URANIUM IMMOBILIZATION BY *CELLULOMONAS* SP. ES6

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of VAIDEESWARAN SIVASWAMY find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

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Removal of uranium (U) from aqueous solution was studied using a Gram-positive facultative anaerobe, *Cellulomonas* sp. strain ES6, under anaerobic, non-growth conditions in bicarbonate and PIPES buffer. During aerobic growth on tryptic soy broth, cells accumulate excess phosphate, which can be hydrolyzed and released as inorganic phosphate (P_i) under anaerobic starvation conditions. Inorganic phosphate released by the cells precipitated U from the medium as uranyl phosphate. The saturation concentration of phosphate required to initiate U precipitation from solution was dependent on the buffer and the amount of U present in solution. A Monod-based kinetic model was used to describe the P_i release process. Examination of the cultures by high-resolution transmission electron microscopy (HR-TEM) and energy dispersive X-ray spectroscopy (EDS) showed both extracellular and intracellular U accumulation. The uranyl phosphate precipitates were nanometer sized needle-like fibrils and EDS analysis suggested a 1:1 molar ratio of U and phosphorus in these precipitates. Studies of U immobilization with strain ES6 and anthraquinone-2,6-disulfonate (AQDS), a model humic substance, showed that U reduction is the predominant mechanism and not precipitation by phosphate ligands. X-ray absorption near-edge spectroscopy (XANES) analysis showed that the predominant oxidation state of U precipitates was +4 in bicarbonate buffer, +6 in PIPES buffer

and +4 in AQDS treatments. Uranium immobilization by *Cellulomonas* sp. was previously reported as reduction, however present work suggests that strain ES6 can precipitate U via both precipitation with phosphate ligands and enzymatic reduction, depending on geochemical conditions. In the presence of AQDS, complete reduction of U(VI) to U(IV) by *Cellulomonas* sp. ES6 was observed. *Cellulomonadaceae* are environmentally relevant subsurface bacteria and for the first time we report U immobilization by multiple mechanisms using the Gram positive subsurface organism *Cellulomonas* sp. ES6.

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DEDICATION

"It is the divine presence that gives value to life. This presence is the source of all peace, all joy and all security. Find this presence in yourself and all your difficulties will disappear."

ANNAI ॐ

This thesis is dedicated to my parents Mrs. Meenakshi and Mr. Sivaswamy, my sisters Mrs. Thaiyalnayagi and Mrs. Anuradha, my brother-in-laws Mr. Girish and Mr. Balaji who have always believed in me, given me their unwavering love & support and are a source of inspiration. I am grateful to god for giving me such a wonderful and loveable family.

CHAPTER 1

INTRODUCTION

Overview

This research was conducted to describe the potential of *Cellulomonas* sp. ES6 to immobilize uranium by precipitation with phosphate and enzymatic reduction of U(VI) to U(IV). In this thesis work, we elucidate the dependence of phosphate release and uranium precipitation on buffer pH and also the ability of *Cellulomonas* sp. ES6 to immobilize uranium using multiple mechanisms namely reductive precipitation and precipitation with phosphate ligands based on environmental conditions. We have also developed kinetics of phosphate release by *Cellulomonas* sp. ES6 and its ability to reductively precipitate U(VI) in the presence of AQDS. The goal for conducting this research was to gain a fundamental understanding of U(VI) immobilization by environmentally relevant bacterium (particularly to the Department of Energy, Hanford, Washington site). This thesis is organized in the Washington State University manuscript format. This introductory chapter is followed by a manuscript that has to be submitted for publication.

Chapter 2 describes U(VI) immobilization by *Cellulomonas* sp. ES6 in two different buffers, PIPES and bicarbonate. This chapter also includes the effect of a model humic acid, Anthroquinone-2,6-disulfonate (AQDS) on phosphate release and U immobilization, elucidated by Transmission Electron Microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), X-ray absorption near-edge spectroscopy (XANES) and a Monod-based kinetic model to describe the P_i release process.

Background

Subsurface Soil and Groundwater contamination

Heavy metals and radionuclide contamination presents a significant environmental problem worldwide. Release of radionuclides to the subsurface has been due to both natural and anthropogenic activities. Natural radionuclides are released to surface and ground waters from rocks and ores by dissolution and desorption, during radioactive decay. Some chemolithotrophic and heterotrophic microorganisms are able to leach uranium and other radioactive elements from minerals in both acidic and alkaline water solutions (Groudev et al. 2001). Uranium (U) is an important radionuclide contaminant in ground water, soils and subsurface sediments at nuclear weapons manufacturing and uranium mining sites, due to processing of uranium ore, mining, milling and tailing operations (Spear et al. 1999, Anderson et al. 2003). Milling and processing of uranium ores is accomplished by crushing the host rock, leaching it in strongly acidic or alkaline solutions to dissolve uraniferous materials, and recovering soluble uranium from solution. The process produces large volumes of acidic or alkaline tailings which are disposed as a slurry to tailing piles, most of which were not lined. Leachates from these tailings contain high concentrations of several metals and radionuclides (Barton et. al. 1994).

A survey by Riley and Zachara (1992) showed that 11 of 18 U.S. Department of Energy sites examined had groundwater and soil contaminated by U. The U.S. Environmental Protection Agency (EPA) promulgated a groundwater concentration limit of 30 pCi/L (approximately 44 μg/L) and a maximum contaminant level goal (MCLG) of 0.0 μg/L for U under the Safe Drinking Water Act to ensure protection of human health and environment near these sites (Health and Environmental Protection Standards for Uranium and Thorium Mill Tailings, Code of Federal Regulations; National Primary Drinking Water Regulation). The

solubility of U is strongly dependent on its chemical oxidation state. Under oxidizing conditions, U usually exists as U(VI) and forms highly soluble uranyl-carbonate complexes [$UO_2CO_3^o$, $UO_2(CO_3)_2^{2-}$, or $UO_2(CO_3)_3^{4-}$] when carbonate is present in the system (Brookins, 1988). However, under reducing conditions, U(VI) is most often reduced to U(IV) forming the insoluble $UO_{2(S)}$ (uraninite) phase (Tucker et al. 1998). U(VI) is highly soluble and mobile within groundwater. Uranium's mobility can threaten down-gradient water resources and can pose difficult remediation challenges. According to the National Research Council (2000), cleanup across the DOE complex is expected to cost at least $200 billion dollars and will take decades to complete.

Passive reactive barriers (PRB)

Physical and chemical methods exist for treatment of U(VI) in soils, including excavation and off-site disposal, soil washing, soil flushing, vitrification, anion exchange, lime softening, conventional coagulation and activated alumina. Some of the above methods namely anion exchange, activated alumina are also used for treating groundwater contaminated with U. Such ex-situ and pump-and-treat technologies are usually very expensive, and involve extensive manual labor. One possible alternative approach is building a permeable reactive barrier (PRB) across the aquifer contaminated with U (Figure 1). A permeable reactive barrier (PRB) is an engineered, subsurface zone of reactive material that treats contaminated groundwater flowing through it using various physical, chemical, or biological reactions (Morrison et al. 2001). When contaminated water passes through the PRB, contaminants are either immobilized or chemically transformed to a less toxic state by the reactive material contained within the barrier (USEPA, 1997). Operational and maintenance costs are lower because water flow across the PRB is driven by the natural hydraulic gradient and the treatment system does not require continual operational

maintenance. The main costs of reactive barriers are in the characterization, design, and construction, after which the primary cost involved is compliance monitoring to characterize contaminant removal. Potential limitations to PRB include re-release of contaminants after aging of reactive material, removal and disposal of the reactive material after breakthrough, and deleterious effects of barrier material on downgradient water quality (Naftz et al. 2000).

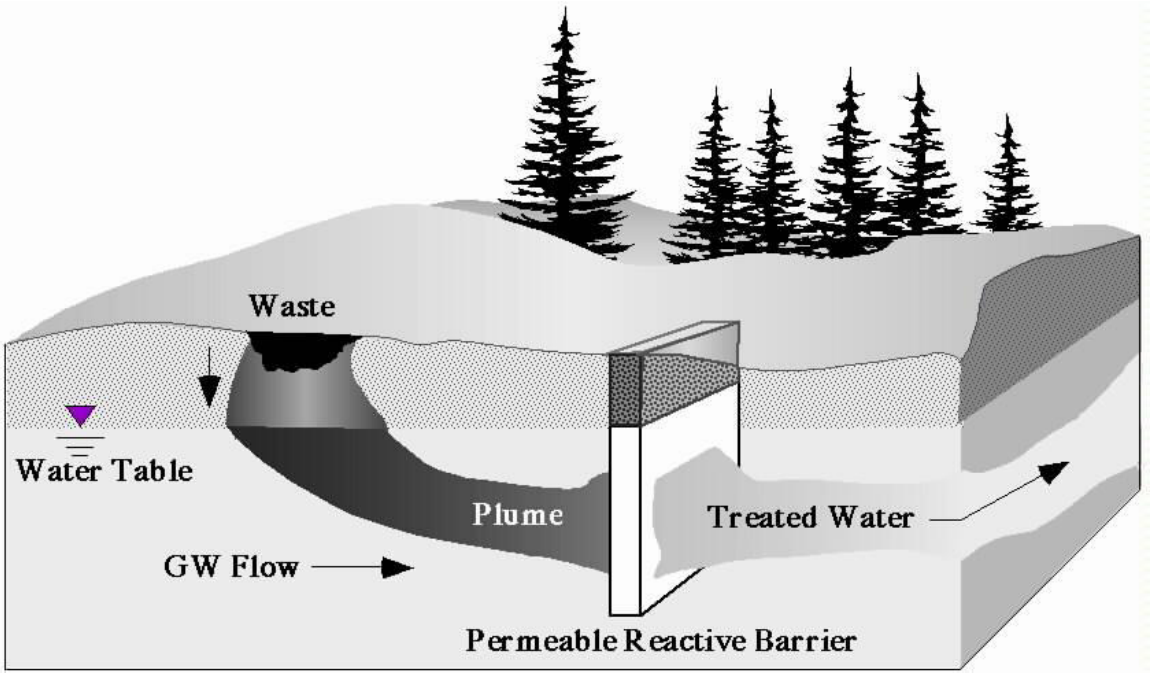

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Figure 1 – Schematic representation of Passive Reactive Barrier

PRBs are beginning to be used to treat groundwater contaminated by U. All PRBs designed for field demonstration of U immobilization have zero valent iron [Fe(0)], amorphous ferric oxyhydroxide (AFO), or phosphate (PO_4^{3-}) as the reactive media. Morrison et al. (2001) and Gu et al. (1998) have demonstrated reductive precipitation of U by zero valent iron. Zero valent iron, a scrap-metal product that is available from the automative industry, is being used as a reactive material in these PRBs. Sufficient contact with Fe(0) causes U concentrations in groundwater to decrease to nondetectable levels (less than 1 µg/L). The potential effectiveness of

phosphate-bearing, reactive barrier systems for U removal from ground water is demonstrated by the work of Fuller et al. (2002) and Naftz et al. (2000). A funnel and gate design was chosen as PRB. In this design, the groundwater was directed by the funnel structure to flow through a gate, which contained reactive material. Some of the advantages of such design are 1) multiple PRB's can be placed side by side, 2) low construction cost, 3) conducive to shallow ground water system, and 4) transferability to other remote, abandoned mine sites with shallow contaminated ground water. The PO_4 barrier material consisted of pelletized bone charcoal used as phosphate source to facilitate formation of insoluble uranyl phosphate compounds. Naftz et al. (2000) has shown that phosphate based PRB and zero-valent iron based PRB removed approximately 99.9% of input uranium, while AFO based PRB removed more than 90% of the input uranium during the first 3 months of operation.

In-situ bioremediation using passive reactive barriers

Biological treatment of groundwater containing uranium offers an alternative to physical/chemical methods. Indigenous subsurface microorganisms are being studied for their potential to immobilize heavy metals and radionuclides by reductive precipitation. Such microorganisms are ubiquitous in the environment, and their populations in the subsurface can be increased by adding electron donors and other nutrients. Bioremediation works by either transforming or degrading contaminants to nonhazardous or less hazardous chemicals. Over the past two decades, bioremediation has become widely accepted as a viable technology to transform and degrade many types of contaminants. It has been shown that many organic contaminants such as hydrocarbon fuels can be biodegraded to relatively harmless products like CO_2. Similarly some microorganisms can change the valence of some heavy metals and

radionuclides (e.g., Cr(VI) to Cr(III) and U(VI) to U(IV)). Natural attenuation, biostimulation, and bioaugmentation are the three basic bioremediation methods widely used.

Natural attenuation means dilution, dispersion, irreversible sorption, volatilization, chemical and biochemical stabilization and/or radioactive decay of contaminants to reduce contaminant toxicity, mobility, or volume effectively to levels that are protective of human health and the ecosystem (Criddle et al. 1990). However rates of natural attenuation are usually slower and insufficient. Biostimulation is the addition of electron donors and acceptors, and/or nutrients to increase the number or activity of naturally occurring microorganisms available for bioremediation. Bioaugmentation is the addition of microorganisms with desired characteristics to the subsurface followed by the addition of electron donors or nutrients for their growth, when indigenous bacteria are unable to mediate the desired transformation. The basic idea of building a biobarrier is that the pumping of nutrients into the subsurface results in formation of a reactive treatment zone or "biocurtain" or "permeable reactive barrier". One of the basic design is a series of evenly spaced wells separated by certain distance arranged across the contaminated aquifer. If the odd numbered wells act as injection wells, the even numbered wells act as extraction wells and vice versa. In this fashion a biocurtain is developed.

To maintain microbial activity within the treatment zone, inorganic nutrients, electron acceptors or electron donors, collectively referred to here, as "substrates" should be delivered at an optimal rate and in the case of bioaugmentation, the microorganism is added along with the nutrients. Inappropriate addition of substrates results in either low/no contaminant degradation or plugging of injection wells due to profuse microbial growth. Biostimulation of indigenous flora may be successful if active organisms are widely distributed at the site of interest, whereas microbes with rare metabolic capabilities whose ecophysiology is compatible with *in situ*

conditions may be suited for bioaugmentation approaches (Barkay and Schaefer, 2001). Although micro-organisms cannot destroy metals, they can alter their chemical properties by different mechanisms such as biosorption, bioleaching, enzyme-catalyzed transformation (bioreduction) and biomineralization (Lloyd JR, 2002). In this thesis, we present enzymatic reduction (bioreduction) of U and microbially mediated U precipitation by phosphate ligands by *Cellulomonas* sp. ES6. Microorganisms can be an effective alternative to zero valent iron or phosphate as reactive media in PRBs. Three basic mechanisms by which bacteria can immobilize U are the following: 1) direct and indirect microbial reduction of U(VI), 2) uptake and accumulation by cells, and 3) precipitation of U as uranyl phosphate with inorganic phosphate released by cells.

Enzymatic reduction of U(VI) to U(IV)

Several organisms common to soil and subsurface environments have been identified to enzymatically reduce U(VI) to U(IV) under anoxic conditions. These include dissimilatory Fe-reducing bacteria, *Geobacter metallireducens* and *Shewanella oneidensis* MR-1 (previously known as *Shewanella putrefaciens* MR-1) (Gorby and Lovley, 1992). In addition to Fe(III), these organisms use U(VI) as a terminal electron acceptor (Lovley et al. 1991). To obtain energy for growth these organisms oxidize acetate with the reduction of U(VI) to U(IV). Various species of sulfate-reducing bacteria, including *Desulfovibrio desulfuricans, D. vulgaris, and D. baculatum*, are able to reduce U(VI) to U(IV) however, these organisms do not grow on uranium (Lovely and Phillips, 1992; Lovley et al. 1993a,b). Tebo and Obraztsova (1998) have shown that a sulfate-reducing bacterium, *Desulfosporosinus* sp. (formerly *Desulfotomaculum reducens* sp.) can grow not only with Fe(III) and also with Cr(VI), U(VI) and Mn(IV).

Apart from Fe-reducing and sulfate-reducing microorganisms, cultures of *Clostridium* sp., *Deinococcus radiodurans* R1, *Pseudomonas* sp. CRB5, and *Thermus* sp. have been shown to reduce U(VI) to U(IV) (Francis et al. 1994; Fredrickson et al. 2000; McLean and Beveridge, 2001; Kieft et al. 1999). Most of these metal-reducing microorganisms couple the oxidation of organic matter to reduction of heavy metals and radionuclides, and this coupling may be an important process affecting the organic and inorganic geochemistry of anaerobic sediments (Nealson and Saffarini, 1994; Lovley 1997).

