International
Fertilizer Industry
Association

IFA Technical Conference

New Orleans, Louisiana, USA
1-4 October 2000

International Fertilizer Industry Association - Secretariat: 28 rue Marbeuf - 75008 Paris - France
Tel. +33 1 53 93 05 00 - Fax +33 1 53 93 05 45/47 - ifa@fertilizer.org - www.fertilizer.org

Bioprocessing Of Rock Phosphate Ore: Essential Technical Considerations For The Development Of A Successful Commercial Technology.

Alan H. Goldstein
Alfred University, New York, USA

Le minerai de phosphate brut (RPO) peut être acidulé et efficacement traité en amenant le minerai au contact de bactéries ou de produits de fermentation bactériens (acides organiques forts) dans les conditions appropriées. Dans ce "procédé bio", les bactéries ou les acides organiques forts viennent au contact des particules de minerai et solubilisent le phosphate aboutissant au produit fini désiré, l'acide phosphorique. Dans la mesure où les produits indésirables restent en arrière dans une matrice non dissoute, le procédé peut être considéré comme bio-lessivage. Des calculs sont présentés qui indiquent que le procédé bio comporte bien plus que la simple dissolution du minerai par l'acide. En plus de l'acidulation, il est probable que, dans un bio-réacteur de contact, les bactéries forment des bio-films à la surface de la particule de minerai. Ces bio-films produisent des conditions physico-chimiques uniques qui engendrent de véritables événements bio-catalytiques qui favorisent fortement la vitesse et le rendement du bio-lessivage de Pi depuis le minerai. Une grande partie de la recherche de base essentielle sur le procédé bio est achevée si bien que la technologie du procédé bio est actuellement au stade de développement. Les constituants techniques restants nécessaires à placer le système en ligne, comportent l'engineering du processus chimique et de fermentation pour optimiser le rendement et les paramètres de production.

Summary:

Rock phosphate ore (RPO) may be acidified and efficiently processed by bringing the ore into contact with bacteria or bacterial fermentation products (strong organic acids) under the appropriate conditions. In this “bioprocess”, the bacteria or the strong organic acids contact the ore particles and solubilize the phosphate resulting in the desired end product, phosphoric acid. To the extent that undesirable materials such as radionuclides are left behind in an undissolved matrix, the process may be considered bioleaching. Calculations are presented to indicate that the bioprocess involves much more than simple acid dissolution of the ore. In addition to acidification, it is probable that, in a contact bioreactor, the bacteria form biofilms on the surface of the ore particle. These biofilms produce unique physicochemical conditions that result in true biocatalytic events that greatly enhance the rate and efficacy of bioleaching of Pi from the ore. Much of the essential basic research on the bioprocess has been completed so that the bioprocess technology is currently in the development stage. The remaining technical components required to bring a system online involve chemical and fermentation process engineering to optimize efficiency and yield parameters.

Fierer Chair of Molecular Cell Biology, and Graduate Program in Biomedical Materials Engineering Science, School of Ceramic Engineering and Materials Science.
E-mail : fgoldste@alfred.edu

Introduction:

From the process engineering standpoint, bioleaching proceeds in two stages:
Stage 1. Large-scale bioconversion of low-value carbohydrate (biomass) to glucose. This is a mature and highly developed branch of bioprocess technology. The carbohydrate (often starch) is fed to microorganisms capable of enzymatically converting it to glucose. Most glucose is used for food, or as the feedstock for ethanol production via fermentation for alcoholic beverages or fuel. Significant progress has been made in the area of solid-phase fermentation of waste biomass to glucose. These applications are large-scale and there are 'turnkey' carbohydrate conversion systems that may be quickly adapted for the bioprocessing of rock phosphate ore (RPO). Estimates from experts in the field indicate that, using current technology, glucose may be produced from waste biomass for 1/2 cent to 2 cents per pound. Further improvements in this technology may be expected over the next decade.

Stage 2. The glucose produced in Stage 1 is used as a feedstock for the bioconversion of RPO. Glucose may be mixed with RPO, and an appropriate Mineral Phosphate Solubilizing positive (MPS+) gram negative bacterium (see below for discussion of the MPS phenotype). In this type of system, the vessel in which mixing occurs is called a contact bioreactor. Many design options exist for the bioreactor depending on a number of parameters. Preliminary development work has used a stirred tank apparatus where the three components may be mixed and aerated at the appropriate temperature. Under these conditions, the bacteria simultaneously contact the ore and oxidize the glucose to powerful organic acids which, in turn, dissolve the calcium phosphate in the RPO. As discussed above, catalytic phenomena (possibly related to formation of bacterial biofilms) enhance the dissolution rate over that achieved with organic acids alone. Alternatively, the glucose may be converted to the appropriate acid by the MPS bacterium in a separate fermentation and the acid mixed with the RPO. This type of process would more closely mimic the current 'green phosphoric acid' processing technology but with the substitution of an organic acid for the currently used mineral acid (sulfuric acid).

Regardless of whether is bioprocess proceeds via a three-component bioreactor or via a fermentation-based organic acid extraction, the ultimate energy source for the bioprocess is biomass. Several advantages immediately follow from the utilization of a bioprocess:

1. There is no phosphogypsum production. The bioreactor output consists of phosphoric acid, the calcium salt of an organic acid, and insoluble matrix material. If gluconic acid is the primary acid used in the extraction, the resulting calcium gluconate has product value as an industrial chemical (detergent, retarding agent in concrete, etc.) which creates a value-added situation over the current wet process. In some acidic soils of the Southeastern United States, aluminum levels are high so that application of calcium gluconate may be useful as a soil amendment. At the very least, this material may be land-farmed with minimal impact to the soil.

2. There is apparently no soluble radioisotope production. According to workers at the DoE, no significant radioactivity is extracted from the ore matrix during the bioprocess
(Dr. Robert Rogers, USDoE, INEL, personal communication).

3. The process is 'environmentally friendly'. In the current wet process, reaction of phosphate ore with sulfuric acid at high temperatures results in almost complete dissolution of the ore. As a result, undesirable ore contaminants are released into gas streams, byproduct streams and into phosphatic products. These contaminants must then be dealt with as potential air and water environmental pollutants (Goldstein, et al, 1993).

4. The bioprocess may become less expensive than current technology because pollution abatement processing represents a substantial portion of capital and processing costs for the industry. These costs are expected to increase in the future. Also, conventional ore processing requires relatively high grade ore whereas the bioprocess can use lower grade ores currently bypassed in the mining process or rejected as tailings in the beneficiation process (Goldstein, et al, 1993).

