed., (eds. F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, C.C.L. Edmund, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger). ASM Press, Washington, D.C. pp. 29-47.

Nikaido H. and Rosenberg E. Y. (1983) Porin channels in *Escherica coli*: studies with liposomes reconstituted from purified proteins. *J. Bacteriol.* **153**, 241-252.

Parfitt R. L., Atkinson R. J., and Smart R. St. C. (1975) The mechanism of phosphate fixation by iron oxides. *Soil Sci. Am. Proc.* **39**, 837-841.

Parkinson J. S. and Kofoid E. C. (1992) Communication modules in bacterial signaling proteins. *Ann. Rev. Genet.* **26** , 71-112.

Paul E. A. and Clark F. E. (1996) *Soil Microbiology and Biochemistry*, 2 ed. Academic Press, San Diego.

Pierzynski G. M., Sims J. T., and Vance G. F. (1994) *Soils and Environmental Quality.* Lewis Publ., Boca Raton.

Poole K. and Hancock R. E. W. (1984) Phosphate transport in *Pseudomonas aeruginosa*. involvement of a periplasmic phosphate-binding protein. *Eur. J. Biochem.* **144**, 607-612.

Qi Y., Kobayashi Y., and Hulett F. M. (1997) The *pst* operon of *Bacillus subtilis* has a phosphate-regulated promoter and is involved in phosphate transport but not in regulation of the pho regulon. *J. Bacteriol.* **179**, 2534-2539.

Rao P. S. C., Bellin C. A., and Lee L. S. (1995) Sorption and biodegradation of organic contaminants in soils: conceptual representations of process coupling. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 263-274.

Ristori G. G. and Fusi P. (1995) Adsorption mechanisms and abiotic catalytic transformations of some agrochemicals by clay minerals. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 337-343.

Robert M. and Berthelin J. (1986) Role of biological and biochemical factors in soil mineral weathering. In *Interactions of Soil Minerals with Natural Organics and Microbes* (eds. P.M. Huang and M. Schnitzer). Soil Science Society of America, Madison. pp. 453-495.

Robinson N. J., Procter C. M., and Guerinot M. L. (1999) A ferric-chelate reductase for iron uptake from soils. *Nature* **397**, 694.

Rogers J. R., Bennett P. C., and Choi W. J. (1998) Feldspar as a source of nutrients for microorganisms. *Am. Miner.* **83**, 1532-1540.

Ronson C. W., Nixon B. T., and Ausbel F. M. (1987) Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. *Cell* **49**, 579-581.

Rosenberg H. (1987) Phosphate transport in prokaryotes. In *Ion Transport in Prokaryotes* (eds. B.P. Rosen and S. Silver). Academic Press, San Diego. pp. 205-248.

Rosenberg H., Gerdes R. G., and Chegwidden K. (1977) Two systems for the uptake of phosphate in *Eschericia coli. J. Bacteriol.* **131**, 505-511.

Runge-Metzger A. (1995) Closing the cycle: obstacles to efficient P management for improved global food security. In *Phosphorous in the Global Environment: Transfers, Cycles, and Management* (ed. H. Tiessen). John Wiley & Sons, Chichester. pp. 27-42.

Ryden J. C., McLaughlin J. R., and Syers J. K. (1977) Mechanisms of phosphate sorption by soils and hydrous ferric oxide gel. *J. Soil Sci.* **8**, 72-92.

Sanyal S. K. and De Datta S. K. (1991) Chemistry of phosphorous transformations in soils. In *Advances in Soil Science* (ed. B.A. Stewart). Springer-Verlag, New York. pp. 1-120.

Scanlan D. J., Silman N. J., Donald K. M., Wilson W. H., Carr N. G., Joint I., and Mann N. H. (1997) An immunological approach to detect phosphate stress in populations and single cells of photosynthetic picoplankton. *Appl. Environ. Microbiol.* **63**, 2411-2420.