Lojou et al. (1998a,b) have shown that metal-reductase activity is exhibited by several c-type cytochromes of bacterial origin in the case of Fe(III), Mn (IV), and Cr(VI). Studies of uranium reduction by DvH cytochrome c_3 indicates that this metalloprotein acts as a U(VI)-reductase in D. *vulgaris* (Lovley et al. 1993). Cytochrome c_3 can be directly reduced electrochemically without the aid of intermediary electron carriers (Niki et al. 1977). Cytochrome c_3 can be readily mass produced and could be employed in fixed-enzyme reactor for U(VI) reduction. Lojou et al. (1999) has demonstrated that the efficiency of the U(VI) electroreduction process originates in the presence of hemin-containing groups, such as low redox-potential polyheme cytochromes. Payne et al. (2002) developed a cytochrome c_3 mutant of *D. desulfuricans* G20 to test the involvement of these proteins in in-vivo reduction of U(VI). It was found that the microorganism was able to reduce U(VI) with lactate or pyruvate as the electron donor at rates about one-half of those of the wild type. With electrons from hydrogen, the rate was more severely impaired. The results showed that cytochrome c_3 may be a part of an in-vivo electron pathway to U(VI), but additional pathways from organic donors can apparently bypass this protein. Payne et al. (2004) suggested that periplasmic cytochrome c_3 of *D. desulfuricans* G20 is unlikely to function as an significant extracellular electron carrier to U(VI).

Studies of U reduction in batch experiments with pure cultures of microorganisms give a fundamental understanding of the mechanism and effect of electron donors, acceptors and geochemical conditions on U reduction. However field conditions are complex and includes mixed culture. Groudev et al. (2001) has demonstrated that an efficient remediation of the soils from agricultural lands (Vromos Bay area, near the Black Sea coast, Southeastern Bulgaria) contaminated with radioactive elements can be achieved by an in-situ treatment method based on the activity of the indigenous soil microflora. It was observed that dissolution of contaminants was connected with the activity of both heterotrophic and chemolithotrophic aerobic microorganisms and the immobilization was due mainly to the anaerobic sulphate-reducing bacteria. The treatment involved dissolution of the contaminants in the upper soil horizons (0 – 25 cm) and their transfer into the deeply located soil horizons (26 – 80 cm) where they were immobilized as different insoluble compounds. Under field conditions, with suitable nutrients, the contents of radioactive elements and toxic heavy metals in the soil at the upper horizon were decreased below the relevant permissible levels within 8 months of treatment.

Anderson et al. (2003) demonstrated that in situ bioremediation of uranium-contaminated groundwater is feasible by stimulating activity of dissimilatory metal-reducing microorganisms in a uranium-contaminated aquifer located in Rifle, Colorado. It was shown that addition of acetate can stimulate microbial growth to consume dissolved oxygen and/or promote active anaerobic respiration resulting in effective reductive precipitation of U(VI). Acetate was used as electron donor and was injected into the subsurface over a 3-month period via a series of injection wells. U(VI) concentrations decreased in as little as 9 days after acetate injection was initiated, and within 50 days U concentration declined from 1.4 μM to 0.18 μM. U(VI) was reduced concurrently with Fe(III) and prior to reduction of sulfate (Finneran et al. 2002a). It was

observed that U(VI) and Fe(III) was associated with an increase in the number of *Geobacteraceae*, by several orders of magnitude. *Geobacter* species were the predominant *Geobacteracea* in groundwaters with freshwater salinities.

Although stimulation of dissimilatory metal reduction to promote reductive precipitation of uranium has been shown to successfully remove uranium from some aquifer sediments, Nevin et al. (2003) has shown that organisms in the family *Geobacteraceae* can grow at high salinities with addition of acetate coupled to U(VI) reduction. Analysis of microorganisms associated with U(VI) reduction using 16S rRNA gene sequencing revealed that most of these microorganisms were closely related to *Pseudomonas* and *Desulfosporosinus* species.

These metal/radionuclide reducing bacteria use several strategies to access extracellular metal terminal electron acceptors (TEA) in the environment (Hernandez and Newman, 2001). Payne et al. (2004) proposed some possible modes to access TEA which include: (1) direct contact of an oxidized metal with an outer-membrane electron transfer component (Beliaev and Saffrini, 1998; Lower et al. 2000; Magnuson et al. 2000); (2) the release of siderophores that complex the metal TEA (Ledyard and Butler, 1997); or (3) the use of extracellular electron-shuttling molecules, such as quinone-containing compounds or c-type cytochromes, to transfer electrons from the cellular electron-transport chain to the metal TEA (Newman and Kolter, 2000; Seeliger et al. 1998). The extracellular electron shuttles may either be already present in the environment due to decomposition of organic material or intentionally produced by cellular processes and excreted into the environment (Payne et al. 2004).

One important type of extracellular electron shuttles are humic substances. Humic substances also called *humus*, are yellow to dark brown polymers formed by microbial mediated

reactions, which can function as catalysts for bacterial metal reduction. Lovley et al. (1996) reported that microorganisms can donate electrons to humic acids, which can shuttle electrons between the microbe and Fe(III) oxide. Anthraquinone 2,6-disulfonate (AQDS) has been proposed as a model humic compound and has been shown to catalyze microbial reduction of Cr(VI), U(VI), Fe(III) and Mn(VI) (Fredrickson et al. 2000; Lovley et al. 1996; Gounot, 1994). Even though microbial reduction of U(VI) offers various advantages over conventional physical/chemical methods, there are some limitations in using this strategy. U(IV) can potentially re-oxidize to U(VI) in the presence of oxygenated water.

Senko et al. (2002) showed that intermediates of dissimilatory nitrate reduction (denitrification or dissimilatory nitrate reduction to ammonia), nitrite, nitrous oxide, and nitric oxide were all capable of oxidizing and mobilizing U(IV). Waste streams from the nuclear industry typically contain high concentrations of anions, heavy metals, organic solvents and chelators (Mackaski et al. 1991; Riley et al. 1992). Nitrate is a common co-contaminant with uranium. Finneran et al. (2002b) showed that nitrate can act as competitive electron acceptor with U(VI) and can anaerobically oxidize U(IV) to U(VI). Generation of dissimilatory nitrate reduction intermediates apparently creates a highly oxidizing environment, leading to the oxidation of U(IV), reversing the reducing conditions required for uranium immobilization. Hence, it will be necessary to stimulate the removal of nitrogen from systems via denitrification before the immobilization of U(VI) can commence (Senko et al. 2002). Brooks et al. (2003) showed that calcium can cause a significant decrease in the rate and extent of bacterial U(VI) reduction as U is a less energetically favorable electron acceptor when $Ca\text{-}UO_2\text{-}CO_3$ complexes are present.

Mn(III/IV) oxides are common secondary phases in soils and sediments. Liu et al. (2002) showed that pyrolusite (β-$MnO_{2(S)}$) can abiotically reoxidize uraninite that was precipitated as a result of microbial reduction. Iron (hydr)-oxides are common in the subsurface and can act as competitive electron acceptor for U reduction. Wielinga et al. (2000) showed that the presence of U(VI) retarded the reduction of crystalline iron (hydr)oxides (goethite and hematite), while the reduction of U(VI) was unaffected or slightly enhanced by the presence of the crystalline Fe (hydr)-oxides. Conversely, the reduction of ferrihydrite appeared to be unaffected by the presence of U(VI), whereas uranyl reduction was inhibited by the amorphous iron hydroxide. Sani et al. (2005) showed that in the absence of electron donor, microbially reduced U(IV) can serve as an electron donor to reduce Fe(III) present in Fe (III) (hydr)oxides resulting in the reoxidation of reduced uranium.

Accumulation of uranium by microorganisms

The second important mechanism of uranium immobilization is uptake, accumulation, and sorption by microbial cells. Bacterial sorption may affect the fate and transport of uranium in many near-surface environments. Laboratory and field studies have demonstrated that microbes have the ability to facilitate the removal of uranium from the aqueous phase through the sorption of U(VI) to bacterial cell walls (Suzuki et al. 1999). Many researchers have investigated uranium sorption onto microbial cell wall surfaces (Friis and Keith, 1986; Cotoras et al. 1992).

Marques et al. (1991) showed that *Pseudomonas* sp. EPS-5028 can take up uranium rapidly. The uptake of uranium was affected by pH, but not by temperature, metabolic inhibitors, culture time or the presence of various cations and anions. Even though the mechanism of U transport into the cell is unknown, transmission electron micrographs of cells treated with U

showed U bound in the cytoplasmic fraction of *Pseudomonas* sp. EPS-5028. The cells were then washed with water and one of the following solutions: Na_2CO_3, sodium citrate, EDTA, potassium oxalate or HNO_3. It was found that Na_2CO_3 was very effective in extracting most uranium from the cells, without an apparent effect on the cell surface (verified by subsequent U uptake) and without loss of viability (verified by reculturing of cells from treated cell preparation).

Similar to Marques et al. (1991), Fowle et al. (2000) also observed a change in U sorption capacity with pH using *Bacillus subtilis*. It was found adsorption increased with increasing pH and solid:solute ratio, presumably due to the deprotonation of cell wall functional groups and the increasing number of surface reactive sites. The adsorption of U was both rapid and reversible. Deprotonation of the cell wall functional groups creates negatively charged surface sites for metal adsorption. The deprotonation also leads to the development of a negative electrical potential associated with the bacterial cell wall. This potential in turn affects the interactions of ions with the bacterial surface sites. Nakajima and Sakaguchi (1986) investigated uranium uptake by 83 species of microorganisms: 32 bacteria, 15 yeasts, 16 fungi and 20 actinomycetes. Of these 83 species of microorganisms tested, extremely high uranium-absorbing ability was found in *Pseudomonas stutzeri, Neurospora sitophila, Streptomyces albus* and *Streptomyces viridochromogenes*. These organisms were also found to be more stable after immobilization and could be used repeatedly. Studies with *Mycobacterium smegmatis* showed that adsorption of U was accompanied by partial release of magnesium from the cell wall, indicating that exchange reactions occurred at magnesium (Mg)-bonding sites (Andres et al. 1993).

Strandberg et al. (1981) used *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* as biosorbents for uranium accumulation. The rate and extent of accumulation was found to be dependent on environmental parameters, such as pH, temperature, and interference by certain anions and cations. Rothstein and Meier (1951) observed that monovalent cations had no effect on U accumulation while divalent cations interfered with uranium uptake by *S. cerevisiae*. However Strandberg et al. (1981) observed no interference of Ca^{2+} on U uptake by *P. aeruginosa*. The accumulation of U can be either intracellular or extracellular depending on the culture. Bacterial adsorption may significantly affect the distribution and, hence, mobility of uranium in groundwater systems.

Precipitation of uranium by microbially mediated phosphate ligands

The third important mechanism of U immobilization is precipitation with inorganic phosphate released by cells. U(VI)-phosphate interactions are important in governing the subsurface mobility of U(VI) in both natural and contaminated environments. Natural immobilization of U in many phosphate minerals as U(VI) phosphates occur extensively at the Kongarra deposit, Australia (Duerden, 1990). Solubility products of U(VI) phosphates vary between 4.73×10^{-47} and 2.14×10^{-11} (Palie, 1970). Arey et al. (1999) reported that addition of phosphate minerals (e.g. hydroxyapatite) can reduce the solubility and bioavailability of U in contaminated soils from the US-DOE Savannah River Site. Jerden et al. (2003) reported that the low solubility of stable U(VI) phosphate minerals can limit U concentrations to less than 15μg l^{-1} and phosphate-based strategies for in situ stabilization of U in oxidizing, fluid rich environments may be effective for long-term containment. Uranyl phosphate minerals are stable over a wide range of solution compositions and there is no re-oxidation problem.

Cultures of *Citrobacter* sp. (Yong and Macaskie, 1998), *Acidithiobacillus ferrooxidans* (Merroun et al. 2002), *Bacillus sphaericus* (Knopp et al. 2003), and *Acinetobacter johnsonii* (Boswell et al. 1999) have been demonstrated to remove uranium from water using a phosphate release mechanism. Aerobically, these microorganisms accumulate phosphate as polyP. Polyphosphate is a phosphate polymer with chain lengths of three to a thousand P_i. Polyphosphate is reversibly synthesized by polyphosphate kinase (PPK) with the addition of a phosphate from a high-energy phosphoryl donor, such as ATP, and hydrolyzed by exopolyphosphatase (PPX) (Kornberg et al. 1999). Subsequent exposure to anaerobic conditions promotes polyP degradation with concomitant release of phosphate into the medium. This release has been coupled to bio-precipitation of heavy metals as cell-bound metal phosphates (Boswell et al. 1998). The release of phosphate via the hydrolysis of an organic phosphate has been shown to be an effective method for the precipitation of metals on cell membranes (Yong and Macaskie, 1995; Boswell et. al. 1999; Renninger et al. 2001).

Yong et al. (1995) showed that localized phosphate release in close proximity to nucleation sites on the cells promotes the bio-crystallization of metals as $MHPO_4$ (M, divalent metal cation). Phosphate groups on the surface of many bacterial species are found to be the main nucleation site for precipitation (Panak et al. 2000). Cell wall components with phosphate residues e.g., polysaccharides, teichoic and teichuronic acids or phospholipid layers of the membranes are responsible for the uranium binding. Joeng et al. (1997) suggested that phospholipid outer and inner membrane bilayers are possibly involved in the formation of metal phosphate nucleation foci. Studies of U removal from solution by *Acinetobacter johnsonii* (Boswell et al. 1999) showed that phosphate release increased with pH between pH 5.5 and 8.0, and also increased with temperature between 4^oC and 37^oC. However, the presence of nitrate at

concentrations of 10mM and above inhibited anoxic phosphate release. Yong et al. (1995) showed that the efficiency of uranium removal by *Citrobacter* sp. can be increased by incorporating ammonium acetate (NH_4Ac) into solution. This was attributed to the generation of a modified form of uranyl phosphate precipitate ($NH_4UO_2PO_4$), which has a lower solubility product (3.6×10^{-27}) than HUO_2PO_4 and $NaUO_2PO_4$.

Sani et al. 2002, previously reported uranium immobilization by *Cellulomonas* sp. as enzymatic reduction. However in this thesis, it is proposed that uranium immobilization by *Cellulomonas* sp. ES6 involves multiple mechanisms namely precipitation with inorganic phosphate released by cells and enzymatic reduction based on geochemical conditions. Immobilizing heavy metals (Cd^{2+})/radionuclides using this precipitation mechanism is advantageous over microbial reduction as this produces thermodynamically and chemically stable form of metals and its use is not limited to reducible metals.

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CHAPTER 2

Uranium Immobilization by *Cellulomonas* sp. ES6

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Abstract

Removal of uranium (U) from aqueous solution was studied using a Gram-positive facultative anaerobe, *Cellulomonas* sp. strain ES6, under anaerobic, non-growth conditions in bicarbonate and PIPES buffer. During aerobic growth on tryptic soy broth, cells accumulate excess phosphate, which can be hydrolyzed and released as inorganic phosphate (P_i) under anaerobic starvation conditions. Inorganic phosphate released by the cells precipitated U from the medium as uranyl phosphate. The saturation concentration of phosphate required to initiate U precipitation from solution was dependent on the buffer and the amount of U present in solution. A Monod-based kinetic model was used to describe the P_i release process. Examination of the cultures by high-resolution transmission electron microscopy (HR-TEM) and energy dispersive X-ray spectroscopy (EDS) showed both extracellular and intracellular U accumulation. The uranyl phosphate precipitates were nanometer sized needle-like fibrils and EDS analysis suggested a 1:1 molar ratio of U and phosphorus in these precipitates. Studies of U immobilization with strain ES6 and anthraquinone-2,6-disulfonate (AQDS), a model humic substance, showed that U reduction is the predominant mechanism and not precipitation by phosphate ligands. X-ray absorption near-edge spectroscopy (XANES) analysis showed that the predominant oxidation state of U precipitates was +4 in bicarbonate buffer, +6 in PIPES buffer and +4 in AQDS treatments. Uranium immobilization by *Cellulomonas* sp. was previously reported as reduction, however present work suggests that strain ES6 can precipitate U via both precipitation with phosphate ligands and enzymatic reduction, depending on geochemical conditions. In the presence of AQDS complete reduction of U(VI) to U(IV) by *Cellulomonas* sp. ES6 was observed. *Cellulomonadaceae* are environmentally relevant subsurface bacteria and for

the first time we report U immobilization by multiple mechanisms using the Gram positive subsurface organism *Cellulomonas* sp. ES6.

INTRODUCTION

Contamination of groundwater, soils and sediments by uranium (U) is a significant environmental problem. Sources of U include natural deposits, as well as cold war-era extraction and processing of U ore (Spear et al. 1999; Anderson et al. 2003). A survey by Riley and Zachara (1992) showed that 11 of 18 U.S. Department of Energy sites examined had groundwater and soil contaminated by U. Uranium at contaminated sites exists predominantly in two forms, U(VI) and U(IV) (Bertsch et al. 1994). U(VI) is the most oxidized valence state (Emsley 1989), and in natural environments, often forms aqueous complexes with high solubility and mobility in water. Reduction of U(VI) to U(IV) (e.g., uraninite) greatly decreases its solubility and mobility in groundwater (Lovley et al. 1991).