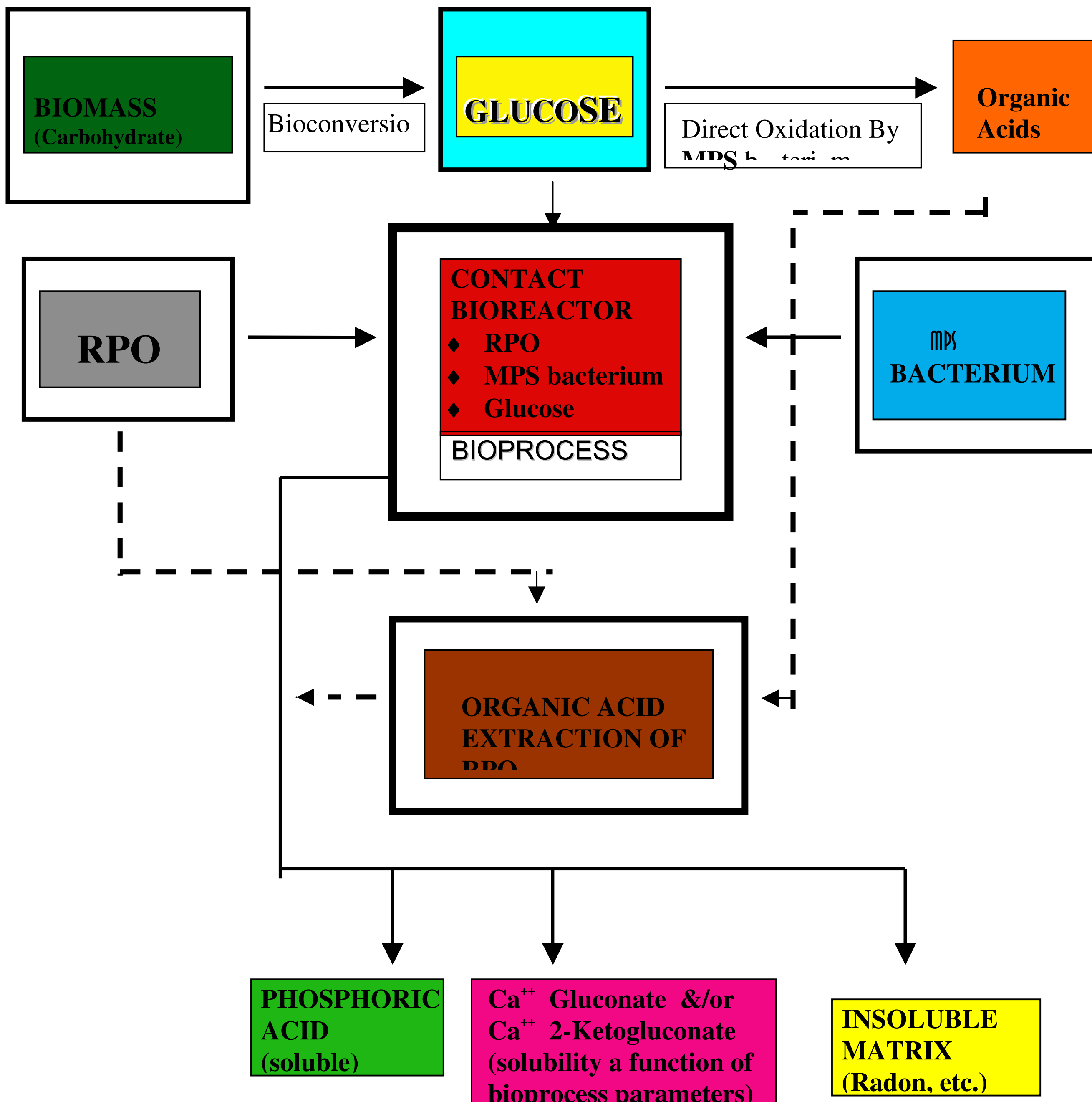


Figure 1. Schematic of the bioprocess for conversion of RPO to phosphoric acid and calcium gluconate. Specific bioprocess events are illustrated for the contact bioreactor (solid lines) or organic acid extraction (dashed lines).

Background: The world's supply of Pi comes from the mining and chemical processing of insoluble rock phosphate ore (RPO). Conventional processing of RPO into agricultural, food, and industrial products poses several technical challenges in our current processing environment. It is well known, for example, that the chemical conversion of insoluble phosphate ores, primarily fluoroapatites, is highly energy intensive. The primary phosphate industry requires nearly 0.5 quads of energy/yr for the processing of RPO which translates into roughly 4 billion dollars annually. In the wet processing of phosphate ores, energy for phosphate extraction and product concentration is supplied by sulfuric acid manufactured on site, where waste process heat can be converted to steam for product concentration. The reaction of phosphate ore with sulfuric acid at high temperatures results in almost complete dissolution of the ore. As a result, undesirable ore contaminants are released into gas streams, byproduct streams and into phosphatic products. These contaminants must then be dealt with as potential air and water environmental pollutants. Pollution abatement processing represents a substantial portion of capital and processing costs for the industry. Also, conventional ore processing requires relatively high grade ore. Substantial quantities of lower grade ore are currently bypassed in the mining process or rejected as tailings in the beneficiation process.

In contrast, bioconversion of RPO to Pi should provide an energy efficient, environmentally desirable alternative to conventional processes. Bioconversion occurs at a low temperature and is more selective to phosphate extraction than conventional processes. Increased selectivity of attack reduces the solubilization of undesirable ore contaminants. Based on data from bench scale bioprocessing tests, large-scale industrial bioprocessing systems will dramatically reduce capital and operating costs for pollution abatement and produce higher quality end products. The bioprocessing of RPO uses carbohydrate as an energy and proton source as opposed to the conventional wet process that uses sulfur, a nonrenewable resource. Finally, the bioprocessing of RPO is not as sensitive to ore quality as are conventional processes and the commercial application of this technology will allow lower grade ore bodies and tailings not presently of any value for processing to be used. Therefore, the shift to a bioprocess-based technology will greatly increase the available phosphate ore reserves.

As the cited review articles indicate (Goldstein, 1987; Goldstein et al, 1993; Goldstein, 1994), it has been known for almost 100 years that certain types of bacteria are highly efficient at dissolving calcium phosphates including RPO. Goldstein's laboratory identified the metabolic pathway by which these bacteria dissolve RPO. In collaboration with workers at the DoE, this lab has shown that use of these bacteria under specified conditions, forms a process for the extraction of phosphoric acid from RPO.

A glossary for bioprocessing: In this section, a brief glossary is provided to define the essential aspects of the bioprocess technology.

MPS: This is an acronym which stands for **M**ineral **P**hosphate **S**olubilizing. Some bacteria cannot dissolve RPO at all, others are extremely efficient. That is to say, different types of bacteria display the **MPS phenotype** to different degrees. The Goldstein laboratory has shown that bacteria with a certain type of metabolism are superior to all other bacteria with respect to

their ability to dissolve RPO. Stated another way, these bacteria exhibit the MPS phenotype to an extremely high level. These bacteria are, in fact, so superior to others that they are often referred to simply as MPS bacteria. All highly efficacious MPS bacteria produce high levels of gluconic acid and/or 2-ketogluconic acid via the direct oxidation pathway.