Schaecter M., Maaløe O., and Kjeldgaard N. O. (1958) Dependency on medium and temperature of cell size and composition during balanced growth of *Salmonella typhimurium. J. Gen. Microbiol.* **19**, 592-606.

Schwertmann U. and Cornell R. M. (1988) Some properties of soil and synthetic iron oxides. In *Iron in Soils and Clay Minerals* (eds. J.W. Stucki, B.A. Goodman, and U. Schwertmann). Reidel Pub. Co., Dordrecht. pp. 203-244.

Schwertmann U. and Cornell R. M. (1991) Goethite. In *Iron Oxides in the Laboratory: Preparation and Characterization.* VCH, Weinheim. pp. 61-80.

Schwertmann U. and Taylor R. M. (1990) Iron oxides. In *Minerals in Soil Environments* (ed. D.A. Weed). Soil Science Society of America, Madison. pp. 379-439.

Schwertmann U., Kodama H., and Fischer W. R. (1986) Mutual interactions between organics and iron oxides. In *Interactions of Soil Minerals with Natural Organics and Microbes* (eds. P.M. Huang and M. Schnitzer). Soil Science Society of America, Madison. pp. 223-250.

Senesi N. and Loffredo E. (1999) The chemistry of soil organic matter. In *Soil Physical Chemistry*, 2 ed. (ed. D.L. Sparks). CRC Press, Boca Raton. pp. 239-370.

Shang C., Caldwell D. E., Stewart J. W. B., Tiessen H., and Huang P. M. (1996) Bioavailability of organic and inorganic phosphates adsorbed on short-range ordered aluminum precipitate. *Microb. Ecol.* **31**, 29-39.

Shulman R. G., Brown T. R., Ugurbil K., Ogawa S., Cohen S. M., and den Hollander J. A. (1979) Cellular applications of ^{31}P and ^{13}C nuclear magnetic resonance. *Science* **205**, 160-166.

Shvets V. I., Stepanov A. E., Schmitt L., Spiess B., and Schlewer G. (1991) Synthesis and complexation properties of inositol-phosphates. In *Inositol Phosphates and Derivatives: Synthesis, Biochemistry, and Theraputic Potential* (ed. A.B. Reitz). American Chemical Society, Washington, D.C. pp. 155-171.

Sibanda H. M. and Young S. D. (1986) Competitive adsorption of humus acids and phosphate on goethite, gibbsite, and two tropical soils. *J. Soil Sci.* **32**, 67-74.

Sigg L. and Stumm W. (1981) The interactions of anions and weak acids with the hydrous goethite (α-FeOOH) surface. *Colloids and Surfaces* **2**, 101-117.

Smith H. L. and Waltman P. (1995) *The Theory of the Chemostat: Dynamics of Microbial Competition.* Cambridge University Press, Cambridge.

Stevenson F. J. (1994) *Humus Chemistry: Genesis, Composition, and Reactions*, 2 ed. John Wiley & Sons, New York.

Stewart J. W. B. and McKercher R. B. (1982) Phosphorous cycle. In *Experimental Microbial Ecology* (eds. R.G. Burns and J.H. Slater). Blackwell, Oxford. pp. 229-238.

Stock J. B., Stock A. M., and Mottonen J. M. (1990) Signal transduction in bacteria. *Nature* **344**, 395-400.

Stone A. T. (1997) Reactions of extracellular organic ligands with dissolved metal ions and mineral surfaces. In *Geomicrobiology: Interactions Between Microbes and Minerals* (eds. J.F. Banfield and K.H. Nealson). Reviews in Mineralogy, vol. 35, Mineralogical Society of America, Washington, D.C. pp. 309-344.

Stone A. T. and Torrents A. (1995) The role of dissolved metals and metal-containing surfaces in catalyzing the hydrolysis of organic compounds. In *Environmental Impact of Soil Component Interactions: Natural and Anthropogenic Organics* (eds. P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill, and A.L. Page). CRC Press, Boca Raton. pp. 275-298.

Stotzky G. (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In *Interactions of Soil Minerals with Natural Organics and Microbes* (eds. P.M. Huang and M. Schnitzer). Soil Science Society of America, Madison. pp. 305-428.