Subsurface environments contaminated with radionuclides pose difficult remediation challenges. According to the National Research Council (2000), cleanup across the DOE complex is expected to cost at least $200 billion dollars and will take decades to complete. Several methods are currently being used to treat U-contaminated groundwater, including anion exchange, lime softening, conventional and activated alumina coagulation, and pump-and-treat (Spear et al. 1999). Pump-and-treat technologies are usually very expensive. An alternative to these technologies is the use of indigenous subsurface bacteria for immobilizing U in contaminated groundwater and soil. Three basic mechanisms by which bacteria can immobilize U are as follows: 1) direct and indirect microbial reduction of U(VI) to U(IV) 2) uptake and accumulation by cells and 3) precipitation of U as uranyl phosphate with inorganic phosphate released by cells.

Cultures of *Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris*, *Geobacter metallireducens*, *Shewanella putrefaciens* MR1, and *Deinococcus radiodurans* , among others have been demonstrated to reduce U(VI) to U(IV) (Lovley and Phillips, 1992; Gorby and Lovley, 1992; Spear et al. 2000; Fredrickson et al. 2000a,b). Reports have shown that U(VI) reduction occurs both directly by enzymatic action in the presence of an electron donor and indirectly with model humic acids (anthraquinone-2,6-disulfonate) as an electron shuttle. Researchers have also shown that cultures of *Saccharomyces cerevisiae, Pseudomonas aeruginosa* (Strandberg et al. 1981)*, Bacillus subtilis (*Fowle et al. 2000) and *Pseudomonas* MGF48 (Malekzadeh et al. 1998) can immobilize U by cellular uptake. This accumulation can be either intracellular or extracellular depending on the culture. The third important mechanism of U immobilization is precipitation with inorganic phosphate released by cells. Cultures of *Citrobacter* sp. (Yong and Macaskie, 1998), *Acidithiobacillus ferrooxidans* (Merroun et al. 2002), *Bacillus sphaericus* (Knopp et al. 2003), and *Acinetobacter johnsonii* (Boswell et al. 1999) have been demonstrated to remove uranium from water using a phosphate release mechanism. Under aerobic growth conditions, these microorganisms can accumulate phosphorus intracellularly in the form of polyphosphate (polyP) granules (Groenestijn et al. 1988; Tandoi et al. 1998). Under anaerobic conditions, the polyP granules are subsequently hydrolyzed producing ATP for the transport and storage of low-molecular weight fatty acids (e.g. acetate, propionate). The polyphosphate hydrolysis is accompanied by simultaneous release of inorganic phosphorus to the bulk liquid (Zafiri et al. 1999; Groenestijn et al. 1987).

The microbial release of inorganic phosphorus has been coupled to bio-precipitation of heavy metals and radionuclides as cell-bound metals or radionuclide phosphates (Boswell et al. 1998; Nakajima and Sakaguchi, 1986). Metal phosphates are often highly insoluble and will

precipitate on cell surfaces (Macaskie et al. 1994; Montgomery et al. 1995). Natural immobilization of U as U(VI) phosphates occur extensively at the Kongarra deposit, Australia (Duerden, 1990). Solubility products of U(VI) phosphates as compiled by Palie (1970) vary between 4.73×10^{-47} and 2.14×10^{-11}. Arey et al. (1999) reported that addition of phosphate minerals (e.g. hydroxyapatite) can reduce the solubility and bioavailability of U in contaminated soils from the US-DOE Savannah River Site. The potential effectiveness of phosphate-bearing, reactive barrier systems for U removal from ground water is demonstrated by the work of Naftz et al. (2000) and Fuller et al. (2002). Jerden et al. (2003) reported that the low solubility of stable U(VI) phosphate minerals can limit U concentrations to less than 15μg l^{-1} and phosphate-based strategies for in situ stabilization of U in oxidizing, fluid rich environments may be effective for long-term containment. In this paper, we will show for the first time that Gram-positive subsurface organism *Cellulomonas* sp. ES6 can immobilize U(VI) by multiple mechanisms namely phosphate precipitation and reduction based on environmental conditions.

Strain ES6 is a Gram positive isolate from subsurface cores obtained from the United States Department of Energy (USDOE) Hanford site in Washington state. Viamajala et al. (2005) showed that a majority of isolates enriched from Hanford cores contaminated with Cr and U, and from uncontaminated overlying sediments, were Gram positive facultative anaerobes in, or closely related to, the genus *Cellulomonas*. Sani et al. (2002) reported that *Cellulomonas* sp. were capable of removing Cr(VI) and U(VI) from solution in the presence and absence of electron donor. Compared to Gram-negative bacteria, only a few Gram-positive organisms have been examined for metal-reduction capabilities as possible contributors to in situ metal bio-immobilization remediation strategies. Thus, the study of metal transformations catalyzed by *Cellulomonas* is environmentally relevant, particularly to the DOE Hanford site, and provides

information on metal biotransformations of Gram positive organisms. Results presented here show for the first time that a subsurface *Cellulomonas* sp. can precipitate U by release of inorganic phosphate and reduce U(VI) via enzymatic reduction. Additional, results quantify *Cellulomonas* capability for U removal in the presence and absence of anthraquinone-2,6-disulfonate (AQDS).

MATERIALS AND METHODS

Cultivation of culture

Frozen stock (-80^{o}C in 20% glycerol) of *Cellulomonas* sp. ES6 was streaked on a tryptic soy agar (TSA) plate and incubated aerobically at 30^{o}C for 3 days. Tryptic soy broth (TSB, 30g/L; Difco, Sparks, MD.) was inoculated with a single colony from the plate. Before inoculation, serum bottles containing TSB were sealed with butyl rubber septa, capped, crimped with an aluminum seal and autoclaved. After inoculation, the serum bottles were incubated aerobically at 30^{o}C on a Lab-line rotary shaker (Barnstead, WI) at 100 rpm for 3 days.

Preparation of cells and experimental design

All experiments were carried out with washed cells of a second generation culture that had been grown aerobically in TSB medium for 3 days. Based on experimental design, either bicarbonate buffer (30mM, pH 7; 1.3mM KCl) or PIPES (Piperazine-1,4-bis(2-ethanesulfonic acid**)** buffer (30mM, pH 7; 1.3mM KCl) was used for washing and re-suspension. Cells were centrifuged at 10,000 g for 20 min. The supernatant was discarded and the cell pellets were suspended in anaerobic bicarbonate or PIPES buffer with all transfers occurring in an anaerobic glove box (90% N_2; 5% H_2; 5% CO_2). This process was performed 3 times and the cells were then re-suspended under non-growth conditions (defined here as the absence of exogenous

nitrogen, phosphorus, vitamins, and other micronutrients) in sterile bicarbonate or PIPES buffer and used for U precipitation experiments.

Anoxic conditions were obtained by bubbling the experimental medium containing bicarbonate and the stock solution (1000 mg/L) of U for 30 minutes with N_2:CO_2 (80:20). The medium containing PIPES buffer was bubbled with ultrapure N_2 for 30 minutes. The final pH of the medium was 7.0. Aliquots of washed-cell suspension were added to the buffered medium contained in 25 ml serum bottles to give a total liquid volume including cells, buffer, and U, of 20 ml. Cultures were incubated at room temperature (25^{o}C) and shaken at 75 rpm. Sodium bicarbonate, potassium chloride and PIPES were obtained from Fisher (Pittsburgh, PA.). Water for all experiments had a resistivity of 18.2 megaohm-cm and was supplied from a Barnstead/Nanopure water system. All glassware was acid washed (2 N HNO_3) and rinsed thoroughly with DI water before being used. For studies with AQDS, cells were re-suspended in autoclaved buffer solution containing 0.1mM AQDS (Fisher, Pittsburg, PA.). Uranium was used in the form of $UO_2Cl_2.3H_2O$ (Bodman, Aston, Pa.). In addition to cell- and uranium-free controls, heat-killed cell controls were included. For heat-killed cell controls, aliquots of washed cell suspension were transferred to an anaerobic serum bottle in the glove box, sealed with butyl rubber septa, capped, crimped with an aluminum seal and autoclaved. Anaerobic conditions in all treatments were verified by a resazurin indicator (0.5 mg/L) changing from pink to clear indicating an $E_h \leq$ -51 mV (Twigg, 1945). Abiotic controls served as indicators that aseptic conditions were maintained during the experiments. Culture purity was also checked by bright field microscopy (Model Leica DMLB, Leica Microsystems, Germany) and by plating aliquots on TSA from individual treatment units. Samples were collected using disposable syringes, which were purged with N_2 to avoid introducing O_2 into the serum bottles.

Analytical Methods

Dry cell weight analyses were performed at the start of the experiment by filtering 0.5 ml of sample through a preweighed 0.2 μm supor® membrane syringe filter (Gelman Acrodisc). Samples from cell free controls were also filtered to ensure no change in weight due to the buffer itself. The filters were dried at 60°C for 3 days, until a constant weight was observed (Gerhardt et al. 1981). Removal of U(VI) from solution was evaluated by monitoring U(VI) concentration in unfiltered samples (0.2 ml) withdrawn by syringe and needle and measured immediately. Samples were diluted 1,000 or 4,000 times based on initial U(VI) concentration. Anoxic nanopure water was used to dilute the samples to avoid matrix effects, and 1 ml of the diluted sample was mixed with 1.5 ml of Uraplex complexing agent (Chemchek, Richland, WA). Samples were analyzed with a kinetic phosphorescence analyzer (Chemchek, Richland, WA), which uses a pulsed nitrogen dye laser to measure U(VI) concentrations in solution (Brina and Miller, 1992). Calibrations were performed using uranyl chloride solutions from 0 to 0.23 μM, yielding a U(VI) detection limit of 0.04 μM with a precision of ±5%. Samples (0.5 ml) for inorganic phosphate (P_i) analysis were withdrawn by syringe and needle and centrifuged at 10,000 g for 8 minutes. Inorganic phosphate (P_i) concentrations were determined on the supernatant spectrophotometrically using Phosver® 3 Phosphate reagent (Hach, Loveland, CO) at 880 nm on a UV-vis spectrophotometer (Milton Roy Company Spectronic® GENESYS 5™, Rochester, NY).

Transmission electron microscopy (TEM)

The embedding procedure, as well as thin sectioning, was conducted in a glove box (Ar:H_2, 95:5; Coy Laboratory Products, Inc.). The visible black precipitates resulting from *Cellulomonas* treated U(VI) were briefly (1 hour) fixed in 2.5% glutaraldehyde, and washed in

anoxic deionized water followed by a gradual ethanol dehydration series and infiltration in LR White embedding resin. Cured blocks were sectioned to 70 nm on an ultramicrotome (Leica Ultracut UCT), and sections were mounted on 200 mesh copper grids coated with formvar support film sputtered with carbon. Sections were examined using a JEOL 2010 high resolution transmission electron microscope (HR-TEM) equipped with a LaB_6 filament operating at 200 kV with resolution of 0.19 nm. Elemental analysis was performed using an Oxford EDS system equipped with a SiLi detector coupled to the TEM, and spectra were analyzed with ISIS software (Oxford Instruments). Images were digitally collected and analyzed using Gatan's Digital Micrograph.

XANES analysis

X-ray absorption near edge structure (XANES) spectroscopy was used to determine the oxidation state of uranium. Filter papers containing the sample filtrate from the experiment were dried in an anaerobic glovebox and sealed between two pieces of Kapton polymide film to prevent oxidation while minimizing X-ray absorption. Samples were stored in the glovebox until analysis. XANES data were collected on beamline 13-BM-C (GSE-CARS) at the Advanced Photon Source (APS). The APS ring operated at 7 GeV with a current of 100 mA. Energy selection was accomplished with a water-cooled Si(111) monochromator. Higher-order harmonics were eliminated by detuning the monochromator ~10%. Fluorescence spectra were recorded by monitoring the U $L_{III\alpha}$ fluorescence with a 13-element Ge semiconductor detector. Incident and transmitted intensities were measured with in-line ionization chambers. The energy range studied was -200 to +500 eV about the $L_{III\alpha}$-edge of U (17.166 keV). All spectra were collected at ambient temperature and pressure and 2 to 4 individual spectra were averaged for each sample.

Spectra were analyzed using IFEFFIT and WinXAS software. Fluorescence spectra were normalized, background subtracted, and the atomic absorption normalized to unity. First derivative XANES spectra were smoothed with a 17.6% Savitsky-Golay algorithm. The extent of downward shift in binding energy for a metal is related to its oxidation state, with a shift towards lower binding energy indicative of a lower oxidation state. The relative amount of reduced uranium in each sample was determined by fitting a series of Gaussian functions to the smoothed derivative spectra using PeakFit v4 (AISN Software Inc). The ratio of the amplitudes of the Gaussian functions centered at the U(IV) and U(VI) first derivative inflection points (17.172 and 17.176 keV, respectively) was related to U(IV)/(VI) proportions using five standards having U(VI) percentages ranging from 10 to 90%. The uncertainty of the fitting routine is ± 10%.

Statistical analysis

Each set of experiments was carried out in duplicate and all critical treatment units were repeated as separate experiments to ensure reproducibility. In each set of experiments, duplicate treatment profiles were similar in P_i concentration and U(VI). Data presented here are the mean of duplicates and error bars represent one standard deviation. One-way analysis of variance (ANOVA) was used to determine the statistical significance of differences in lag times of U removal among treatments. The threshold level of statistical significance for this study was $\alpha = 0.05$.

RESULTS AND DISCUSSION

U(VI) precipitation experiments with PIPES buffer

Figure 1a shows soluble inorganic phosphate (P_i) concentration profiles at two cell concentrations. Initially, the P_i concentration was approximately zero in all treatments (except the heat killed cell control). With heat-killed cells there was a measurable initial P_i concentration,

which decreased with time as U precipitated. In all other cell containing treatments, P_i concentrations increased over time. The P_i concentration in the cell free control was approximately zero throughout the experiment indicating that the buffer itself contained no measurable P_i. Treatments with U(VI) also showed a P_i concentration increase, however the increase was less than the corresponding U(VI)-free treatment. Like most other Gram-positive bacteria, the cell walls of *Cellulomonas* consist of secondary polymers, which often include teichoic acids and teichuronic acids and contain phosphate and carboxylate residues, respectively (Panak et al. 2000). The initial amount of P_i observed in heat-killed cells is likely from these polymers, nucleic acids, and other phosphate rich cellular components that may have been released in the heating process.

Figure 1b shows concentration profiles of soluble U(VI) observed for the cell suspensions and cell-free controls under non-growth anaerobic conditions in PIPES buffer. Since precipitation of U(VI), as metaschoepite, occurs in PIPES buffer for U(VI) concentrations greater than 0.125 mM, only 0.1mM U(VI) was used with PIPES buffer (Fredrickson et al. 2000b). In all treatments containing cells, including the heat-killed cell control, soluble U(VI) concentrations decreased over time. No change in soluble U(VI) concentration was observed in cell-free controls. In the treatment with heat-killed cells, the decrease in U(VI) concentration occurred immediately after inoculation, while with viable cells there was a considerable lag time before U(VI) started precipitating. The immediate onset of U(VI)-precipitation with heat-killed cells was likely caused by the availability of dissolved phosphate due to the heat induced cell lysis and release of P_i into solution. As there are likely no enzymes in heat-treated cells, the P_i concentration did not increase above the initial concentration, which was sufficient to initiate precipitation. In treatments with viable cells, the P_i concentration was initially zero, but increased

with time (Figure 1a). It appears that once the saturation concentration of P_i was reached in the solution, precipitation of U(VI) began. Yong and Macaskie, (1995) observed similar results with *Citrobacter* sp. and attributed the delay in onset of uranyl phosphate removal to the solubility product and the time required for the formation of nucleation sites and precipitation foci. Both cell treatments and abiotic tests (data not shown) showed that the saturation concentration of P_i in PIPES buffer to initiate U (0.1 mM) precipitation was approximately 0.03 ± 0.01 mM . Precipitation of U(VI) in heat-killed cell treatments was observed only when P_i concentration was greater than saturation concentration.

U(VI) precipitation experiments with bicarbonate buffer

Figure 2a shows P_i release by ES6 in bicarbonate buffer at two different concentrations in the presence and absence of U(VI). As in PIPES buffer, cells were under non-growth conditions and two different cell concentrations were used to study the kinetics of phosphate release by ES6. Higher cell concentrations were used, since preliminary results showed that U(VI) removal occurred at a slower rate in the bicarbonate buffered systems. These experiments show that as compared to PIPES buffer, the rate of increase of P_i concentration was slower in bicarbonate buffer. Similar to the PIPES buffered cultures, in all experiments containing viable cell suspensions, P_i concentrations increased over time until a stable value was reached or the experiment was stopped. In addition to the heat-killed control with U(VI), an additional control with heat-killed cells and no U(VI) showed no increase in P_i concentration over time. This result suggests that only active enzymes were responsible for phosphate release into the solution. In bicarbonate buffer, the concentration of P_i (0.11 ± 0.01 mM) required to initiate uranium precipitation was observed to be higher than that required in PIPES buffer. This is likely due to complexation of U in bicarbonate buffer thereby decreasing the concentration of U in solution

available for precipitation. The solubility product of uranyl phosphate calculated from experiments in PIPES (K_{SP} = 3 x 10^{-9}) and bicarbonate (K_{SP} = 1.1 x 10^{-8}) were less than the values reported by Palie (1970). This was expected as the values reported by Palie (1970) were obtained in system with only water. Since the solubility product of uranyl phosphate is constant in a specific medium, a lower concentration of U in solution requires a higher concentration of P_i to initiate precipitation.