Gram Negative Bacteria And The Direct Oxidation Pathway: From the standpoint of the bioprocess, the important aspect is that the outer perimeter of gram negative bacteria is composed of two membranes separated by a porous gel-like material. This metabolic pathway is essential for RPO solubilization and therefore for the MPS phenotype. This pathway is also known as the 'nonphosphorylating oxidation' pathway. The direct oxidation pathway involves the enzymatic conversion of glucose to gluconic acid and/or 2-ketogluconic acid on the outer face of the inner membrane. It is important to note that acid production actually occurs in the periplasmic space. Glucose can enter and move freely through the periplasmic space. Likewise, gluconic acid and 2-ketogluconic acid can move freely out of the periplasmic space to the ore surface. Glucose dehydrogenase (GDH) is the enzyme that oxidizes glucose to gluconic acid (pK_a ~3.6). This enzyme is anchored in the inner membrane but the catalytic surface is in the periplasmic space. Because the catalytic surface of glucose dehydrogenase is in the periplasmic space and because glucose is freely diffusible from the external solution to the catalytic surface, production of gluconic acid occurs functionally at the cell surface. Gluconic acid produced from glucose oxidation will freely diffuse out of the periplasmic space and contact the RPO. This eliminates many of the problems associated with some other commercial bioprocessing systems where the substrate must be transported into and out of the cell for efficient bioactivity. Gluconic acid dehydrogenase (a.k.a. gluconate dehydrogenase; GADH) is the second enzyme in the direct oxidation pathway. GADH converts gluconic acid to 2-ketogluconic acid (pK_a ~ 2.4). Like glucose dehydrogenase, this enzyme is anchored in the inner membrane but the catalytic surface is in the periplasmic space. As a result, 2-ketogluconic acid will also freely diffuse out of the periplasmic space and make direct contact with the RPO. Gluconic acid (pK_a ~3.4) and 2-ketogluconic acid (pK_a ~ 2.6) are two of the strongest naturally occurring organic acids and the first two products in the direct oxidation pathway (Duine, 1991). In addition, both gluconic acid and 2-ketogluconic acid are capable of acting as Ca^{++} chelators under appropriate physico-chemical conditions.

A brief history of research on biosolubilization of calcium phosphates: The ability of some bacteria to dissolve poorly soluble mineral phosphates, specifically ground bone, was one of the first phenotypes to be described by agricultural microbiologists (reviewed by Goldstein, 1986). Interest in this phenotype was a natural consequence of accumulated information about the role of phosphorus in plant growth especially with respect to agricultural crop production. Because of its potential for the enhancement of crop growth and/or the development of alternative fertilizer technologies, microbial biotransformation of poorly soluble mineral phosphates to Pi has been studied intensively by agricultural microbiologists. However, until recently, little was known about the genetic and biochemical bases for the mineral phosphate solubilizing (MPS) phenotype. This discussion will be limited to dissolution of these poorly soluble calcium phosphates by gram negative bacteria where, during the past few years, significant progress in our understanding of MPS metabolism has been achieved. Table 1 shows milestones in the study of the mineral phosphate solubilizing (MPS) phenotype in gram negative bacteria.

Table 1. Milestones in the study of the mineral phosphate solubilizing (MPS) phenotype in gram negative bacteria.

Date	Worker(s)	Observation	Significance
1908	Sackett et al	36 of 50 different bacteria isolated from soil gave visible solvent action on raw bone meal and/or phosphate rock.	First report in the published Scientific literature on the MPS phenotype in bacteria
1948	Gerretsen	Development of insoluble phosphate agar plates for visualization of the MPS phenotype in bacteria.	Demonstrated a role forbacteria in enhancing the availability of P in the root zone.
1957	Sperber	Characterization of organic acids produced by MPS bacteria isolated from soils. 84 of 291 bacterial isolates showed the MPS phenotype. All MPS bacteria produced organic acids and lowered the pH of their growth medium.	First demonstration that the MPS phenotype was the result of extracellular organic acid production.
1958	Sperber	Demonstrated that MPS bacteria were present in the root zone (rhizosphere) in higher numbers and proportions than in adjacent soil.	First demonstration of a potential role for MPS bacteria in vivo.
1959	Duff and Webley	Proposed that 2-ketogluconic acid played an important role in mineral phosphate solubilization in soils.	First evidence that the direct oxidation pathway provides a metabolic basis for the MPS phenotype.
1962	Katznelson et al	Showed that MPS bacteria from the root surface (rhizoplane) of wheat were more active in glucose oxidation than either rhizosphere or soil bacteria	Further evidence to support the role of the direct oxidation pathway in the MPS phenotype.
1978	Moghimi et al	Confirmed that 2-ketogluconic acid was the major solubilizinmg compound released by rhizobacteria growing on wheat roots.	Further evidence to support the role of the direct oxidation pathwayin the MPS phenotype.
1987	Goldstein and Liu Liu	Produced a genetically engineered E. coli strain capable of dissolving hydroxyapatite by cloning and expressing a MPS gene from Erwinia herbicola .	First MPS bacteria produced by recombinant DNA technology (genetic engineering).

1992	Liu et al	Analysis via DNA sequencing showed that that the MPS gene cloned in 1987 was, in fact, a PQQ synthase gene. PQQ is the essential redox cofactor for glucose dehydrogenase, the enzyme that oxidizes glucose to gluconic acid in gram negative bacteria	First demonstration that the direct oxidation' metabolic pathway is responsible for the MPS phenotype in gram negative bacteria.
1993	Goldstein et al	First publication in the peer reviewed scientific literature showing the efficacious bioprocessing of rock phosphate ore by the gram negative bacteria Pseudomonas cepacia in bioreactor.	Preliminary demonstration of the feasibility of bio- processing of RPO
1994	Goldstein et al	A P. cepacia mutant lacking a functional glucose dehydrogenase gene lowers the pH of the medium to the same level as the wild-type but fails to show hydroxyapatite solubilization.	First conclusive proof that the direct oxidation pathway is essential for the dissolution of calcium phosphates by gram negative bacteria.
1999	Goldstein & Braverman	*Enterobacter cloacae* isolated from roots of plant growing in Mojave desert shown to require root exudate for induction of MPS activity	First direct demonstration of mutualism between MPS bacteria and plant in natural ecosystem.

The bacterial mineral phosphate solubilizing (MPS) phenotype has historically been associated with the production of low molecular weight organic acids (Goldstein, 1986). Goldstein and colleagues have identified the metabolic and genetic bases for high efficiency solubilization of poorly soluble calcium phosphates (Liu, et al, 1992). These workers have shown that solubilization is the result of acidification of the periplasmic space (and ultimately the external medium) by the direct oxidation of glucose (nonphosphorylating oxidation) or other aldose sugars by the quinoprotein glucose dehydrogenase. Glucose-derived gluconic acid often undergoes one or two additional periplasmic oxidations to 2-ketogluconic or 2,5-diketogluconic acid (Anderson et al, 1985). The enzymes of the direct oxidative pathway are oriented in the outer face of the cytoplasmic membrane so that they oxidize their substrates in the periplasmic space (Duine, 1991). The products of direct periplasmic oxidation are taken up by specific phosphotransferase or other transport systems. GDH oxidizes a broad range of aldose sugars.

Little is known about the regulation of the genes coding for quinoproteins or how quinoprotein-mediated oxidative metabolism is regulated. Nonphosphorlyating oxidation is one of the four major metabolic pathways for glucose (aldose) utilization by bacteria (Gottschalk, 1986). Most species have at least two of these pathways. The quinoprotein GDH controls the unique step in direct oxidation. GDH transfers electrons from aldose sugars to the electron transport chain via

two electron, two proton oxidations mediated by the cofactor pyrrolo-quinoline-quinone (PQQ, Duine, 1991).