Stumm W. and Morgan J. J. (1981) *Aquatic Chemistry*, 2 ed. John Wiley & Sons, New York.

Sugawara E. and Nikaido H. (1992) Pore-forming activity of OmpA protein of *Eschericia coli. J. Biol. Chem.* **267**, 2507-2511.

Sun X. and Doner H. E. (1996) An investigation of arsenate and arsenite bonding structures on goethite by FTIR. *Soil Sci.* **161**, 865-872.

Suter D., Banwart S., and Stumm W. (1991) Dissolution of hydrous iron(III) oxides by reductive mechanisms. *Langmuir* **7**, 809-813.

Sutherland I. W. (1991) *Biotechnology of Microbial Exopolysaccharides.* Cambridge University Press, Cambridge.

Tate R. L. I. (1995) The sulfur and related geochemical cycles. In *Soil Microbiology*. John Wiley & Sons, New York. pp. 359-387.

Taylor S. R. and McLennan S. M. (1985) *The Continental Crust: Its Composition and Evolution.* Blackwell Scientific Publ., Oxford.

Tejedor-Tejedor M. I. and Anderson M. A. (1986) "In situ" attenuated total reflection Fourier transform infrared studies of the goethite (α-FeOOH)-aqueous solution interface. *Langmuir* **2**, 203-210.

Tempest D. W. and Neijssel O. M. (1978) Eco-physiological aspects of microbial growth in aerobic nutrient limited environments. In *Advances in Microbial Ecology* (ed. M. Alexander). Plenum Press, New York. pp. 105-153.

Tempest D. W. and Neijssel O. M. (1992) Physiological and energetic aspects of bacterial metabolite overproduction. *FEMS Microbiol. Lett.* **100**, 169-176.

Torriani-Gorini A. (1994) Introduction: The Pho regulon of *Escherichia coli.* In *Phosphate in Microorganisms: Cellular and Molecular Biology* (eds. A. Torriani-Gorini, E. Yagil, and S. Silver). ASM Press, Washington, D.C. pp. 1-4.

Van Der Ley P., Bekkers A., Van Meersbergen J., and Tommassen J. (1987) A comparative study on the *phoE* genes of three enterobacterial species: implications for structure-function relationships in a pore-forming protein of the outer membrane. *Eur. J. Biochem.* **164**, 469-475.

Van Der Zee S. E. A. T. M. and Van Riemsdijk W. H. (1991) Model for the reaction kinetics of phosphate with oxides and soil. In *Interactions at the Soil Colloid-Soil Solution Interface* (ed. G.H. Bolt). Kluwer, Dordrecht. pp. 205-239.

Van Veen H. W., Abee T., Kortstee J. J., Konings W. N., and Zehnder A. J. B. (1993) Characterization of two phosphate transport systems in *Acinetobacter johnsonii* 210A. *J. Bacteriol.* **175**, 200-206.

Van Veen H. W., Abee T., Kortstee J. J., Konings W. N., and Zehnder A. J. B. (1994) Phosphate inorganic transport (Pit) system in *Escherichia coli* and *Acinetobacter johnsonii.* In *Phosphate in Microorganisms: Cellular and Molecular Biology* (eds. A. Torriani-Gorini, E. Yagil, and S. Silver). ASM Press, Washington, D.C. pp. 43-49.

Veldkamp H. (1977) Ecological studies with the chemostat. In *Advances in Microbial Ecology,* Vol. 1 (ed. M. Alexander). Plenum Press, New York. pp. 59-94.

Violante A. and Gianfreda L. (1993) Competition in adsorption between phosphate and oxalate on an aluminum hydroxide montmorillonite complex. *Soil Sci. Soc. Am. J.* **57**, 1235-1241.

Voegele R. T., Bardin S., and Finan T. M. (1997) Characterization of the *Rhizobium* (*Sinorhizobium*) *meliloti* high- and low-affinity phosphate uptake systems. *J. Bacteriol.* **179**, 7226-7232.