Similar to treatments in PIPES buffer, viable cells treated with U(VI) in bicarbonate buffer also removed U(VI) from solution after P_i was released into solution. Figure 2b shows the respective concentration profiles of soluble U(VI) observed for cell suspensions and cell-free controls under non-growth anaerobic conditions in bicarbonate buffer. U(VI) concentrations decreased over time in all treatments with viable cells. No change in soluble U(VI) concentration was observed in cell-free controls or in heat-killed cell control. In heat-killed controls, active enzymes responsible for phosphate release were very likely denatured and the initial P_i concentration was less than the saturation concentration of P_i required to initiate U precipitation, so no decrease in soluble U(VI) concentration was measured.

Similar to PIPES buffer, a lag time was observed before U started precipitating in bicarbonate buffer treatments containing cells. Observations from both PIPES and bicarbonate experiments showed that the lag time before the onset of U(VI) precipitation was inversely proportional to cell concentration. The proportionality constants (PIPES = 7.8 ± 0.6 mg h/L; bicarbonate = 130.5 ± 32.8 mg h/L) defined as the ratio of cell concentration over lag time were statistically different ($\alpha = 0.05$) between PIPES and bicarbonate buffer for equal U concentration (0.01 mM). This may be the result of two factors: 1) high saturation P_i concentration in bicarbonate buffer, and 2) slower rate of P_i release in bicarbonate buffer than in PIPES. The lag

time for cell suspensions treated with 0.25 mM U(VI) was less than in the 0.01 mM treatment. This was expected because the solubility product of uranylphosphate is a constant in a specific medium (Eq 1), such that U(VI) and P_i concentrations should be inversely related to each other.

$$UO_2(HPO_4) \longrightarrow UO_2^{2+} + PO_4^{3-} + H^+ \quad (1)$$

Assuming a molar ratio of 1:1 of U:P in the precipitated uranylphosphate, with 0.25 mM U(VI), 0.25 mM P_i should have co-precipitated. However, after complete precipitation of 0.25 mM U(VI), the difference between the total amount of soluble P_i in treatment with 0.25 mM U(VI) and without U(VI) is 0.20 mM which is less than 0.25 mM (Figure 2a). This shows that there may be another mechanism, most likely enzymatic reduction, acting in addition to uranyl phosphate precipitation for loss of U from solution in bicarbonate buffer. Sani et al. (2002) reported that treatments of U(VI) with *Cellulomonas* spp., showed insignificant difference between filtered and unfiltered U(VI) concentrations and hence concluded that cell-associated U(VI) was not in significant amount. The KPA can measure only soluble U(VI), and hence analysis of filtered and unfiltered U samples cannot differentiate between U(VI) reduction and abiotic precipitation as uranyl phosphate.

Figure 3 shows soluble U(VI) concentrations and corresponding P_i concentrations in both PIPES and bicarbonate buffer. From the figure, it is clear that a small concentration of P_i is sufficient to start precipitation of U(VI) in PIPES buffer, while a much higher P_i concentration is required to initiate precipitation in bicarbonate buffer. Only after the soluble P_i reached a saturation concentration, could precipitation of U(VI) begin. The initial concentration of P_i (0.095 ± 0.05 mM/(g/L)) in heat-killed cells in bicarbonate buffer was approximately equal to that measured in PIPES buffer after normalizing for the higher cell concentration. This initial concentration of P_i in heat-killed cells in bicarbonate buffer was less than the saturation

concentration of P_i required to initiate uranium precipitation. Hence unlike PIPES buffer, no decrease in P_i or U concentration was observed in uranium treated heat-killed cells. Subsurface systems have significant amount of humic acids, that play a major role in fate, and transport of metals/radionuclides and hence it is necessary understand their potential effects on P_i release and U removal.

Effects of AQDS on P_i release and U(VI) removal

One of the most important soil properties that influence the transport of contaminants is naturally occurring organic matter, mainly humic materials. Humic substances also called *humus*, are yellow to dark brown polymers formed by microbial mediated reactions. Humic substances are heterogeneous high-molecular-weight organic materials and are widely distributed on the earth's surface (Benz et al. 1998). Humic materials are thermodynamically stable and predominant in most of the subsurface (Watts, 1997). They function as catalysts for bacterial metal reduction. Lovley et al. (1998) reported that relatively low concentrations of humic substances are sufficient to facilitate reduction. Humic acids, because of their recalcitrance to biodegradation, are common to many soils and sediments. Lovley et al. (1996) reported that microorganisms can donate electrons to humic acids, which can shuttle electrons between the microbe and Fe (III) oxide. 2,6-anthraquinone disulfonate (AQDS) has been proposed as a model humic compound and has been shown to catalyze microbial reduction of Cr(VI), U(VI), Fe(III) and Mn(VI) (Fredrickson et al. 2000a,b; Lovley et al. 1996; Gounot, 1994). Because of the ubiquitous nature of humic substances in the subsurface, it is important to understand the effects of AQDS on P_i release rates and soluble U(VI) removal rates.

Experiments were performed in PIPES and bicarbonate buffer with treatments containing 0.1 mM AQDS, and ES6, with and without U(VI). During the experiments, the media turned

yellow indicating the reduction of AQDS by *Cellulomonas*. In both PIPES and bicarbonate buffer, AQDS treatments (PIPES – Figure 1b, bicarbonate – Figure 2b) showed a different soluble U(VI) profile compared to treatments without AQDS. This might be due to the reduction of U(VI) to U(IV) by reduced AQDS rather than precipitation by P_i. Reduction of U(VI) by AQDS has been reported previously (Fredrickson et al. 2000 a, b). In addition, reduction of U(VI) in the presence of AQDS in PIPES buffer was much faster than in bicarbonate buffer. $UO_{2(aq)}^{2+}$ forms a series of strong aqueous complexes with CO_3^{2-} [e.g., $UO_2(CO_3)_{3(aq)}^{4-}$, $UO_2(CO_3)_{2(aq)}^{2-}$ and $UO_2CO_{3(aq)}^{o}$). The equilibrium speciation in PIPES buffer is predominantly hydroxo complexes $UO_2OH_{(aq)}^{+}$ or $UO_2(OH)_{2(aq)}^{o}$. Thermodynamically $UO_2(OH)_{2(aq)}^{o}$ is the most strongly oxidizing species and hence tends to be reduced first compared to other carbonate complexes based on potential free energy change (Fredrickson et al. 2000b; Scott and Morgan, 1990; Zehnder and Stumm, 1988).

Figure 4 shows the comparison of P_i release by cells in treatments with/without AQDS and with/without soluble U(VI). In the absence of uranium, there is no difference in the rate and extent of P_i release with or without AQDS with either buffer. This indicates that AQDS does not enhance phosphate release by cells. Single-factor ANOVA ($\alpha = 0.05$) with P_i concentrations in AQDS treatments with and without 0.1 mM U(VI) showed that P_i concentrations were not statistically different. However a significant decrease in soluble U(VI) concentrations was observed in AQDS treatments in both PIPES (Figure 1b) and bicarbonate (Figure 2b) buffered systems. If U(VI) had been precipitated by P_i, a decrease in P_i concentration would have been observed. Reduction of U by AQDS was confirmed by analysis of these precipitates by XANES. This shows that *Cellulomonas* can utilize a combination of direct and indirect U(VI) precipitation to effectively immobilize U(VI) in subsurface environments. Fredrickson et al.

(2000a) has reported that *Deinococcus radiodurans* can oxidize lactate coupled to the reduction of Fe(III) and Cr(VI), but cannot reduce U(VI) directly without AQDS. Such an inability to directly reduce U was attributed to enzyme substrate specificity or enzyme inhibition.

Characterization of microbially mediated U precipitates: (A) XANES Spectra

The U L_3-edge XANES spectra for U(VI) treated with ES6 in PIPES buffer in the absence of AQDS was similar to that of U(VI) standard (uranyl nitrate). XANES analysis of U precipitates in PIPES treatment showed that the oxidation state of U was predominantly +6 (Figure 5). This confirms that in PIPES buffer with no AQDS, precipitation by phosphate and not reduction is the predominant mechanism of U immobilization. How ever the U L_3-edge XANES spectra for U(VI) treated with ES6 in bicarbonate buffer with and without AQDS and in PIPES buffer with AQDS were similar to that of natural uraninite. No decrease in phosphate concentration was observed in both PIPES and bicarbonate buffered treatments with AQDS while U concentration decreased from 0.1 mM to approximately zero mM. This observation along with XANES spectra showed that reductive precipitation is the predominant mechanism of U removal in AQDS treatments. XANES analysis performed on U precipitates in bicarbonate buffer showed that the oxidation state of U in these precipitates was predominantly +4 (Figure 5). EDAX analysis showed that molar ratio of U and P in these precipitates is approximately 1:1. This shows that ES6 may have the capacity to reduce U(VI) to U(IV). However this process is very slow but gets induced due to long residence time of U(VI) in bicarbonate buffered system. The absence of both phosphate release enzyme and U reducing enzyme in heat-killed cell treatments, resulted in no decrease in U concentration by either mechanisms. In contrast, with PIPES buffer, the low saturation concentration, rapid rate of phosphate release and U precipitation would have overwhelmed any observed effects of enzymatic reduction.

(B) TEM and EDS analysis

Electron dense granules were observed during TEM analysis of whole cells that had not been exposed to U (Figure 6a). Such metachromatic granules are considered to be masses of volutin, a polymetaphosphate (Mutsunori et al. 1999; Nielsen et al. 1998; Liu et al. 1996; Nester et al. 2004). Inorganic polyphosphates are linear polymers of P_i residues linked by phosphoanhydride bonds (Merroun et al. 2002) with chain lengths varying between 3 and 1000 P_i residues, depending on the organism, its growth, and other physiological conditions (Van Veen et al. 1993). Polyphosphate has many biochemical functions, for example, as a substitute for ATP, sugar and adenylate kinases, an energy source, and a chelator of bivalent metals ions (Keasling and Hupf, 1996). The presence of electron dense granules in ES6 and release of excessive phosphate shows that polyphosphate is likely the source of phosphate release in ES6.

Figure 6b shows a typical TEM image of ES6 after exposure to uranium in bicarbonate buffer. Irregularly shaped electron-dense granules were observed in TEM images. The darkening of cellular surface is attributed to electron dense uranyl phosphate precipitation. To verify the cellular location of uranyl phosphate precipitates, TEM was performed with thin sections of ES6 treated with U (Figure 7a). It can be seen from Figure 7a that uranylphosphate precipitates are bound not only to the cell surface (areas of high contrast) but are also present as intracellular granules. The corresponding EDS spectrum of uranylphosphate precipitates is shown in Figure 7(c). EDAX analysis confirmed the presence of U and P in these precipitates with a molar ratio of approximately 1:1. Such a trend of both intra and extra-cellular U association was observed in *Mycobacterium smegmatis* (Andres et al. 1993 and 1994). Krueger et al. (1993) reported that *Pseudomonas fluorescens* accumulates uranium in the periplasm along its plasma and outer membranes as fine-grained, uranium-crystals. Francis et al. (2004) suggested that precipitates in

Bacillus subtilis were originally surface associated, but were then brought into the cell by microtome.

Figure 7b shows that uranylphosphate precipitates were nanometer size needle-like fibrils. Such needle-like fibrils were reported by Marques et al. (1991) with *Pseudomonas sp*. Francis et al. (2004) reported that extracellular association of uranium with bacterial cell surfaces is primarily due to physical and chemical interactions involving adsorption, ion exchange, and complexation. Carboxylate, hydroxyl, amino, and phosphate are the predominant functional groups in bacterial cell walls, exopolymers and lipids. These functional groups are capable of forming U complexes. Macaskie et al. 2000 reported that lipopolysaccharide (LPS) in *Citrobacter* serves as a major site of UO_2^{2+} binding and also of uranyl phosphate nucleation. But Langley and Beveridge, (1999) concluded that carboxylic acid groups on the O side-chains of LPS do not contribute significantly to metal binding (gold, copper, iron, and lanthanum) and, instead hypothesized a role for the phosphate groups in mineral formation.

Panak et al. (2000) showed that *Bacillus* sp. cell wall components with phosphate residues (e.g., polysaccharides, teichoic and teichuronic acids or phospholipid layers of the membranes) can bind U. While cellular functional groups can be responsible for extracellular association of U, Strandberg et al. (1981) proposed that passive transport mechanisms were responsible for the formation of dense U deposits inside *Pseudomonas aeruginosa*. Figure 8 (a,b) shows TEM images of uranylphosphate precipitates that did not appear to be cell-associated, while Figure 8 (c) shows the respective EDS spectrum of the precipitates. Figure 8b shows that these precipitates were also nanometer size needle-like fibrils, and these also had a 1:1 U:P ratio (Figure 8c). The general formula of uranylphosphate precipitates is $M(UO_2PO_4)_2.nH_2O$ in which M may be a mono or divalent cation. These compounds have a typical structure of negatively

charged layers of $(UO_2PO_4)n^n$ separated by staggered layers of water molecules and compensating cations. In the absence of cations such as Na^+, or NH_4^+, H^+ can substitute (Yong and Macaskie, 1995). Our experimental results show that *Cellulomonas* sp. ES6 can immobilize U by both precipitation with phosphate ligands as well as reduction in buffered systems in the absence of AQDS, a model humic substance. Absence of significant phosphate release in heat-killed treatments showed that this process is probably enzyme mediated. The rate and onset of U(VI) removal in PIPES and bicarbonate buffered systems appears to be controlled by the uranylphosphate solubility product and the rate of P_i released by the cells. To better understand the influence of *Cellulomonas* on soluble U(VI) concentrations, it was necessary to model the release of P_i.

Kinetic modeling

Nongrowth Monod kinetic models have been used to model enzyme mediated heavy metal or radionuclide reduction (Truex et al. 1997; Guha et al. 2001). Pauli and Kaitala (1996) used the Michaelis-Menton model to quantify phosphate uptake kinetics by *Acinetobacter* isolates. In this study, a Monod-based kinetic expression (Eq. 2) was used to describe P_i release under non-growth conditions.

$$\frac{dP_i}{dt} = \frac{V_{MAX}S}{K_M + S} \qquad (2)$$

where V_{MAX} is the maximum specific P_i release rate in mM P_i/h; K_M is Monod half-saturation coefficient in mM P_i; S is phosphate source that acts as substrate defined in Eq. 3.

$$S = P_F - P_i;\ P_F = X * G \qquad (3)$$

where P_F is maximum/final concentration of soluble P_i observed in the system; P_i is the inorganic phosphate concentration at time point of interest; X represents dry weight of cells in mg/L; G = Maximum phosphate yield (mM P_i / (mg dry weight cells/L)). Since the substrate for enzymatic

phosphate release is intracellular, P_F is proportional to biomass concentration. When soluble P_i concentration equals P_F, substrate concentration (S) becomes zero and no more release of P_i by the cells is possible. Equation 2 can be solved for P_i by Euler's method. Due to large number of data points over a short time interval, it is valid to use Euler's method to solve Equation 2. The kinetic parameters V_{MAX}, K_M, G were estimated using the Solver function in Microsoft Excel™, which uses a generalized reduced gradient nonlinear optimization code. Initial guesses for the parameters were provided and used to minimize the sum of the squared differences between the experimentally determined and predicted P_i concentrations. The Monod model was applied to all experimental data sets for both PIPES buffer and bicarbonate buffer to determine individual values for the three model parameters. The estimated mean parameter values and corresponding standard deviations are shown in Table 1.

To test the model, average values of V_{MAX}, K_M, and G were used to calculate P_i concentration as a function of time for PIPES and bicarbonate treatments. These results are shown as solid lines in Figure 9 in which simulated P_i concentrations are imposed over the experimental data points. It can be seen that the model fits correspond closely to experimental observations. Statistical comparisons were made between the experimentally determined data and the model defined by the three constants by using coefficients of determination, r^2. In all the experiments, r^2 was greater than 0.96. Single-factor ANOVA showed that mean values of V_{MAX}, K_M, and G were statistically different between the two buffers at 95% confidence limit. Both the maximum specific P_i release rate, V_{MAX} and Monod half-saturation coefficient, K_M was significantly higher in PIPES buffer than in bicarbonate buffer. This indicates that phosphate release rates were greater in PIPES buffer than in bicarbonate buffer. This resulted in a slower uranium removal rate in bicarbonate buffer.