The biochemical complexity of aldose utilization is, of course, the result of the interactive and dynamic nature of cell growth. Metabolic capabilities must be tuned for environmental conditions including organic and mineral nutrient availability. It is now known that periplasmic oxidation of aldose sugars contributes electrons directly to the respiratory electron transport pathway. In addition, protons generated from these oxidations contribute directly to the transmembrane PMF (Reviewed by Goldstein, 1994). A significant body of evidence exists to suggest that GDH plays a key regulatory and bioenergetic role in this aspect of energy metabolism (reviewed by Goldstein, 1994). In several bacterial species using the direct oxidation pathway, it has further been shown that uptake of solutes such as alanine, lactose and proline are directly controlled by electron transfer activity in the respiratory chain . The molecular mechanism(s) whereby periplasmic oxidation is coupled to respiratory electron transport vary between genera and even species. The biochemical or genetic regulatory mechanisms by which a given species switches between the direct phosphorylative and periplasmic oxidative mode remain unknown.

While GDH can have a bioenergetic raison d'etre in some species under certain conditions, it has been difficult to assess the utility of this pathway in terms of microbial ecology (reviewed by Goldstein, 1994). The gluconic acid phenotype is widely distributed among the gram negative genera but it has not been possible to identify a major bioenergetic or ecological advantage for this trait. For example, many pseudomonads express the direct oxidation pathway in the presence of glucose but oxidize less than 1% of the glucose present. The bioenergetic purpose of such a "dissimilatory bypass" is obscure. Conversely, some species of *Acinetobacter* (eg. *A. calcoaceticus* LMD 79.41) can stoichiometrically convert glucose to gluconic acid at concentrations of 1 mol per liter or higher but are incapable of uptake of glucose or gluconic acid for energy metabolism (reviewed by Goldstein, 1994). As will be discussed shortly, a metabolic rationale for at least some of these phenotypes can be found in the role of periplasmic (and ultimately extracellular) acidification in the dissolution of poorly soluble mineral phosphates.

The complexity of the direct oxidation phenotype is reflected in the molecular genetics of GDH and related quinoprotein dehydrogenases. There is no information on the genetic or biochemical mechanisms that regulate the synthesis or assembly of the GDH/PQQ holoenzyme. A substantial amount of data exist to show that significant differences in regulation of this system exist between bacterial genera and even species. Virtually every combinatorial form of expression is observed, i.e. constitutive apoGDH expression coupled with inducible PQQ biosynthesis vs constitutive PQQ biosynthesis coupled with inducible biosynthesis of the GDH apoenzyme. In *P. aeruginosa*, GDH is inducible by glucose, gluconate, mannitol and glycerol, whereas in *A. calcoaceticus*, the enzyme is synthesized constitutively. *A. lowffii* and *E. coli* do not show acid production in the presence of glucose without the addition of exogenous PQQ. Cell free extracts of these two bacteria also show glucose oxidation upon addition of PQQ indicating that GDH apoenzyme was produced constitutively. *A. lowffi* does not metabolize glucose at all, but nevertheless synthesizes apoGDH constitutively. It has been postulated that, for organisms such as *E. coli* and *A. lowffii*, PQQ present in the external environment may be considered nutritionally as a vitamin . The location of the GDH apoenzyme on the outer face of

the cytoplasmic membrane facilitates binding of exogenous PQQ to form the holoenzyme. Conversely, in *P. stutzeri*, PQQ can be detected only when ethanol is present in the culture medium as an inducing agent . The biosynthetic pathway for PQQ has not yet been elucidated (for details and references on the above review, see Goldstein, 1994).

While the GDH pathway may make a contribution to the energy status of some cells by generating a transmembrane proton-motive force (PMF), it seems improbable that this role can justify the stoichiometric conversion of glucose to gluconic acid at concentrations such as those discussed above. However, in Pi-limited, high calcium phosphate soil ecosystems, the GDH-mediated dissimilatory bypass system can play a vital role by increasing the availability of Pi. In fact, we have recently shown that high efficiency solubilization of RPO by *Pseudomonas cepacia* and *Erwinia herbicola* is the result of gluconic acid produced in the periplasmic space by the GDH-catalyzed direct oxidation of glucose. Initially, the GDH pathway was identified via cloning of functional mineral phosphate solubilizing (*MPS*) genes (Goldstein and Liu, 1987; Liu et al, 1992). These studies ultimately demonstrated that activation of the GDH pathway resulted in both gluconic acid production and mineral phosphate solubilization. Recent studies have confirmed that gluconic acid is, in fact, capable of mediating dissolution of RPO via direct acidification.

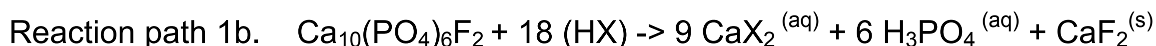
Goldstein's laboratory has recently provided a conclusive demonstration of the necessity of the direct oxidation pathway for the dissolution of calcium phosphates by gram negative bacteria. *Pseudomonas cepacia* strain 249-100 was isolated by the laboratory of Professor T.G. Lessie (U. Mass. Amhurst) and generously provided to AHG along with the wild type parent strain 249-VM. 249-100 was deleted in the glucose dehydrogenase gene and therefore did not produce gluconic acid. Both strains lowered the pH to the same degree in glucose minimal liquid medium but, when spotted onto hydroxyapatite plates, only 249-VM was capable of dissolving the calcium phosphate (data not shown).

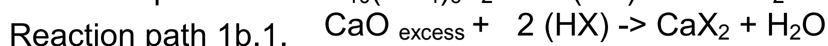
The mechanism(s) by which gram negative bacteria solubilize rock phosphate ore:

Physicochemical Considerations:

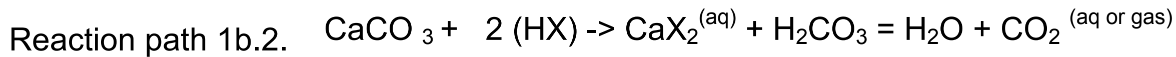
Calcium phosphate compounds have a wide range of solubilities which, in general, follow an inverse relationship with the Ca/P ratios. For example, monocalcium phosphate [$Ca(H_2PO_4)_2$, Ca/P = 0.50] has a water solubility of 150,000 ppm at pH 7. Whereas fluroapatite [$Ca_{10}(PO_4)_6F_2$ Ca/P=1.66] has a water solubility of 0.003 ppm. Poorly soluble mineral phosphates such as fluroapatite or hydroxyapatite can only be effectively dissolved in aqueous solution under acidic conditions. This dissolution is the result of acid-mediated proton substitution for calcium as shown in Equation 1 for fluroapatite and a generic acid HX that dissociates to form $H^+ + X^-$:

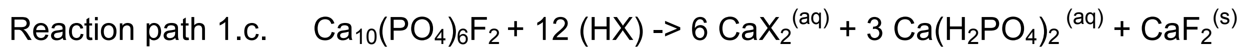
Reaction path 1a. $Ca_{10}(PO_4)_6F_2 + 20\ (HX) \rightarrow 10\ CaX_2^{(aq)} + 6\ H_3PO_4^{(aq)} + 2\ HF^{(aq)}$