Voet D. and Voet J. G. (1995) *Biochemistry*, 2 ed. John Wiley & Sons, Inc., New York.

Wanner B. L. (1994) Multiple controls of the *Escherichia coli* Pho regulon by the P_i sensor PhoR, the catabolite regulatory sensor CreC, and acetyl phosphate. In *Phosphate in Microorganisms: Cellular and Molecular Biology* (eds. A. Torriani-Gorini, E. Yagil, and S. Silver). ASM Press, Washington, D.C. pp. 13-21.

Wanner B. L. (1995) Signal transduction and cross regulation in the *Escherichia coli* phosphate regulon by PhoR, CreC, and acetyl phosphate. In *Two-Component Signal Transduction* (eds. J.A. Hoch and T.J. Silhavy). ASM Press, Washington, D.C. pp. 203-221.

Wanner B. L. (1996) Phosphorous assimilation and control of the phosphate regulon. In *Eschericia coli and Salmonella: Cellular and Molecular Biology*, 2 ed. (eds. F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, C.C.L. Edmund, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger). ASM Press, Washington, D.C. pp. 1357-1381.

Wanner B. L., Jiang W., Kim S., Yamagata S., Haldimann A., and Daniels L. L. (1996) Are the multiple signal transduction pathways of the Pho regulon due to cross talk or cross regulation? In *Regulation of Gene Expression in Escherichia coli* (eds. E.C.C. Lin and A.S. Lynch). Chapman & Hall, New York. pp. 297-315.

Webb D. C. and Cox G. B. (1994) Proposed mechanism for phosphate translocation by the phosphate-specific transport (Pst) system and the role of the Pst system in phosphate regulation. In *Phosphate in Microorganisms: Cellular and Molecular Biology* (eds. A. Torriani-Gorini, E. Yagil, and S. Silver). ASM Press, Washington, D.C. pp. 37-42.

Weiss M. S. and Schulz G. E. (1992) Structure of porin refined at 1.8 Å resolution. *J. Mol. Biol.* **227**, 493-509.

White D. (1995) Growth. In *The Physiology and Biochemistry of Prokaryotes*. Oxford University Press, Oxford. pp. 34-48.

Willett I. R. (1982) Phosphorous availability in soils subjected to short periods of flooding and drying. *Aust. J. Soil Res.* **20**, 131-138.

Willett I. R. (1985) The reductive dissolution of phosphated ferrihydrate and strengite. *Aust. J. Soil Res.* **23**, 237-244.

Willett I. R. (1989) Causes and prediction of changes in extractable phosphorous during flooding. *Aust. J. Soil Res.* **27**, 45-54.

Willsky G. R. and Malamy M. H. (1980a) Characterization of two genetically separable inorganic phosphate transport systems in *Eschericia coli*. *J. Bacteriol.* **144**, 356-365.

Willsky G. R. and Malamy M. H. (1980b) Effect of arsenate on inorganic phosphate transport in *Eschericia coli*. *J. Bacteriol.* **144**, 366-374.

Wrangstadh M., Szewzyk U., Östling J., and Kjelleberg S. (1990) Starvation-specific formation of a peripheral exopolysaccharide by a marine *Pseudomonas* sp., strain S9. *Appl. Environ. Microbiol.* **56**, 2065-2072.

Wubolts M. G. and Witholt B. (1998) Selected industrial biotransformations. In *Pseudomonas* (ed. T.C. Montie). Plenum Press, New York. pp. 271-329.

Yates D. E. and Healy T. W. (1975) Mechanism of anion adsorption at the ferric and chromic oxide/water interface. *J. Colloid Interface Sci.* **52**, 222-228.

Yoshimura F., Zalman L. S., and Nikaido H. (1983) Purification and properties of *Pseudomonas aeruginosa* porin. *J. Biol. Chem.* **258**, 2308-2314.

Zinder B., Furrer G., and Stumm W. (1986) The coordination chemistry of weathering: II. Dissolution of Fe(III) oxides. *Geochim. Cosmochim. Acta* **50**, 1861-1869.