Kortstee et al. (1994) reported that on average, growth of cells requires 5 – 10 mg P_i per g dry weight. Groenestijn et al. (1988) reported that *Acinetobacter* strain 210A can accumulate polyphosphate between 40 to 100 mg P_i per g dry weight of cell under different growth conditions. Experimental studies showed that phosphate accumulation (as measured by phosphate release) by strain ES6 varied between 53.6 ± 0.4 (PIPES buffer) and 37.6 ± 0.003 (bicarbonate buffer) mg P_i per g dry weight of cell. The maximum phosphate yield, G, in PIPES buffer is higher than in bicarbonate buffer. Since cells for all experiments were prepared same, the specific uptake of phosphate should also be the same. It was anticipated that the value of G would be independent of the buffer system. But G was found to be statistically different. One plausible explanation for different G in different buffers might be the buffer effect on phosphate release enzymes similar to slow phosphate release rates. Even though the ionic strength of two buffers was the same, the enzymes responsible for phosphate release may be sensitive to buffer salts and hence the buffer type could have caused a significant change in enzymatic phosphate release. Different G can also be reasoned that same amount of phosphate could have accumulated during growth, but due to long duration of experimental study under non-growth in bicarbonate buffer which in turn is a result of slow phosphate release rate, cell lysis would have occurred thereby limiting the total amount of phosphate that can be released. The parameters determined could be used to predict inorganic phosphate release rates, which in turn can be used to predict precipitation of uranium and other heavy metal phosphate complexes.

In conclusion, the results presented here demonstrate the potential of *Cellulomonas* in precipitating U(VI) as uranyl phosphate as well as U(IV) in the absence of humic substances and as U(IV) in the presence of electron shuttles (e. g. AQDS). This is the first report of an environmentally relevant subsurface microorganism capable of uranium immobilization by two

different mechanisms (reductive precipitation or direct precipitation with phosphate ligands) based on environmental conditions. Our results offer a first step toward understanding and quantifying the phosphate release and uranium removal by strain ES6. Research is currently underway in our laboratories to identify the effects of other co-contaminants and various environmental factors on cellular metabolism, and on P_i release. The ability of *Cellulomonas* sp. to reduce Cr(VI), and to precipitate U(VI) as U(IV) and uranyl phosphate indicates the potential long-term application of in situ biological barriers for mixed heavy metal and radionuclide removal.

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Table 1 – Kinetic values determined from experimental data (mean ± standard deviation)

Parameter	PIPES	Bicarbonate
V_{MAX} mM P_i h^{-1}	0.0058 ± 0.0011	0.0021 ± 0.0005
K_M mM P_i	0.3863 ± 0.0347	0.2928 ± 0.0237
G mM P_i (mg DW Cells/ml)$^{-1}$	0.4093 ± 0.0164	0.3151 ± 0.0347

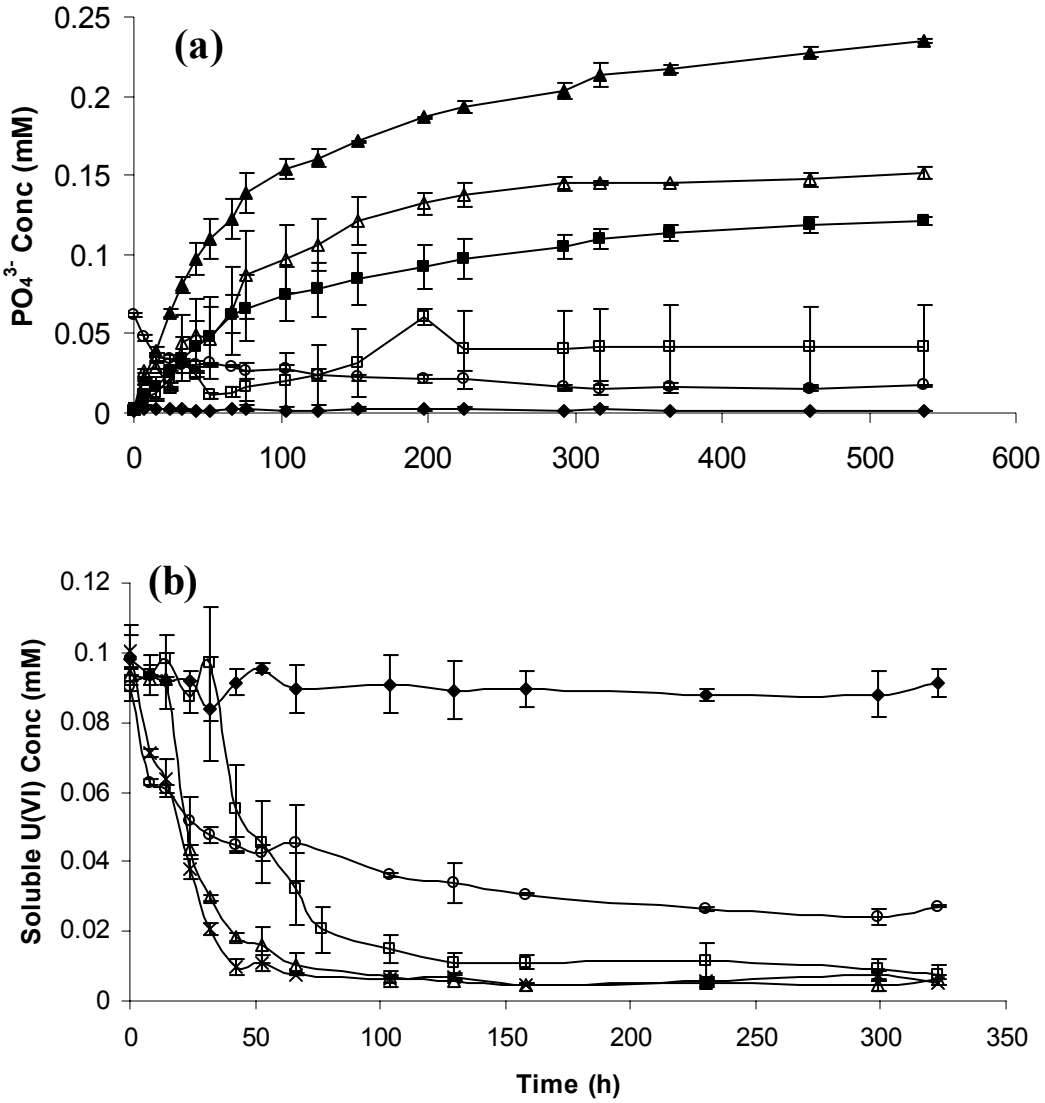

Figure 1 – (a) – Concentration profile of inorganic phosphate (P_i) released by cells in PIPES buffer over time; (b) - Concentration profile of soluble U(VI) in PIPES buffer; ◆ - Cell free control + 0.1 mM U(VI), ○ - Heat killed cells (equivalent to 520 mg DW cells/L) + 0.1 mM U(VI), ■ - 260 mg DW cells/L, □ - 260 mg DW cells/L + 0.1 mM U(VI), ▲ - 520 mg DW cells/L, △ - 520 mg DW cells/L + 0.1 mM U(VI), × - 520 mg DW cells/L + 0.1 mM AQDS + 0.1 mM U(VI); DW – Dry Weight

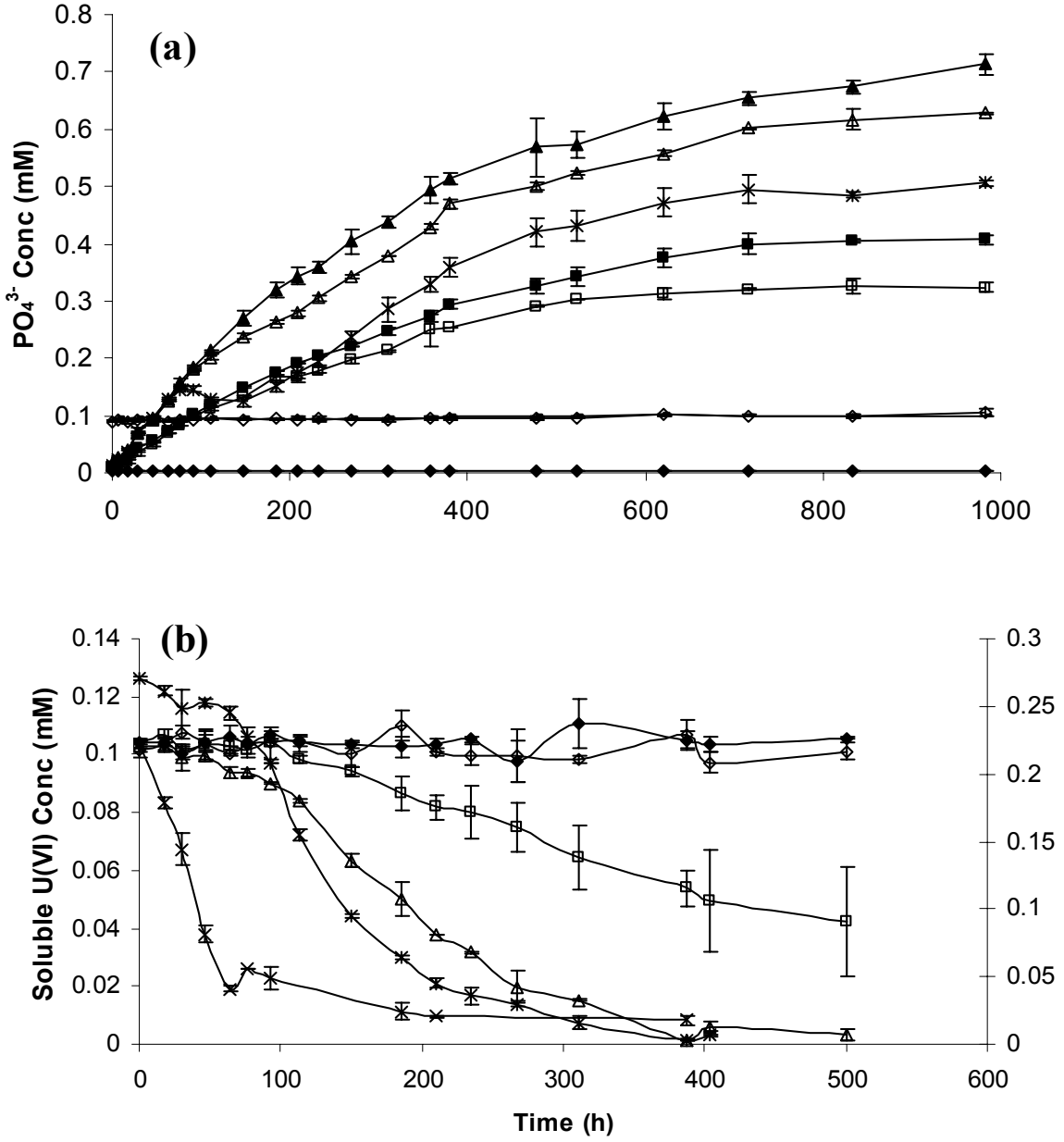

Figure 2 – (a) - Concentration profile of inorganic phosphate (P_i) released by cells in bicarbonate buffer over time; (b) - Concentration profile of soluble U(VI) in bicarbonate buffer; ◆ - Cell free control + 0.1 mM U(VI), ◇ - Heat killed cells (equivalent to 1150 mg DW cells/L) + 0.1 mM U(VI), **--** Heat killed cells (equivalent to 1150 mg DW cells/L), ■ - 1150 mg DW cells/L, □ - 1150 mg DW cells/L + 0.1 mM U(VI), ▲ - 2300 mg DW cells/L, △ - 2300 mg DW cells/L + 0.1 mM

U(VI), × - 1150 mg DW cells/L + 0.1 mM AQDS + 0.1 mM U(VI), ∗ - 2300 mg DW cells/L + 0.25 mM U(VI); DW – Dry Weight

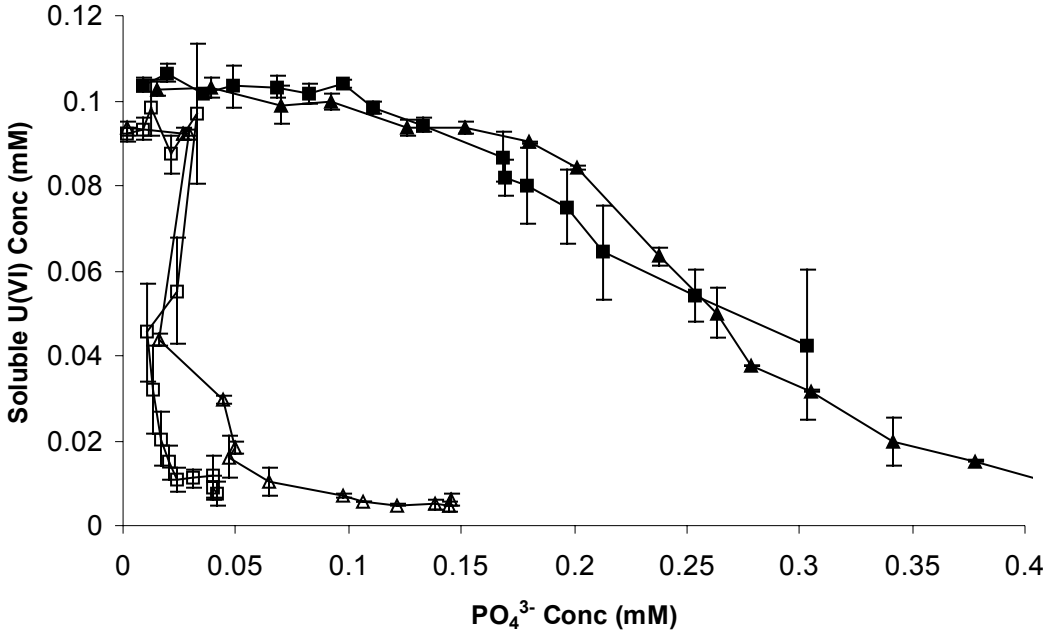

Figure 3 – Comparison of soluble U(VI) removal profile in two different buffers; □ - PIPES + 260 mg DW cells/L, △ - PIPES + 520 mg DW cells/L, ■ - Bicarbonate + 1150 mg DW cells/L, ▲ - Bicarbonate + 2300 mg DW cells/L; DW – Dry Weight

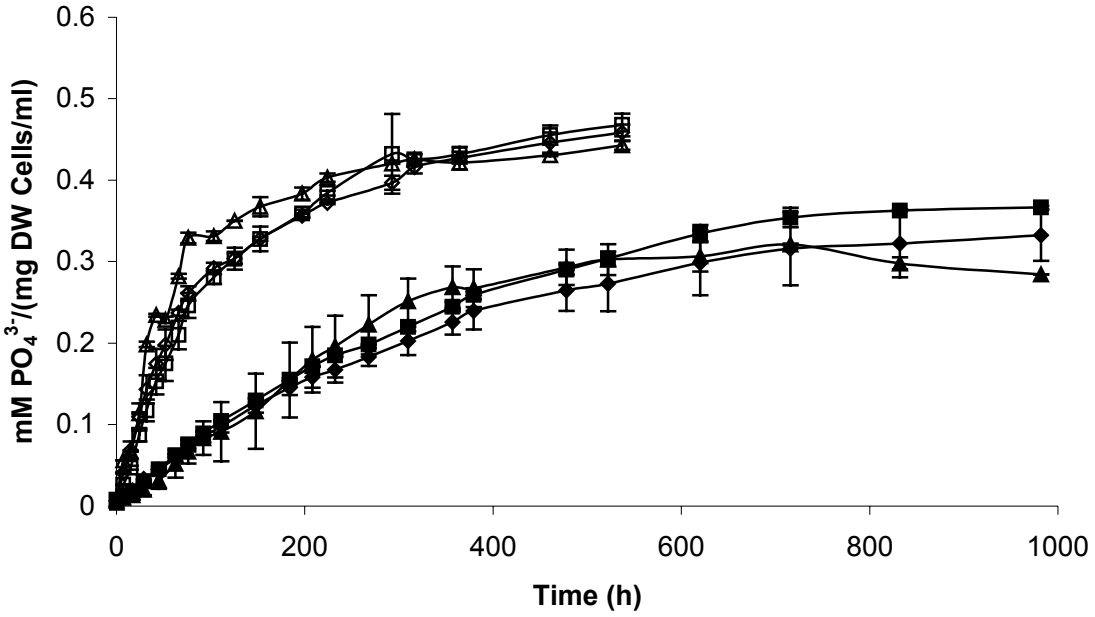

Figure 4 – Comparison of P_i concentration profile in PIPES and bicarbonate buffer with normalized biomass, with/without AQDS and with/without U(VI); ◇- PIPES + Cells, □ - PIPES + Cells + 0.1 mM AQDS, △ - PIPES + Cells + 0.1 mM AQDS + 0.1 mM U(VI), ◆ - Bicarbonate + Cells, ■ - Bicarbonate + Cells + 0.1 mM AQDS, ▲ - Bicarbonate + Cells + 0.1 mM AQDS + 0.1 mM U(VI)