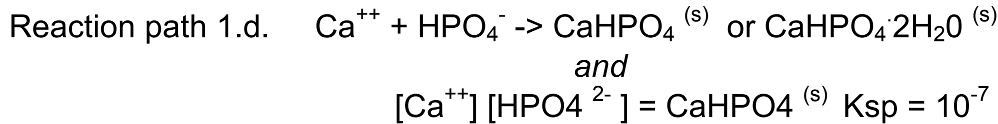

Reaction path 1b. $Ca_{10}(PO_4)_6F_2 + 18\ (HX) \rightarrow 9\ CaX_2^{(aq)} + 6\ H_3PO_4^{(aq)} + CaF_2^{(s)}$


Reaction path 1b.1. $CaO_{excess} + 2\ (HX) \rightarrow CaX_2 + H_2O$

equivalent to

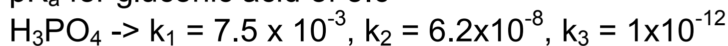

Reaction path 1b.2. $CaCO_3 + 2\ (HX) \rightarrow CaX_2^{(aq)} + H_2CO_3 = H_2O + CO_2^{\ (aq\ or\ gas)}$


Reaction path 1.c. $Ca_{10}(PO_4)_6F_2 + 12\ (HX) \rightarrow 6\ CaX_2^{(aq)} + 3\ Ca(H_2PO_4)_2^{\ (aq)} + CaF_2^{(s)}$


Reaction path 1.d. $Ca^{++} + HPO_4^{-} \rightarrow CaHPO_4^{\ (s)}$ or $CaHPO_4 \cdot 2H_20^{\ (s)}$

and

$[Ca^{++}]\ [HPO4^{\ 2-}] = CaHPO4^{\ (s)}\ \ Ksp = 10^{-7}$

As discussed above, given:
Fluroapatite Ca/P mole ratio of 1.66
pK_a for gluconic acid of 3.6

$H_3PO_4 \rightarrow k_1 = 7.5 \times 10^{-3}$, $k_2 = 6.2 \times 10^{-8}$, $k_3 = 1 \times 10^{-12}$

pH	$[H^+]$	$[Gluconate^-]/[Gluconic\ acid]$	$[H_2PO_4^-]/[H_3PO_4]$
1.0	10^{-1}	2.51×10^{-3}	7.5×10^{-2}
1.5	3.16×10^{-2}	7.94×10^{-3}	0.24
2.0	10^{-2}	2.51×10^{-2}	0.75
2.4	3.98×10^{-3}	6.30×10^{-2}	1.88
3.0	10^{-3}	0.25	7.5

Sample calculation assuming 1000 g of RPO reacts

460 g Ca   8.20 moles Ca
308 g P_2O_5 4.34 moles P

4.34 x 1.667 moles Ca = 7,23 moles Ca react via path 1.b. and 1.c. as dictated by the final pH and the ratios as shown above.

Therefore (8.20 - 7.23) = 0.97 moles of Ca reacts by path 1.b.1/1.b.2
Precipitation can occur via 1.d. when solubility product is exceeded, controlled by k_1 & k_2 of phosphoric acid and k_{sp} $CaHPO_4$

Most probable Reaction Paths	moles Ca dissolved/mole HX	moles P dissolved/mole HX
1b.	0.50	0.33
1.b.2	0.50	0.00
1c	0.75	0.50

Laboratory Simulation Data Shows That Gluconic Acid-Mediated Dissolution Alone Cannot Account For The Efficacy Of The Bioprocess:

Reaction components :

10g RPO (mined from the Vernal Reserve, < 200 mesh, 24% P_2O_5, 40.3% CaO)
60 ml of 50% (wt:wt) gluconic acid solution (Sigma Chemical # G1139)
30 ml H_2O

Step 1. Mix 30 ml water with 60 ml gluconic acid. pH 1.3
Step 2. Add 10g RPO at T_o and maintain slurry in a 125 250 ml Ehrlenmeyer flask with continuous stirring as per the contact reactor described in Goldstein et. al. (1993)
Step 3. Measure change in pH, Pi (PO_4) and Ca^{++} over time.

Time after T_o (h)	pH	PO_4 (ppm)	Ca^{++} (ppm)
0.17 (10 min)	1.9	N.D.	N.D.
0.33 (20 min)	2.0	N.D.	N.D.
1.0	2.0	N.D.	N.D.
24.0	2.3	7,726	8,539
112.0	2.3	9,896	N.D.
160.0	2.3	8,770	N.D.
240.0	2.3	8,500	N.D.

Analysis of the data:

Reactions 1b. or 1c. assumed as above
Initial molarity of gluconic acid in 90 ml solution = 1.9M
Dissociation of gluconic acid => HGA = H^+ + GA^-

At 24h: Reached apparent pH equlibrium at 2.3, with a pKa of 3.6 =>
95 millimoles GA^- / liter
At 24h: 7,726 mg/95 = 81 millimoles PO_4 /liter
At 24 h: 8,539 mg/40 = 213 millimoles Ca^{++}/liter
Mole ratio in solution = 2.63
Mole ratio in the ore = 2.13 (assuming CaO/P_2O_5 = 1.68)
24% P_2O_5 accounting for 1.317 x 24% = 31.6% of CaO
40.3% CaO

10 g RPO =>	33.82 millimoles P
	79.0 millimoles Ca-apatite
	21.75 millimoles Ca-excess
90 ml of 1.9M HGA =>	171.0 millimoles HGA

Requirements from Reaction path 1.b. and 1.b.1.

	HX required	Dissolved Ca	Dissolved P
	79 x [18/10] = 142.2	71.1	47.4
	21.75 x 2 = 43.5	21.8	0.0
Model yield (millimoles)	185.7	92.8	47.4

Requirements from reaction path 1.c. and 1.b.1.

	HX required	Dissolved Ca	Dissolved P
	79 x [12/10] = 94.8	71.1	47.4
	21.75 x 2 = 43.5	21.8	0.0
Model yield (millimoles)	138.3	92.8	47.4
Actual Yield (millimoles)		***19.2***	***7.3***

Assuming RPO is 10.6% P => 1.06 g P in 10g RPO
7.3 millimoles P = 0.27g
Direct extraction with 171 millimoles gluconic acid yields ~ 25.5% solubilization after 10 days
Contact bioreactor efficiency (Data from Goldstein et al, Figure 1)
> Throughput of 400 mL day = 4L in 10 days contact bioreactor yields ~10% solubilization of 10g RPO.
> The feedstock is 1% glucose of which 1% is converted to gluconic acid. Therefore (.01) (4L feedstock) = (.04 L glucose) (.01) = .0004 L gluconic acid.
(0.4 mL) (1.23 g/mL*) = (0.49 g gluconic acid) (1 mole gluconic acid/196.6 g) = 2.5 millimoles gluconic acid.

Yield (as dissolved P/millimoles acid)

Bioprocess => 2.86/0.25 = 1.140
Acid dissolution => 7.30/171 = 0.043
Bioprocess is ~ 30x as efficient as gluconic
acid dissolution on a 'per millimoles acid' basis.