APPENDIX

A.1. Chemostat (Continuous) Growth

A microbial culture can be maintained in an unvarying physiological condition by operating as an open system in which nutrient input is constant with time and system volume is held constant by balancing the system influent and effluent flows. Under these conditions, the concentrations of all chemical and biological constituents in the culture are constant with time and the system is said to be operating at steady state (White, 1995). The dilution rate D^* (h^{-1}) of the culture due to constant inflow is given by:

$$D^* = \frac{F}{V} \qquad \text{(A.1.1)}$$

where F ($mL \cdot h^{-1}$) is the influent flow rate and V (mL) is the system volume. At steady state microbial growth rate is controlled by the dilution rate. Equating the expressions for microbial growth rate and rate of cell loss due to washout in the system effluent at steady state, given by:

$$\frac{dx}{dt} = \mu x \qquad \text{(growth)} \qquad \text{(A.1.2)}$$

and

$$\frac{dx}{dt} = D^* x \qquad \text{(washout)} \qquad \text{(A.1.3)}$$

where x is a measure of microbial population and μ (h^{-1}) is the specific growth rate constant, results in:

$$\mu = D^* \qquad \text{(A.1.4)}$$

A.2. Physicochemical and Kinetic Properties of Bacterial P_i Transport Proteins

Table A.2.1. Physicochemical properties of transport related outer membrane proteins (porins) of several Gram-negative bacterial species.

Protein	Organism	Pore Diameter (nm)	Reference
	Non-specific Outer Membrane Transport Proteins		
Omp A	*Escherichia coli*	1.1-1.2	(Sugawara and Nikaido, 1992)
Omp C	*Escherichia coli*	1.08	(Nikaido and Rosenberg, 1983)
Omp F	*Escherichia coli*	1.16	(Nikaido and Rosenberg, 1983)
Orp F	*Pseudomonas aeruginosa*	2.0	(Yoshimura et al., 1983)
Porin	*Rhodobacter capsulatus*	0.8-0.9	(Weiss and Schulz, 1992)
Porin	*Rhodopseudomonas blastica*	1.2	(Kreusch et al., 1994)
	Outer Membrane Transport Proteins Induced by P_i Limitation		
Pho E	*Escherichia coli*	1.0-1.2	(Benz, 1984; Jap and Walian, 1990)
	Salmonella typhimurium	1.4	(Benz, 1984)
Orp P	*Pseudomonas aeruginosa*	0.5-0.7	(Hancock et al., 1982; Benz and Hancock, 1987; Benz et al., 1993)

Table A.2.2. Kinetic parameters for energy dependent bacterial cytoplasmic membrane P_i transport systems.

Bacterial Species	V_{max} (nmol/mg DW·min)†	K_M (μM)	Reference
	Low Affinity Pit System Proteins		
Eschericia coli	55	38.2	(Willsky and Malamy, 1980a)
	60	25	(Rosenberg et al., 1977)
Pseudomonas aeruginosa	6.1‡	19.3	(Poole and Hancock, 1984)
	22.2	10	(Lacoste et al., 1981)
	High Affinity Pst System Proteins		
Eschericia coli	15.9	0.43	(Willsky and Malamy, 1980a)
	10.9	0.6	(Willsky and Malamy, 1980b)
	69	0.16	(Rosenberg et al., 1977)
Pseudomonas aeruginosa	3.0‡	0.46	(Poole and Hancock, 1984)
	12.5	1.1	(Lacoste et al., 1981)
Micrococcus lysodeikticus	73‡	4.3	(Friedberg, 1977)
Methanobacterium thermoaototrophicum	58	0.025	(Krueger et al., 1986)

†DW is dry weight of cell constituents

‡Based on 0.55 mg protein / mg DW (Neidhardt et al., 1990)

V_{max} is the maximum reaction rate when the enzyme is saturated by substrate

K_M is the Michaelis-Menton half-saturation constant (substrate concentration at 0.5 V_{max})