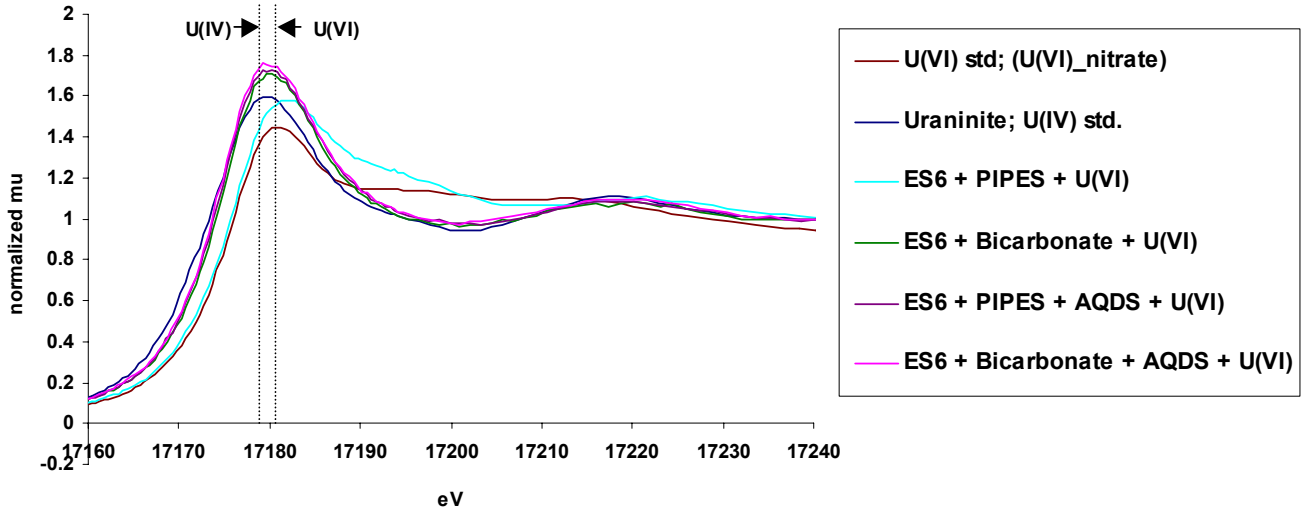

Figure 5 – XANES spectra of uranium precipitates

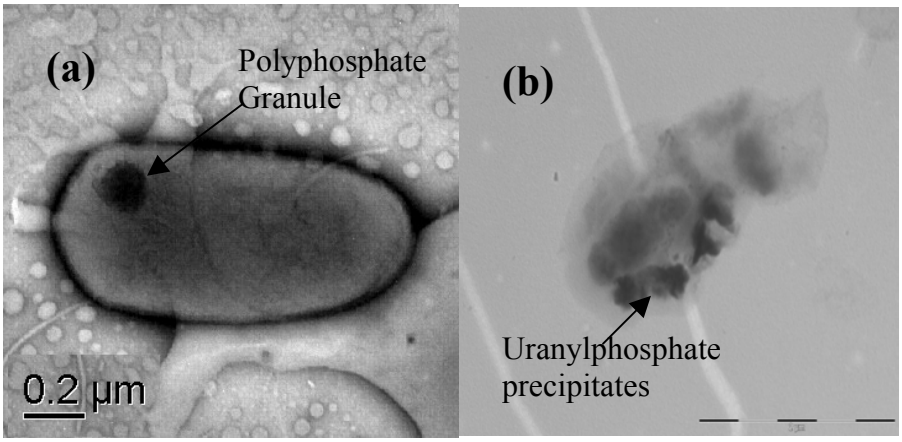

Figure 6 – Transmission electron micrograph of whole cell of strain ES6 (a) before and (b) after treatment with uranium

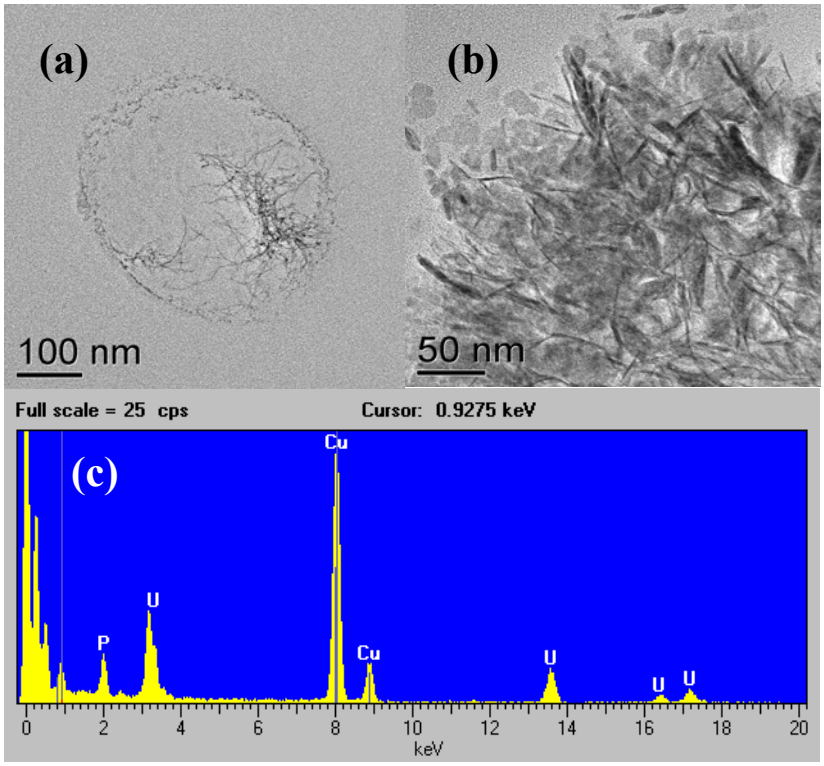

Figure 7 - Transmission electron micrograph of thin section of strain ES6 cells challenged with uranium (a); (b) Nanometer size fiber like uranylphosphate precipitates; (c) EDS spectrum of cell associated uranylphosphate precipitates

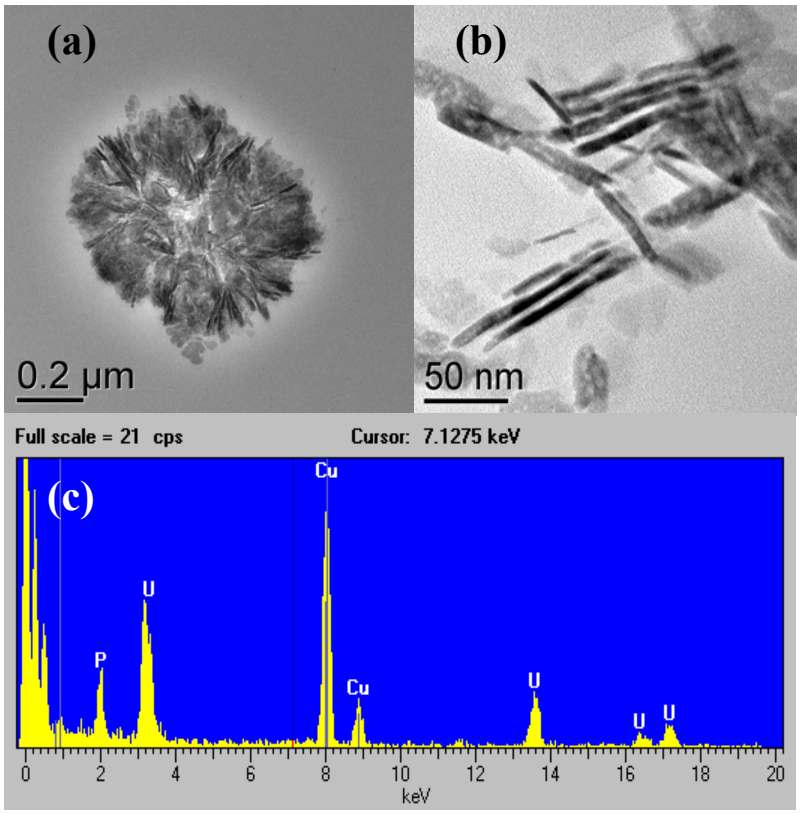

Figure 8 – a, b – Transmission electron micrograph of uranylphosphate precipitates unassociated with cells; (c) EDS spectrum of uranylphosphate precipitates

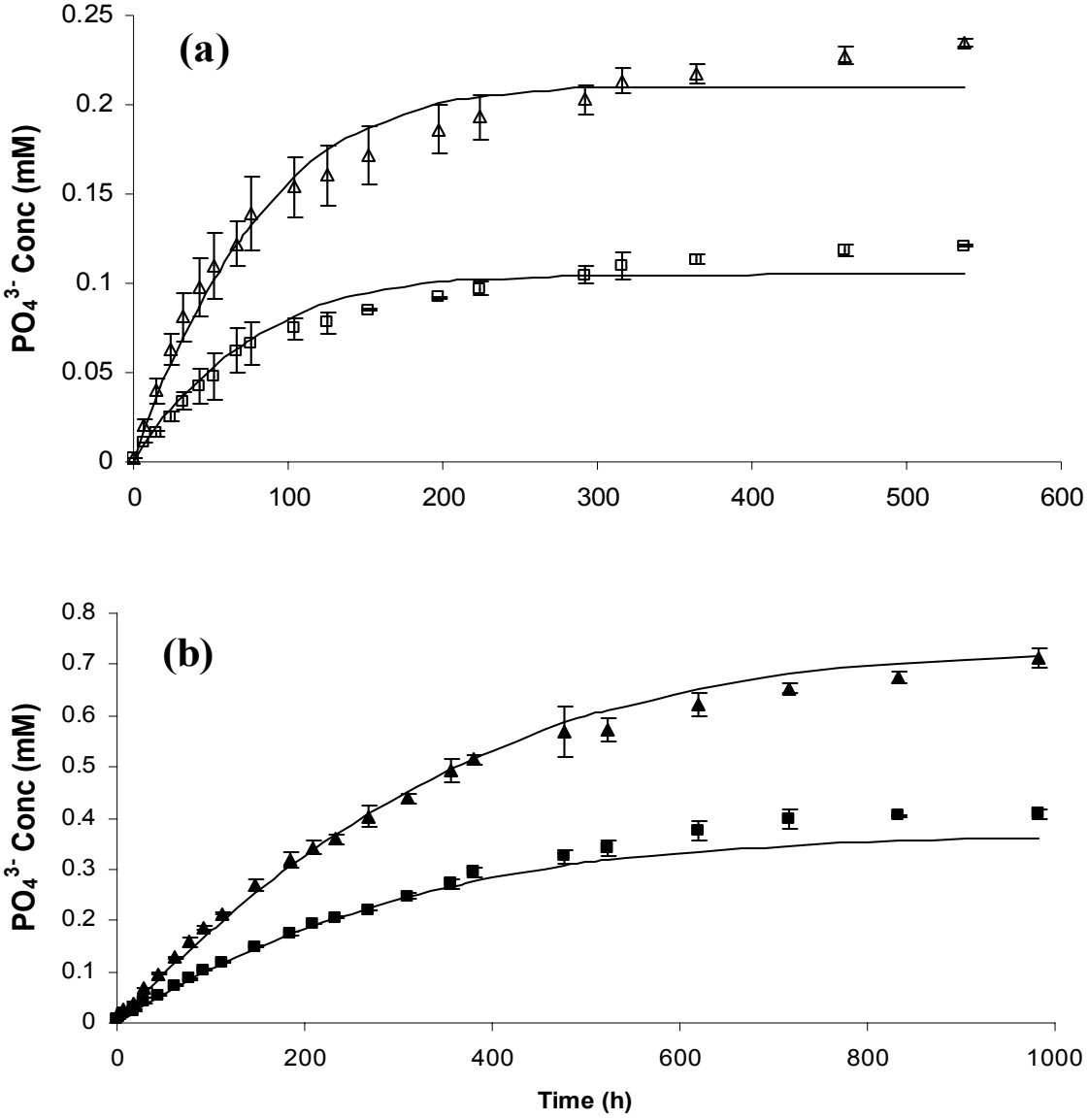

Figure 9 – (a) – Experimental data and model of P_i concentration profile in PIPES buffer; (b) – Experimental data and model of P_i concentration profile in bicarbonate buffer. □ - PIPES + 260 mg DW cells/L, △ - PIPES + 520 mg DW cells/L, ■ - Bicarbonate + 1150 mg DW cells/L, ▲ - Bicarbonate + 2300 mg DW cells/L; Data points represent averaged experimental data sets; Solid line represents model predicted outcomes for those points; DW – Dry Weight

FUTURE WORK

The results presented in this thesis work were based on growth of *Cellulomonas* sp. ES6 on nutrient rich medium (TSB). It is necessary to evaluate the ability of strain ES6 to accumulate phosphate aerobically on minimal nutrient medium. Phosphate accumulation depends on growth medium composition, growth conditions, pH and cations [1,2]. This can be tested by growing the cells on simulated ground water medium (SGM) with excess phosphate (multiple concentrations) and varying concentrations of field applicable carbon sources namely sucrose, xylose and molasses. Phosphate uptake can be measured by two potential ways, 1) measuring phosphate and cell concentration in growth medium with time, 2) re-suspension of washed cells in buffered medium under non-growth conditions and measuring concentration of inorganic phosphate released by cells. From the results of these experiments, it is possible to identify optimal phosphate concentration, sugar source, and its concentration required during growth to accumulate maximum phosphate.

Uptake of phosphate is usually accompanied by simultaneous uptake of cations such as Mg^{2+}, Ca^{2+} or K^{+}. It is possible to identify which of these cations are important for phosphate accumulation by *Cellulomonas* sp. ES6 by growing cells on minimal medium with excess phosphate in the presence and absence of cations. The decrease in cation concentration with maximum phosphate uptake could be an indicator of crucial cation and its concentration for phosphate accumulation. This can also be tested by measuring phosphate uptake during growth in the absence of cations.

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[1]Groenestijn JW, et al., 1988, Appl. Environ. Microbiol., 54(12), 2894-2901
[2]Pauli ASL, Kaitala S, 1995, Appl. Microbiol. Biotechnol., 43, 746-754

Most of the industrial waste streams and sludges have either low pH or high pH based on treatment conditions [3]. Hence it is necessary to elucidate the effect of pH on phosphate release process. This can be tested by growing the cells on either nutrient rich medium or minimal medium to accumulate excess phosphate and then re-suspending the washed cells on buffered non-growth medium with specific pH. The rate and amount of phosphate release can be compared over varying pH. This study would help us better understand the effect of pH on phosphate release by ES6 and hence whether the organism is applicable for a particular case study.

Apart from pH, waste streams from nuclear industry typically contain high concentrations of anions, heavy metals, organic solvents and chelators [4]. These co-contaminants may affect phosphate release process and hence metal/radionuclide immobilization. Van Neil (1998) has shown that nitrate can inhibit phosphate release and hence it is necessary to evaluate the effect of other co-contaminants such as sulphate, nitrite, heavy metals (chromium, copper, nickel) on phosphate uptake as well as release process.

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APPENDIX A

The data in this appendix is the raw data used for the results presented in Chapter 2

Table A 1: Inorganic phosphate data in PIPES buffer

The following tables contain data for inorganic phosphate released by *Cellulomonas* sp. ES6 in PIPES buffer under non-growth conditions. Inorganic phosphate concentrations were measured by reading the absorbance at 880 nm with time.