These data indicate that acidification per se cannot provide the basis for the efficacy of biosolubilization of RPO. Even given an order of magnitude variation in actual production of gluconic vs. the level of soluble gluconic acid measured in the bioreactor lixiviant*, the amount of HX production falls far short of that required by any of the physicochemical reaction paths modeled above. Likewise, the batch experiment showed that gluconic acid alone was not a strong enough acid to dissolve more than 25.5% of the RPO even with 92% (Reaction path 1.b.)

or 124% (Reaction path 1.c.) of the HX required by the physicochemical models. 2-ketogluconic acid (pK_a of ~2.4 vs. 3.6 for gluconic acid) is commercially available but has not been used for similar simulations yet.

* As published in a subsequent erratum (BIO/TECHNOLOGY, January 1994), analysis of the lixiviant stream showed that only 1% of the 1% glucose feedstock is converted to gluconic acid. Likewise, we have made direct measurements of gluconic acid produced by *Pseudomonas cepacia* E-37 growing in shake flasks in unbuffered minimal medium containing 1% glucose (i.e. 50 mM glucose). We usually measure ~ 0.5 mM gluconic acid. 0.5/50 = 0.01% which is consistent with a 1% conversion of the 1% glucose in the medium (.01 x .01 = .0001 or .01%).

<u>The molecular mechanism(s) of biodegredation of rock phosphate ore by gram negtive bacteria</u>: As discussed in the previous section, preliminary data indicate that the highly efficacious bioconversion of rock phosphate ore to Pi involves a great deal more than simple acidification. Based on detailed analyses of and experimental work with this system, it is proposed that there are at least four additional metabolic variables of state for the biodegredation system (there are, of course, engineering variables of state for the bioreactor as well). These variables of state are not independent but, rather, form a dynamic interactive system that may be manipulated as a part of a total system design and engineering plan to optimize the yield of the bioprocess:

- ***Biofilm Formation*** It is highly probable that gram negative MPS bacteria grow as a biofilm on the surface of the ore particle. Biofilms create a unique growth environment in which microscopic domains or regions of space may exist where physicochemical conditions are extremely different from the average values measured in the bulk solution of the reactor tank.
- ***Specific Microdomain Effects Within The Biofilm*** At the interface between a bacterial biofilm and the ore particle, the physicochemical environment may be extreme. For example, while the macroscopic pH of the bioreactor is often held at ~3.4, the effective proton activity (true acidity) at the surface of the ore particle may be much stronger. This is especially true if the bacteria is expressing both enzymes of the direct oxidation pathway so that a significant percentage of the gluconic acid (pK_a~3.6) is immediately oxidized to 2-ketogluconic acid (pK_a~2.4). The electrochemical potential of protons at the ore surface may be further modified by the unique surface electrochemistry of the biofilm and the polyanionic matrix that forms the actual adhesion layer between the bacterium and the ore surface.
- ***Surface Electrochemistry & Biocatalysis At The Bacteria/Ore Particle Interface*** Theoretical studies of crystalline hydroxyapatite (HAP) dissolution have shown that, for nonstoichiometric solutions, the rate of dissolution of HAP is controlled by surface processes and depends more on the concentration of calcium than the concentration of phosphate as given by the function $[Ca]_x$ [PO4] where x may vary from 1.67 to 3.3 depending on solution conditions and stoichiometry. The initial event in solubilization is the reaction of phosphate groups with hydrogen ions to form hydrogen phosphate groups at the crystal surface. The acidity constant for this surface complex has been experimentally determined and found to depend strongly on the electrical potential difference between the crystal surface and the solution. In the bioreactor, it is probable that there is no interface between the crystal surface

and the solution but rather an interphase region between the crystal surface and the porus polyanionic matrix of the adherent gram negative bacterium in the biofilm.

- ***Calcium chelation and/or electrostatic binding*** As discussed above, the rate of dissolution of HAP depends on the actual values of both the calcium and phosphate ion activities in the phase adjacent to the crystal surface but depends more on the concentration (activity) of the calcium ions. Under nonequlibrium conditions, even a small amount of Ca^{++} chelation/binding by free organic acids <u>or</u> the polyanionic matrix would be expected to dramatically enhance the rate of dissolution. Several studies have shown that sugar acids such as 2-ketogluconic acid have multiple conformations and, therefore, may chelate cations via unusual molecular mechanisms not predicted from equlibrium binding studies carried out in bulk solutions. In addition, polyanions can be highly effective at binding Ca^{++}.

The unique physicochemical implications for the biofilm mode of growth have been recently reviewed by Costerton et al (1994). We now know that, in both natural and artificial systems, adherent (i.e. biofilm) populations generally contain many more cells than the planktonic population growing in the bulk fluid. Application of confocal scanning laser microscopy clearly shows that biofilm bacteria grow in matrix-enclosed microcolonies interspersed with less dense regions of the matrix that include highly permeable water channels. The water channels that anastomose throughout microbial biofilms provide direct high-permeability access from the bulk fluid to the colonized surface. It has further been shown that convective flow patterns are operative within the water channels.
From the standpoint of the physicochemical variables discussed above, the ultrastructure of the biofilm provides a system almost perfectly designed for the biodegredation process. Assuming extreme environmental conditions at the ore particle surface produce the solubilization products, these products will be carried into the bulk phase of the solution by convective currents within the biofilm. The polyanionic matrix in which the biofilm cells are embedded could play a direct role in the surface electrochemistry of the dissolution process. Christofferson and Christofferson (1982) have calculated the electrochemical activation energy for transport of Ca^{++} ions from the HAP surface to the solution (the rate-limiting step in HAP dissolution). According to this work, adherence of a polyanionic material would favorably affect the energetics of solvation of the Ca^{++} ions by lowering the electrical potential and therefore the free energy for Ca^{++} in the solution (actually interphase space) directly adjacent to the mineral surface. Once in solution, any chelation by gluconate, 2-ketogluconate and/or Ca^{++} binding by the polyanionic matrix would effectively decrease the activity coefficient of the solvated Ca^{++} . Likewise, microdomains of high acidity would drive the dissolution reaction since (according to these same authors) hydrogen ions catalyse the exchange of phosphates between the crystal surface and the solution.

The possibility of Ca^{++} chelation within microdomains of the biofilm cannot be ignored. Van Bekkum and coworkers (c.f. van Duin, 1989) have used oxygen-17 NMR shifts to develop a general coordination-ionization scheme for polyhydroxy carboxylic acids such as gluconic acid and 2-ketogluconic acid. Their data clearly show that both gluconic and 2-ketogluconic acid are capable of Ca^{++} coordination at low pH. This is especially true for gluconic acid where, under acidic conditions, bidentate coordination of the cation occurs via interaction with the hydroxy and carboxylic acid moieties.
Taken in combination, these two affects are equivalent to a true catalytic event so that the enhanced efficiency of solubilization seen in the contact bioreactor may, in part, result from the