PIPES + 0.1 mM U(VI) [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0	0.0018	0.0018
7	0.002	0.003	0.0027	0.0031
15	0.001	0.002	0.0023	0.0027
24	0	0.001	0.0018	0.0023
32	0.002	0	0.0027	0.0018
42	0	0	0.0018	0.0018
52	0	0	0.0018	0.0018
66	0.001	0.001	0.0023	0.0023
76	0.005	0	0.0040	0.0018
103.5	0	0	0.0018	0.0018
125.5	0	0	0.0018	0.0018
152.5	0	0.004	0.0018	0.0036
197	0.001	0	0.0023	0.0018
224	0.001	0.003	0.0023	0.0031
292.5	0	0	0.0018	0.0018
316.5	0.005	0.001	0.0040	0.0023
364.5	0	0	0.0018	0.0018
460.5	0	0	0.0018	0.0018
537	0	0	0.0018	0.0018
686	0	0.006	0.0018	0.0044

PIPES + 0.26 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.001	0	0.0023	0.0018
7	0.021	0.01	0.0108	0.0061
15	0.032	0.017	0.0155	0.0091
24	0.054	0.035	0.0250	0.0168
32	0.071	0.075	0.0322	0.0339
42	0.054	0.05	0.0250	0.0232
52	0.025	0.018	0.0125	0.0095
66	0.032	0.021	0.0155	0.0108
76	0.05	0.02	0.0232	0.0104
103.5	0.072	0.015	0.0327	0.0083
125.5	0.083	0.02	0.0374	0.0104
152.5	0.105	0.033	0.0468	0.0160
197	0.128	0.145	0.0566	0.0639
224	0.13	0.048	0.0575	0.0224
292.5	0.13	0.049	0.0575	0.0228
316.5	0.132	0.054	0.0583	0.0250
364.5	0.136	0.05	0.0601	0.0232
460.5	0.134	0.05	0.0592	0.0232
537	0.137	0.051	0.0605	0.0237
686	0.139	0.055	0.0613	0.0254

PIPES + 0.52 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.001	0	0.0023	0.0018
7	0.061	0.056	0.0280	0.0258
15	0.068	0.061	0.0309	0.0280
24	0.029	0.039	0.0143	0.0185
32	0.068	0.129	0.0309	0.0571
42	0.074	0.149	0.0335	0.0656
52	0.064	0.148	0.0292	0.0652
66	0.101	0.192	0.0451	0.0840
76	0.153	0.245	0.0673	0.1067
103.5	0.188	0.258	0.0823	0.1123
125.5	0.217	0.27	0.0947	0.1174
152.5	0.254	0.304	0.1106	0.1320
197	0.292	0.316	0.1268	0.1371
224	0.306	0.331	0.1328	0.1435
292.5	0.326	0.341	0.1414	0.1478
316.5	0.332	0.338	0.1439	0.1465
364.5	0.334	0.333	0.1448	0.1444
460.5	0.347	0.333	0.1504	0.1444
537	0.356	0.343	0.1542	0.1487
686	0.355	0.347	0.1538	0.1504

PIPES + 0.52 mg DW cells/ml + 0.1 mM U(VI) + 0.1mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.002	0	0.0027	0.0018
7	0.064	0.064	0.0292	0.0292
15	0.078	0.077	0.0352	0.0348
24	0.133	0.135	0.0588	0.0596
32	0.234	0.24	0.1020	0.1046
42	0.279	0.282	0.1213	0.1225
52	0.27	0.277	0.1174	0.1204
66	0.341	0.336	0.1478	0.1457
76	0.39	0.401	0.1688	0.1735
103.5	0.393	0.403	0.1701	0.1743
125.5	0.421	0.421	0.1820	0.1820
152.5	0.432	0.452	0.1868	0.1953
197	0.468	0.455	0.2022	0.1966
224	0.49	0.481	0.2116	0.2077
292.5	0.506	0.507	0.2184	0.2189
316.5	0.513	0.512	0.2214	0.2210
364.5	0.509	0.507	0.2197	0.2189
460.5	0.518	0.52	0.2236	0.2244
537	0.538	0.529	0.2321	0.2283
686	0.44	0.529	0.1902	0.2283

PIPES + Heat-killed cell (equivalent to 0.52 mg/ml) + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.138	0.142	0.0609	0.0626
7	0.103	0.11	0.0459	0.0489
15	0.082	0.073	0.0369	0.0331
24	0.08	0.069	0.0361	0.0314
32	0.07	0.064	0.0318	0.0292
42	0.07	0.064	0.0318	0.0292
52	0.069	0.067	0.0314	0.0305
66	0.064	0.066	0.0292	0.0301
76	0.067	0.05	0.0305	0.0232
103.5	0.064	0.059	0.0292	0.0271
125.5	0.059	0.046	0.0271	0.0215
152.5	0.051	0.044	0.0237	0.0207
197	0.049	0.042	0.0228	0.0198
224	0.053	0.036	0.0245	0.0172
292.5	0.037	0.03	0.0177	0.0147
316.5	0.04	0.025	0.0190	0.0125
364.5	0.038	0.028	0.0181	0.0138
460.5	0.035	0.03	0.0168	0.0147
537	0.036	0.036	0.0172	0.0172
686	0.037	0.044	0.0177	0.0207

PIPES + 0.26 mg DW cells/ml				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0	0.0018	0.0018
7	0.016	0.026	0.0087	0.0130
15	0.022	0.044	0.0113	0.0207
24	0.04	0.07	0.0190	0.0318
32	0.053	0.098	0.0245	0.0438
42	0.067	0.121	0.0305	0.0536
52	0.077	0.139	0.0348	0.0613
66	0.121	0.162	0.0536	0.0712
76	0.117	0.184	0.0519	0.0806
103.5	0.142	0.198	0.0626	0.0866
125.5	0.149	0.206	0.0656	0.0900
152.5	0.167	0.221	0.0733	0.0964
197	0.189	0.233	0.0827	0.1016
224	0.202	0.243	0.0883	0.1059
292.5	0.227	0.254	0.0990	0.1106
316.5	0.241	0.263	0.1050	0.1144
364.5	0.252	0.27	0.1097	0.1174
460.5	0.264	0.28	0.1148	0.1217
537	0.275	0.282	0.1195	0.1225
686	0.284	0.297	0.1234	0.1290

PIPES + 0.52 mg DW cells/ml				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.001	0.001	0.0023	0.0023
7	0.044	0.045	0.0207	0.0211
15	0.091	0.086	0.0408	0.0387
24	0.139	0.147	0.0613	0.0648
32	0.177	0.193	0.0776	0.0845
42	0.207	0.24	0.0904	0.1046
52	0.231	0.274	0.1007	0.1191
66	0.26	0.302	0.1131	0.1311
76	0.3	0.341	0.1303	0.1478
103.5	0.346	0.366	0.1499	0.1585
125.5	0.361	0.38	0.1564	0.1645
152.5	0.395	0.397	0.1709	0.1718
197	0.432	0.43	0.1868	0.1859
224	0.44	0.453	0.1902	0.1957
292.5	0.479	0.462	0.2069	0.1996
316.5	0.482	0.507	0.2082	0.2189
364.5	0.498	0.507	0.2150	0.2189
460.5	0.522	0.532	0.2253	0.2296
537	0.543	0.546	0.2343	0.2355
686	0.565	0.548	0.2437	0.2364

PIPES + 0.52 mg DW cells/ml + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0.002	0.0018	0.0027
7	0.028	0.027	0.0138	0.0134
15	0.055	0.051	0.0254	0.0237
24	0.109	0.095	0.0485	0.0425
32	0.15	0.127	0.0660	0.0562
42	0.196	0.168	0.0857	0.0738
52	0.227	0.19	0.0990	0.0832
66	0.266	0.236	0.1157	0.1029
76	0.308	0.282	0.1337	0.1225
103.5	0.344	0.33	0.1491	0.1431
125.5	0.376	0.353	0.1628	0.1529
152.5	0.407	0.381	0.1761	0.1649
197	0.428	0.436	0.1850	0.1885
224	0.46	0.465	0.1987	0.2009
292.5	0.479	0.563	0.2069	0.2428
316.5	0.507	0.518	0.2189	0.2236
364.5	0.518	0.523	0.2236	0.2257
460.5	0.539	0.559	0.2326	0.2411
537	0.552	0.576	0.2381	0.2484
686	0.577	0.585	0.2488	0.2522

Table A 2: Soluble U(VI) data in PIPES buffer

The following tables contain data for soluble U(VI) precipitated by inorganic phosphate released by *Cellulomonas* sp. ES6 in PIPES buffer under non-growth conditions. Soluble U(VI) concentrations were measured using unfiltered samples from serum bottle by KPA.

PIPES + 0.1 mM U(VI) [Cell free control]				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	21.833	25.001	0.0917	0.1050
8	21.315	23.276	0.0896	0.0978
14	20.474	23.244	0.0860	0.0977
24	21.436	22.364	0.0901	0.0940
32	17.435	22.412	0.0733	0.0942
42	21.147	22.43	0.0889	0.0942
52.5	22.46	22.959	0.0944	0.0965
66.5	20.115	22.437	0.0845	0.0943
77	N/A	N/A	N/A	N/A
104	20.237	23.044	0.0850	0.0968
129.5	20.359	22.112	0.0855	0.0929
158	20.953	21.579	0.0880	0.0907
230	19.835	22.023	0.0833	0.0925
298.5	20.28	21.629	0.0852	0.0909
322.5	21.699	21.704	0.0912	0.0912

PIPES + 0.26 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	22.196	21.658	0.0933	0.0910
8	21.763	22.696	0.0914	0.0954
14	24.515	22.294	0.1030	0.0937
24	21.563	20.046	0.0906	0.0842
32	25.805	20.272	0.1084	0.0852
42	11.023	15.279	0.0463	0.0642
52.5	8.858	12.785	0.0372	0.0537
66.5	5.851	9.345	0.0246	0.0393
77	3.787	5.953	0.0159	0.0250
104	2.83	4.238	0.0119	0.0178
129.5	2.087	3.084	0.0088	0.0130
158	3.022	2.274	0.0127	0.0096
230	3.574	1.925	0.0150	0.0081
298.5	2.628	1.634	0.0110	0.0069
322.5	2.223	1.327	0.0093	0.0056

PIPES + 0.52 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	21.958	22.548	0.0923	0.0947
8	21.675	22.227	0.0911	0.0934
14	22.093	21.856	0.0928	0.0918
24	10.137	10.617	0.0426	0.0446
32	7.203	6.907	0.0303	0.0290
42	4.172	4.612	0.0175	0.0194
52.5	2.993	4.73	0.0126	0.0199
66.5	1.953	3.005	0.0082	0.0126
77	N/A	N/A	N/A	N/A
104	1.614	1.768	0.0068	0.0074
129.5	1.35	1.343	0.0057	0.0056
158	1.113	1.188	0.0047	0.0050
230	1.369	1.015	0.0058	0.0043
298.5	1.302	0.83	0.0055	0.0035
322.5	1.729	1.246	0.0073	0.0052

PIPES + 0.52 mg DW cells/ml + 0.1 mM U(VI) + 0.1mM AQDS				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.137	24.652	0.0972	0.1036
8	16.686	17.079	0.0701	0.0718
14	14.335	16.113	0.0602	0.0677
24	9.45	8.543	0.0397	0.0359
32	4.628	5.193	0.0194	0.0218
42	2.648	1.885	0.0111	0.0079
52.5	2.973	2.153	0.0125	0.0090
66.5	1.836	1.762	0.0077	0.0074
77	N/A	N/A	N/A	N/A
104	1.131	1.859	0.0048	0.0078
129.5	1.697	1.583	0.0071	0.0067
158	1.113	1.159	0.0047	0.0049
230	1.171	1.466	0.0049	0.0062
298.5	1,964	1.631	8.2521	0.0069
322.5	1.131	1.452	0.0048	0.0061

PIPES + Heat-killed cell (equivalent to 0.52 mg/ml) + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	22.012	20.761	0.0925	0.0872
8	15.115	14.774	0.0635	0.0621
14	14.303	14.629	0.0601	0.0615
24	13.434	11.093	0.0564	0.0466
32	11.751	10.975	0.0494	0.0461
42	11.018	10.392	0.0463	0.0437
52.5	10.481	9.67	0.0440	0.0406
66.5	12.613	8.912	0.0530	0.0374
77	N/A	N/A	N/A	N/A
104	8.718	8.468	0.0366	0.0356
129.5	9.061	7.112	0.0381	0.0299
158	7.328	7.282	0.0308	0.0306
230	6.239	6.398	0.0262	0.0269
298.5	6.096	5.378	0.0256	0.0226
322.5	6.525	6.439	0.0274	0.0271

Table A 3: Inorganic phosphate data in bicarbonate buffer

The following tables contain data for inorganic phosphate released by *Cellulomonas* sp. ES6 in bicarbonate buffer under non-growth conditions. Inorganic phosphate concentrations were measured by reading the absorbance at 880 nm with time.

Note:

a) Between 148 and 232 hours, sample from treatments 7, 8, 19, 20 were diluted 2 times

b) Data at 268 hours is after 2 times dilution of sample from treatments 7, 8, 17, 18, 19, 20, 21, and 22

c) Data at 309.5 and 357 hours is after 2 times dilution of sample from treatments 5, 6, 7, 8, 9, 10, 11, 12, 17, 18, 21, 22 and after 4 times dilution of sample from treatments 19 and 20

d) Between 379.5 and 982 hours, sample from treatments 5, 6, 9, 10, 11, 12, 17, 18, 21, 22 were diluted 2 times and sample from treatments 7, 8, 19, and 20 were diluted 4 times

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0.001	0.0018	0.0023
7	0	0.002	0.0018	0.0027
17	0	0	0.0018	0.0018
29	0	0	0.0018	0.0018
45	0.003	0.004	0.0031	0.0036
62.5	0.001	0.001	0.0023	0.0023
76	0.001	0.001	0.0023	0.0023
92	0.001	0.002	0.0023	0.0027
111	0.001	0	0.0023	0.0018
148	0.001	0.001	0.0023	0.0023
184	0	0	0.0018	0.0018
208	0	0	0.0018	0.0018
232	0	0	0.0018	0.0018
268	0.001	0.001	0.0023	0.0023
309.5	0	0	0.0018	0.0018
357	0.001	0.002	0.0023	0.0027
379.5	0.001	0.001	0.0023	0.0023
478	0.001	0.004	0.0023	0.0036
522	0	0	0.0018	0.0018
620	0.001	0.001	0.0023	0.0023
716	0.001	0.002	0.0023	0.0027
832	0.001	0.005	0.0023	0.0040
982	0.001	0.001	0.0023	0.0023

Bicarbonate + 0.1 mM U(VI) + 0.1 mM AQDS [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0	0.0018	0.0018
7	0.001	0.005	0.0023	0.0040
17	0	0	0.0018	0.0018
29	0.001	0.001	0.0023	0.0023
45	0.002	0.003	0.0027	0.0031
62.5	0.001	0.002	0.0023	0.0027
76	0	0.001	0.0018	0.0023
92	0	0.002	0.0018	0.0027
111	0.002	0.001	0.0027	0.0023
148	0	0.001	0.0018	0.0023
184	0	0	0.0018	0.0018
208	0.003	0.001	0.0031	0.0023
232	0	0	0.0018	0.0018
268	0.001	0.001	0.0023	0.0023
309.5	0.001	0.001	0.0023	0.0023
357	0.004	0.002	0.0036	0.0027
379.5	0.002	0.004	0.0027	0.0036
478	0.001	0.001	0.0023	0.0023
522	0	0	0.0018	0.0018
620	0.001	0.001	0.0023	0.0023
716	0.001	0.001	0.0023	0.0023
832	0.012	0.001	0.0070	0.0023
982	0.003	0.002	0.0031	0.0027

Bicarbonate + 1.15 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.012	0.019	0.0070	0.0100
7	0.019	0.036	0.0100	0.0172
17	0.035	0.048	0.0168	0.0224
29	0.071	0.087	0.0322	0.0391
45	0.107	0.114	0.0476	0.0506
62.5	0.155	0.154	0.0682	0.0678
76	0.188	0.187	0.0823	0.0819
92	0.223	0.226	0.0973	0.0986
111	0.259	0.249	0.1127	0.1084
148	0.307	0.304	0.1332	0.1320
184	0.373	0.406	0.1615	0.1756
208	0.397	0.384	0.1718	0.1662
232	0.421	0.408	0.1820	0.1765
268	0.464	0.446	0.2004	0.1927
309.5	0.243	0.245	0.2117	0.2134
357	0.264	0.31	0.2297	0.2691
379.5	0.293	0.291	0.2545	0.2528
478	0.334	0.338	0.2896	0.2930
522	0.35	0.351	0.3033	0.3042
620	0.368	0.354	0.3187	0.3067
716	0.368	0.371	0.3187	0.3213
832	0.365	0.388	0.3161	0.3358
982	0.369	0.38	0.3196	0.3290

Bicarbonate + 2.3 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.032	0.029	0.0155	0.0143
7	0.056	0.057	0.0258	0.0262
17	0.087	0.087	0.0391	0.0391
29	0.161	0.156	0.0708	0.0686
45	0.211	0.211	0.0922	0.0922
62.5	0.292	0.288	0.1268	0.1251
76	0.352	0.347	0.1525	0.1504
92	0.421	0.411	0.1820	0.1778
111	0.474	0.46	0.2047	0.1987
148	0.277	0.27	0.2408	0.2348
184	0.302	0.305	0.2622	0.2648
208	0.324	0.318	0.2810	0.2759
232	0.347	0.357	0.3007	0.3093
268	0.393	0.397	0.3401	0.3435
309.5	0.437	0.437	0.3778	0.3778
357	0.492	0.502	0.4249	0.4334
379.5	0.267	0.274	0.4645	0.4765
478	0.291	0.286	0.5056	0.4970
522	0.3	0.302	0.5210	0.5244
620	0.319	0.323	0.5535	0.5604
716	0.346	0.348	0.5998	0.6032
832	0.363	0.349	0.6289	0.6049
982	0.363	0.362	0.6289	0.6272

Bicarbonate + 2.3 mg DW cells/ml + 0.25 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.024	0.029	0.0121	0.0143
7	0.04	0.058	0.0190	0.0267
17	0.074	0.089	0.0335	0.0399
29	0.156	0.179	0.0686	0.0785
45	0.217	0.225	0.0947	0.0981
62.5	0.291	0.297	0.1264	0.1290
76	0.335	0.336	0.1452	0.1457
92	0.329	0.343	0.1427	0.1487
111	0.29	0.304	0.1260	0.1320
148	0.27	0.304	0.1174	0.1320
184	0.332	0.363	0.1439	0.1572
208	0.376	0.431	0.1628	0.1863
232	0.439	0.464	0.1897	0.2004
268	0.534	0.565	0.2304	0.2437
309.5	0.312	0.347	0.2708	0.3007
357	0.368	0.392	0.3187	0.3393
379.5	0.402	0.43	0.3478	0.3718
478	0.466	0.507	0.4026	0.4377
522	0.476	0.52	0.4112	0.4488
620	0.526	0.567	0.4540	0.4891
716	0.553	0.595	0.4771	0.5130
832	0.567	0.558	0.4891	0.4814
982	0.583	0.591	0.5028	0.5096