bacteria and their associated biofilm matrix acting as biocatalystic system. At the present time, the effective pH or partial pressure of O_2 at the ore particle surface is not known. Fluorescent probes and microelectrodes have been used to measure these parameters in model biofilm systems including *Pseudomonas aeruginosa* (a close relative of *Pseudomonas cepacia* now classified as *Burkholderia cepacia*; the bacterium currently used in the contact bioreactor). These data show that biofilms are generally aerobic which, in turn, should allow the direct oxidation of glucose to proceed. In the microcolony itself, both the concentration of acid and the pK_a will be a function of interphase conditions which may be viewed as a continuum that moves from the periplasm through the extracellular polyanionic matrix to the surface of the ore particle. Adhesion to a surface and/or the formation of biofilms is now known to trigger changes in bacterial metabolism including changes in gene expression. As discussed in previously, the degree to which the direct oxidation pathway is expressed and, consequently, the extent to which gluconic acid and/or 2-ketogluconic acid is produced in the 'dissimilatory bypass' mode is highly variable. In the bioreactor, this level may be much higher in the biofilm bacteria than the bacteria growing in the planktonic population within the bulk fluid of the stirred tank bioreactor. As a result, the average level of gluconic acid production (as measured by % conversion of glucose in the feedstock) may not represent the effective concentration of acid at the ore surface. Roles for some of the candidate variables of state identified in this section may be validated within the variation of parameters format that would be expected as part of an optimized implementation of the bioprocess and metabolic engineering systems necessary to develop a successful commercial production facility. Therefore, design of a pilot-scle bioreactor to test the commercial feasibility of the bioprocess must include strong chemical engineering and fermentation engineering components.

Biomass conversion technologies for glucose production with special consideration of low value (waste or byproduct) feedstocks: Currently most glucose comes directly from enzymatic conversion of starch. On average 8-9 billion bu of corn are produced in the U.S. per year which converts to ~ 0.5 trillion lbs of grain. Industrial processing of this grain results in the production of purified starch which is then enzymatically saccharified to glucose via industrial-scale enzyme processing. The enzymes involved in this process; alpha-amylase, glucose amylase and glucose isomerase are produced mainly by Genencor (U.S.A.) and Novo Nordisk (Norway). Glucose produced from saccharified grain starch is generally sold as bulk commodity in the form of concentrated syrup (~70% glucose). As with all commodities, the price fluctuates as a function of raw materials cost. In recent years, the price of a bushel of corn has fluctuated from $2.50/bu to a current price of $4.50/bu (which represents something close to a record high). Based on an average value of $2.50 -$3.00 per bushel, corn starch-derived glucose may be purchased for between $0.13 and $0.15 per lb. An attractive alternative to this purified product is the production of glucose from cellulosic materials including agricultural waste biomass, paper, wood chips, etc. There is no doubt that bioconversion technologies provide the cheapest route for production of solid-phase fermentation quality glucose from waste biomass. As with agricultural commodities, waste raw materials prices are highly variable. However, according to reliable sources in the field of renewable resource engineering it should be possible to obtain glucose of sufficient quality for use as a feedstock for solid-phase fermentation for between $0.005 and $0.02 per lb. Using available technology, the glucose feedstock for the bioprocessing of RPO may be obtained from cellulosic waste materials for between 1/2 cent and 2 cents per pound. Further cost reductions could result from future enhancements in biomass

conversion technology and/or special business relationships between fertilizer and agricultural production companies or operating companies within the same parent corporation.

Bioreactor design strategies with special consideration of maintenance of maximum metabolic activity per unit cell and per unit volume: The bioremediation of contaminated soils, bacterial leaching of metals from ores, biodesulfurization of coal, and the direct fermentation of biomass to sugars or alcohols are all relatively new processes in which the microbial substrate is a solid (Andrews, 1990). Economic viability for these types of high volume low value products will depend, to a large extent on appropriate bioreactor design. These reactors differ from most previous biochemical engineering and fermentation hardware in that they must be true 'three-phase' systems where the substrate is a solid. Minimizing the capital and operating costs of a bioreactor used for large-scale bioprocessing of solids is extremely important because these costs represent a large fraction of the total production cost. It is of interest to note that the efficient bioprocessing of RPO may involve coupling two 'three-phase' systems; the first involving the solid-phase fermentation of waste biomass to glucose, the second involving solid-phase bioleaching of the soluble phosphate from the ore matrix (the question of bioleaching vs. biodegredation depends to a large extent on the amount of matrix remaining after the Pi has been efficiently extracted). High volume low value bioreactors must be designed to provide minimum cost and maximum effectiveness.

Design of these three-phase bioprocessing systems is complex but new technologies and design principles are rapidly emerging. In fact, there can be no doubt that sufficient technology currently exists to conduct pilot-scale studies of the bioprocessing of RPO. In general, solid-phase bioprocessing occurs in slurry bioreactors, in which the solids are kept in suspension either by mechanical agitation, aeration or a combination of both. Specific designs include airlift fermenters (Bos et al,1988), aerated troughs (Andrews, 1990), various modifications of the fluidized-bed (e.g. Asif et. al., 1993), and various modifications of the slurry agitator (e.g. Griffin et. al., 1990). The contact bioreactor developed at INEL and described by Goldstein et al (1993) may be considered to be a microscale version of a modified slurry agitator.

Production of gluconic and 2-ketogluconic acids is an aerobic process so that essential bioreactor design parameters will need to include interactions between particle size, recycling and/or growth of microorganisms, rate of particle consumption, oxygen supply, substrate supply and removal of inhibitory end products. As discussed above, the presence of biofilms on the particles may modify design considerations further. This analysis assumes that initial pilot-scale studies will involve slurry reactors containing finely ground solids such as the spiral concentrate product or flotation concentrate product from standard industry beneficiation plants or equivalent material produced by crushing pebble product. All other parameters being equal, finer particles mean a larger surface area which should imply higher process rate which, in turn, demands higher oxygen and nutrient demand and greater heat production. Of course, heat energy capture and recycling will be incorporated into the bioprocessor design. Coarser products can be processed in fluidized beds, which are inherently cheaper (Andrews, 1990).

Pile reactors are not a viable option for gram negative bacteria using glucose as substrate because, in the open air, these organisms would not be able to compete with other (nonMPS) microorganisms. As discussed in the previous section, variables of state for the solubilization process should initially be studied in the fine particle slurry reactor. Once the functional

relationship of these state variables has been worked out, a coarse particle fluidized-bed system should be explored to define the trade-off between process rate, extraction efficiency and operating costs.

Genetic and metabolic engineering strategies to complement bioreactor design and maximize biosolubilization activity per unit cell and per unit volume: As discussed in a previous section, a high degree of genetic and metabolic variability exists among gram negative bacteria expressing the dissimilatory bypass mode of direct glucose oxidation. This variability exists both between and within genera. Many of the genes of the direct oxidation pathway have been cloned and sequenced, some by my laboratory. While genetic engineering may hold the key to long-term optimization of the bioprocessing of RPO, Goldstein's laboratory has also demonstrated that strain selection via classical mutagenesis can result in mutant *Pseudomonas cepacia* cells that express the direct oxidation pathway to 10X the level of E-37 (Goldstein et al, 1993). 2-ketogluconic acid overproducing strains of *Erwinia herbicola* have also been developed at Genentech as part of a fermentation process for ascorbic acid production (Anderson et al, 1991). Bench-scale studies with these overproducing strains will allow determination of whether enhanced acid production at the bacterial surface results in enhanced rates and/or efficiencies of ore bioprocessing.

The second area of obvious importance is the development of strains that produce efficacious biodegredation without the need for 1% glucose in the feedstock. As discussed above, contact bioreactor studies have shown that only ~1% of the 1% glucose feedstock is oxidized during the biodegredation of the ore. From the viewpoint of bacterial metabolism, it is probable that E-37 only expresses the dissimilatory bypass mode of direct glucose oxidation in the presence of luxuriant levels of exogenous glucose (i.e. the 1%). From an economic standpoint, it is absolutely essential that the remaining glucose be used to release phosphate from the ore. While one strategy might be to recover and recycle the glucose, the more obvious and effective strategy would be to develop or select strains that operate effectively with much lower glucose feedstock concentrations. For pilot plant development, rapid classical mutagenesis and strain selection procedures may be applied to produce mutants with the desired phenotype. Development (and/or acquisiton) of these strains should allow testing of the significance of amount and type of acid produced and minimize the amount of feedstock glucose necessary for efficient bioprocessing. Studies carried out with a glucose dehydrogenase minus strain of *Pseudomonas cepacia* have already shown that expression of the direct oxidation pathway is essential for efficacious mineral phosphate solubilization by gram negative bacteria. Classical mutagenesis and strain selection strategies should allow rapid testing and optimization of the metabolic variables of state that contribute to bioreactor efficiency. It is probable that strains can be developed that give maximum bioleaching with minimal (0.1% or less) glucose in the feedstock. Strain development and selection will play a major role in the overall economic performance of the bioprocess. In the longer term, metabolic and genetic engineering techniques should be employed to develop ultra-efficient strains for use in second generation RPO bioprocessing systems.

Bioreactor design aspects relating to product recovery and nutrient recycling: The efficiency of product recovery will depend on a number of variables. One of the most important is the fate of the radionuclides during bioleaching of soluble Pi from the ore matrix. Assuming that the radionuclides stay in the insoluble ore matrix, major product recovery activities will

center on the phosphoric acid and Ca^{++} salts of the organic acids produced during the oxidative metabolism that underlies the bioleaching process. These calcium salts would be expected to be mainly calcium gluconate and calcium 2-ketogluconate. Calcium gluconate is already marketed as a bulk chemical under the trade name 'Dissolvine'. This material is produced via large-scale bioprocessing. The process involves stoichiometric oxidation of 30% glucose solutions to gluconic acid using the gram negative bacterium *Gluconobacter oxydans* . Gluconic acid and its sodium salt are capable of forming highly stable complexes with metal ions at all pHs but, at higher pHs are known to act as true chelating agents. Gluconates are sold for metal treatment, retarding agents for concrete and mortar, cleaners, corrosion inhibition, detergents, as a metal carrier for micronutrients in agricultural fertilizers and many other applications. Van Beynum and Roels (1985) estimated worldwide consumption of all forms of gluconic acid to be about 45,000 st. tons.

In addition to its uses as an industrial chemical, it is possible that calcium gluconate could be applied as an amendment on some of the acidic agricultural soils of the Southeastern United States. At the very least, calcium gluconate and calcium 2-ketogluconate could easily be 'land-farmed' to avoid the current 'gyp-stack' problems associated with the radioactive phosphogypsum created by the wet process. A significant number of soil bacteria and fungii are capable of using both gluconate and 2-ketogluconate as carbon sources and, in acidic soils, the residual calcium not incorporated into microbial or plant biomass would probably not pose a serious threat to the physical structure of the soil in the relatively short term. Multiple sites and/or site rotation would further increase the amount of byproduct that could be land farmed in this manner. The fate(s) of these end product streams would depend on the radioisotope content and other process parameters that would be set as part of the engineering that occurs during pilot plant development and testing.

Bioreactor design aspects in relation to end product removal to enhance the rate of bioleaching: As discussed by Wyman and Goodman (1993), accumulation of end products may severely inhibit the rate of solid-phase fermentation processes. This phenomenon of feedback inhibition is a well known property of many enzymes. Because of the energy requirements of the stirred tank technology, a key will be to achieve high efficiency with minimum residency time within the tank. The possibility exists for simultaneous saccharification and bioleaching in a manner similar to the simultaneous saccharification and fermentation (SSF) scheme described by Wyman and Goodman for ethanol production. In this scenario, solid-phase fermentation of biomass to glucose is conducted in the same tank as the oxidation of glucose to gluconic and 2-ketogluconic acid and the subsequent bioleaching of Pi from the RPO. As with the SSF technology, constant removal of end product (glucose) maximizes the rate of enzyme-mediated saccharification. A key consideration will be whether the saccharification enzymes will retain catalytic function at the low pH created by operation of the direct oxidation pathway. The other option is, of course, sequential saccharification and bioleaching. This is basically the two-tank scenario shown in the flowchart produced in the next section.

It is probable that constant removal of end products plays a significant role in the enhanced efficacy of the the contact bioreactor system developed at INEL and described by Goldstein et al (1993) when compared to the batch experiments conducted with 30% gluconic acid described

in this report. However, the end product removal possibilities for the continuous flow system are far from optimized and it is recommended that, as part of pilot plant development, several end product removal systems be analyzed.

Other microbial applications:

- **Direct bioleaching of ore (or enriched tailings) in "bioreactor heaps" may be economically feasible.** This technology is currently in use for the biomining of several metals including gold and copper and for removal of both organosulfur and pyritic sulfur from coal (in pilot-scale projects, see Andrews et. al. 1993). It is unlikely that gram negative bacteria using glucose as a carbon source would compete well in this type of 'open', nonsterile environment. However a number of other microbial candidates exist including citric and gluconic acid producing fungi, acidophilic eubacteria and acidophilic archaeobacteria. For example, 'mixed acid fermentation' bacteria capable of using pyruvic acid to produce acetic acid might be useful. Likewise, 'acetic acid bacteria' capable of oxidizing ethanol to acetic acid might work in a 'heap bioreactor' of the type being tested for the large-scale desulfurization of coal (Andrews, et al,1993). The ethanol used to spray the "heaps" could be produced from waste biomass via solid-phase fermentation in a manner similar to that proposed for the generation of glucose for the bioreactor. The ethanol produced from the solid-phase fermentation would be mixed with appropriate levels of bacterial innoculum and sprayed onto the ore heaps.
- **Polyphosphate accumulation by bacteria**. A few types of gram negative bacteria have the ability to take up soluble orthophosphate from solution, polymerize it into long chain polyphosphates and store the polyphosphate as granules inside the cell There are already **numerous** reports of enhanced polyphosphate accumulation via genetic engineering for overexpression of polyphosphate kinase. Some of the new strains are extremely efficient at Pi uptake.
- **The polyphosphate accumulation trait might be coupled to the newly discovered trait of positive phosphate taxis.** Bacteria showing positive phosphate taxis swim towards Pi. Presumably, this trait allows these bacteria to achieve enhanced phosphate nutrition in phosphate-limited environments. Coupling of the two traits would result in bacteria that 'swim around' scavenging soluble phosphate and accumulate it as polyphosphate granules inside the cell. These types of bacteria may be useful in treating certain holding ponds associated with current wet process phosphoric acid plants .
- **Magnesium accumulation and storage.** Microbial bioleaching of Mg^{++} occurs in nature via both chelation and sequestion. Exploration of this phenotype and its potential role in solid-phase bioprocessing and/or heap bioreactors could form the basis of a separate report.

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