Bicarbonate + 1.15 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.013	0.014	0.0074	0.0078
7	0.018	0.029	0.0095	0.0143
17	0.021	0.043	0.0108	0.0202
29	0.037	0.063	0.0177	0.0288
45	0.061	0.1	0.0280	0.0446
62.5	0.103	0.168	0.0459	0.0738
76	0.148	0.203	0.0652	0.0887
92	0.181	0.259	0.0793	0.1127
111	0.172	0.31	0.0755	0.1345
148	0.221	0.396	0.0964	0.1713
184	0.324	0.499	0.1405	0.2154
208	0.402	0.555	0.1739	0.2394
232	0.45	0.594	0.1945	0.2561
268	0.525	0.663	0.2266	0.2856
309.5	0.307	0.36	0.2665	0.3119
357	0.333	0.381	0.2888	0.3298
379.5	0.333	0.377	0.2888	0.3264
478	0.369	0.41	0.3196	0.3547
522	0.384	0.42	0.3324	0.3632
620	0.39	0.425	0.3375	0.3675
716	0.426	0.428	0.3684	0.3701
832	0.389	0.403	0.3367	0.3487
982	0.378	0.378	0.3273	0.3273

Bicarbonate + Heat-killed cell (equivalent to 1.15 mg/ml)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.213	0.213	0.0930	0.0930
7	0.218	0.214	0.0952	0.0934
17	0.211	0.212	0.0922	0.0926
29	0.218	0.217	0.0952	0.0947
45	0.222	0.224	0.0969	0.0977
62.5	0.213	0.212	0.0930	0.0926
76	0.22	0.217	0.0960	0.0947
92	0.222	0.219	0.0969	0.0956
111	0.219	0.219	0.0956	0.0956
148	0.213	0.217	0.0930	0.0947
184	0.213	0.21	0.0930	0.0917
208	0.223	0.219	0.0973	0.0956
232	0.212	0.214	0.0926	0.0934
268	0.219	0.22	0.0956	0.0960
309.5	0.219	0.22	0.0956	0.0960
357	0.223	0.217	0.0973	0.0947
379.5	0.225	0.223	0.0981	0.0973
478	0.224	0.224	0.0977	0.0977
522	0.224	0.225	0.0977	0.0981
620	0.235	0.228	0.1024	0.0994
716	0.225	0.23	0.0981	0.1003
832	0.233	0.221	0.1016	0.0964
982	0.228	0.225	0.0994	0.0981

Bicarbonate + Heat-killed cell (equivalent to 1.15 mg/ml) + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.202	0.206	0.0883	0.0900
7	0.204	0.216	0.0892	0.0943
17	0.195	0.21	0.0853	0.0917
29	0.206	0.216	0.0900	0.0943
45	0.207	0.216	0.0904	0.0943
62.5	0.204	0.206	0.0892	0.0900
76	0.209	0.206	0.0913	0.0900
92	0.212	0.214	0.0926	0.0934
111	0.216	0.214	0.0943	0.0934
148	0.211	0.214	0.0922	0.0934
184	0.218	0.215	0.0952	0.0939
208	0.213	0.211	0.0930	0.0922
232	0.222	0.209	0.0969	0.0913
268	0.218	0.211	0.0952	0.0922
309.5	0.22	0.209	0.0960	0.0913
357	0.213	0.223	0.0930	0.0973
379.5	0.222	0.215	0.0969	0.0939
478	0.219	0.214	0.0956	0.0934
522	0.224	0.219	0.0977	0.0956
620	0.232	0.229	0.1011	0.0999
716	0.228	0.225	0.0994	0.0981
832	0.23	0.219	0.1003	0.0956
982	0.25	0.231	0.1088	0.1007

Bicarbonate + 1.15 mg DW cells/ml				
Time (Hrs)	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.019	0.014	0.0100	0.0078
7	0.047	0.031	0.0220	0.0151
17	0.056	0.048	0.0258	0.0224
29	0.104	0.086	0.0464	0.0387
45	0.126	0.122	0.0558	0.0541
62.5	0.168	0.162	0.0738	0.0712
76	0.2	0.193	0.0874	0.0845
92	0.239	0.235	0.1041	0.1024
111	0.278	0.268	0.1208	0.1166
148	0.345	0.341	0.1495	0.1478
184	0.41	0.397	0.1773	0.1718
208	0.444	0.447	0.1919	0.1932
232	0.48	0.472	0.2073	0.2039
268	0.252	0.252	0.2194	0.2194
309.5	0.28	0.289	0.2434	0.2511
357	0.306	0.322	0.2656	0.2793
379.5	0.332	0.347	0.2879	0.3007
478	0.365	0.386	0.3161	0.3341
522	0.382	0.408	0.3307	0.3530
620	0.42	0.449	0.3632	0.3881
716	0.447	0.478	0.3863	0.4129
832	0.466	0.47	0.4026	0.4060
982	0.465	0.479	0.4018	0.4137

Bicarbonate + 2.3 mg DW cells/ml				
Time (Hrs)	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.027	0.028	0.0134	0.0138
7	0.056	0.055	0.0258	0.0254
17	0.086	0.091	0.0387	0.0408
29	0.154	0.156	0.0678	0.0686
45	0.218	0.223	0.0952	0.0973
62.5	0.293	0.299	0.1273	0.1298
76	0.35	0.378	0.1517	0.1636
92	0.42	0.432	0.1816	0.1868
111	0.49	0.496	0.2116	0.2141
148	0.3	0.321	0.2605	0.2785
184	0.357	0.381	0.3093	0.3298
208	0.384	0.409	0.3324	0.3538
232	0.407	0.424	0.3521	0.3667
268	0.449	0.485	0.3881	0.4189
309.5	0.247	0.256	0.4303	0.4457
357	0.275	0.294	0.4782	0.5107
379.5	0.292	0.3	0.5073	0.5210
478	0.307	0.348	0.5330	0.6032
522	0.321	0.34	0.5570	0.5895
620	0.35	0.368	0.6066	0.6374
716	0.373	0.382	0.6460	0.6614
832	0.385	0.394	0.6665	0.6819
982	0.405	0.42	0.7008	0.7265

Bicarbonate + 1.15 mg DW cells/ml + 0.1 mM AQDS				
Time (Hrs)	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.02	0.013	0.0104	0.0074
7	0.037	0.022	0.0177	0.0113
17	0.049	0.04	0.0228	0.0190
29	0.08	0.075	0.0361	0.0339
45	0.119	0.116	0.0528	0.0515
62.5	0.161	0.167	0.0708	0.0733
76	0.189	0.209	0.0827	0.0913
92	0.23	0.24	0.1003	0.1046
111	0.274	0.279	0.1191	0.1213
148	0.339	0.35	0.1469	0.1517
184	0.409	0.416	0.1769	0.1799
208	0.456	0.459	0.1970	0.1983
232	0.489	0.496	0.2111	0.2141
268	0.262	0.262	0.2280	0.2280
309.5	0.286	0.297	0.2485	0.2579
357	0.319	0.33	0.2768	0.2862
379.5	0.339	0.349	0.2939	0.3024
478	0.38	0.392	0.3290	0.3393
522	0.395	0.412	0.3418	0.3564
620	0.435	0.455	0.3761	0.3932
716	0.46	0.483	0.3975	0.4172
832	0.484	0.482	0.4180	0.4163
982	0.49	0.486	0.4232	0.4197

Table A 4: Soluble U(VI) data in bicarbonate buffer

The following tables contain data for soluble U(VI) precipitated by inorganic phosphate released by *Cellulomonas* sp. ES6 in bicarbonate buffer under non-growth conditions. Soluble U(VI) concentrations were measured using unfiltered samples from serum bottle by KPA.

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.857	24.618	0.1044	0.1034
18	25.127	24.339	0.1056	0.1023
30	24.166	23.585	0.1015	0.0991
47	25.428	24.306	0.1068	0.1021
64	25.872	24.688	0.1087	0.1037
77	25.021	24.115	0.1051	0.1013
93	25.689	25.264	0.1079	0.1062
113	25.267	24.176	0.1062	0.1016
150	24.897	24.434	0.1046	0.1027
186	25.074	23.85	0.1054	0.1002
210	24.962	24.204	0.1049	0.1017
235	24.854	25.205	0.1044	0.1059
267	22.057	24.429	0.0927	0.1026
311	27.776	24.925	0.1167	0.1047
387	25.516	24.401	0.1072	0.1025
403	24.163	25.024	0.1015	0.1051
500	25.214	25.084	0.1059	0.1054

Bicarbonate + 0.1 mM U(VI) + 0.1 mM AQDS [Cell free control]				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	25.367	24.735	0.1066	0.1039
18	25.017	24.737	0.1051	0.1039
30	25.116	24.763	0.1055	0.1040
47	25.168	25.049	0.1057	0.1052
64	26.173	24.956	0.1100	0.1049
77	24.946	24.276	0.1048	0.1020
93	25.21	24.816	0.1059	0.1043
113	24.761	23.142	0.1040	0.0972
150	24.968	24.571	0.1049	0.1032
186	25.269	24.689	0.1062	0.1037
210	24.685	24.745	0.1037	0.1040
235	25.158	25.725	0.1057	0.1081
267	24.518	24.957	0.1030	0.1049
311	24.538	23.962	0.1031	0.1007
387	26.701	25.806	0.1122	0.1084
403	25.789	23.658	0.1084	0.0994
500	23.924	25.421	0.1005	0.1068

Bicarbonate + 1.15 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.439	24.942	0.1027	0.1048
18	25.013	25.717	0.1051	0.1081
30	24.06	24.379	0.1011	0.1024
47	23.774	25.463	0.0999	0.1070
64	24.116	24.972	0.1013	0.1049
77	23.785	24.614	0.0999	0.1034
93	24.593	24.879	0.1033	0.1045
113	23.684	23.201	0.0995	0.0975
150	22.705	22.184	0.0954	0.0932
186	21.637	19.666	0.0909	0.0826
210	20.221	18.792	0.0850	0.0790
235	20.581	17.514	0.0865	0.0736
267	19.285	16.381	0.0810	0.0688
311	17.141	13.444	0.0720	0.0565
387	13.898	11.806	0.0584	0.0496
403	14.69	8.78	0.0617	0.0369
500	13.314	6.921	0.0559	0.0291

Bicarbonate + 2.3 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.726	24.158	0.1039	0.1015
18	24.923	24.16	0.1047	0.1015
30	24.293	22.842	0.1021	0.0960
47	23.439	24.073	0.0985	0.1011
64	22.638	21.992	0.0951	0.0924
77	22.532	22.085	0.0947	0.0928
93	21.377	21.522	0.0898	0.0904
113	20.076	19.914	0.0844	0.0837
150	15.429	14.72	0.0648	0.0618
186	12.897	10.941	0.0542	0.0460
210	8.942	8.963	0.0376	0.0377
235	7.57	7.492	0.0318	0.0315
267	5.64	3.786	0.0237	0.0159
311	3.559	3.647	0.0150	0.0153
387	0.305	0.284	0.0013	0.0012
403	1.716	1.223	0.0072	0.0051
500	1.065	0.442	0.0045	0.0019

Bicarbonate + 2.3 mg DW cells/ml + 0.25 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	32.325	32.084	0.2716	0.2696
18	30.858	31.405	0.2593	0.2639
30	28.458	30.719	0.2391	0.2581
47	29.866	30.241	0.2510	0.2541
64	28.794	29.622	0.2420	0.2489
77	27.644	26.653	0.2323	0.2240
93	24.73	24.746	0.2078	0.2079
113	18.071	18.764	0.1519	0.1577
150	11.353	11.11	0.0954	0.0934
186	7.544	7.704	0.0634	0.0647
210	5.093	5.684	0.0428	0.0478
235	4.761	3.736	0.0400	0.0314
267	3.737	3.375	0.0314	0.0284
311	2.298	1.461	0.0193	0.0123
387	0.412	0.305	0.0035	0.0026
403	0.866	0.732	0.0073	0.0062
500	N/A	N/A	N/A	N/A

Bicarbonate + 1.15 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.902	24.395	0.1046	0.1025
18	20.157	19.51	0.0847	0.0820
30	16.938	15.094	0.0712	0.0634
47	9.486	8.497	0.0399	0.0357
64	4.397	4.731	0.0185	0.0199
77	6.142	6.16	0.0258	0.0259
93	4.783	6.123	0.0201	0.0257
113	N/A	N/A	N/A	N/A
150	N/A	N/A	N/A	N/A
186	3.237	2.181	0.0136	0.0092
210	2.226	2.293	0.0094	0.0096
235	N/A	N/A	N/A	N/A
267	N/A	N/A	N/A	N/A
311	N/A	N/A	N/A	N/A
387	2.258	1.72	0.0095	0.0072
403	N/A	N/A	N/A	N/A
500	N/A	N/A	N/A	N/A

Bicarbonate + Heat-killed cell (equivalent to 1.15 mg/ml) + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.69	24.704	0.0995	0.1038
18	24.501	24.758	0.1029	0.1040
30	25.273	25.999	0.1062	0.1092
47	24.278	25.237	0.1020	0.1060
64	24.152	23.726	0.1015	0.0997
77	24.637	24.698	0.1035	0.1038
93	23.822	25.717	0.1001	0.1081
113	24.631	25.143	0.1035	0.1056
150	23.112	24.54	0.0971	0.1031
186	25.273	27.005	0.1062	0.1135
210	23.846	24.222	0.1002	0.1018
235	24.372	23.125	0.1024	0.0972
267	22.152	25.24	0.0931	0.1061
311	23.595	23.142	0.0991	0.0972
387	24.607	26.254	0.1034	0.1103
403	23.743	22.546	0.0998	0.0947
500	23.579	24.535	0.0991	0.1031

Table A 5: Model predicted P_i data in PIPES buffer

The following table contains inorganic phosphate concentration in PIPES buffer predicted by model using parameters in table 1

PO_4^{3-} concentration (mM)				
	PIPES + 0.26 mg DW cells/ml		PIPES + 0.52 mg DW cells/ml	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0	0	0
7	0.0062	0.0098	0.0145	0.0176
15	0.0129	0.0202	0.0304	0.0366
24	0.0201	0.0310	0.0473	0.0565
32	0.0260	0.0396	0.0614	0.0727
42	0.0330	0.0495	0.0779	0.0914
52	0.0395	0.0581	0.0931	0.1081
66	0.0478	0.0688	0.1125	0.1289
76	0.0530	0.0751	0.1246	0.1412
103.5	0.0663	0.0901	0.1545	0.1705
125.5	0.0744	0.0976	0.1712	0.1846
152.5	0.0824	0.1038	0.1862	0.1957
197	0.0921	0.1100	0.2020	0.2054
224	0.0955	0.1112	0.2054	0.2065
292.5	0.1016	0.1129	0.2105	0.2079
316.5	0.1022	0.1129	0.2103	0.2077
364.5	0.1032	0.1128	0.2102	0.2076
460.5	0.1041	0.1128	0.2101	0.2076
537	0.1041	0.1128	0.2101	0.2076

Table A 6: Model predicted P_i data in bicarbonate buffer

The following table contains inorganic phosphate concentration in bicarbonate buffer predicted by model using parameters in table 1

PO_4^{3-} concentration (mM)				
	Bicarbonate + 1.15 mg DW cells/ml		Bicarbonate + 2.3 mg DW cells/ml	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.019	0.014	0.0100	0.0078
7	0.047	0.031	0.0220	0.0151
17	0.056	0.048	0.0258	0.0224
29	0.104	0.086	0.0464	0.0387
45	0.126	0.122	0.0558	0.0541
62.5	0.168	0.162	0.0738	0.0712
76	0.2	0.193	0.0874	0.0845
92	0.239	0.235	0.1041	0.1024
111	0.278	0.268	0.1208	0.1166
148	0.345	0.341	0.1495	0.1478
184	0.41	0.397	0.1773	0.1718
208	0.444	0.447	0.1919	0.1932
232	0.48	0.472	0.2073	0.2039
268	0.252	0.252	0.2194	0.2194
309.5	0.28	0.289	0.2434	0.2511
357	0.306	0.322	0.2656	0.2793
379.5	0.332	0.347	0.2879	0.3007
478	0.365	0.386	0.3161	0.3341
522	0.382	0.408	0.3307	0.3530
620	0.42	0.449	0.3632	0.3881
716	0.447	0.478	0.3863	0.4129
832	0.466	0.47	0.4026	0.4060
982	0.465	0.479	0.4018	0.4137

APPENDIX B

The data in this appendix is the raw data of experiments repeated as part of the results presented in Chapter 2

Table B 1: Inorganic phosphate data in PIPES buffer

The following tables contain data for inorganic phosphate released by *Cellulomonas* sp. ES6 in PIPES buffer under non-growth conditions. Inorganic phosphate concentrations were measured by reading the absorbance at 880 nm with time.

PIPES + 0.1 mM U(VI) [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0	0.0018	0.0018
8	0	0	0.0018	0.0018
16	0	0.001	0.0018	0.0023
26	0.002	0	0.0027	0.0018
35	0	0	0.0018	0.0018
42	0.001	0	0.0023	0.0018
60	0.001	0	0.0023	0.0018
70	0.001	0.002	0.0023	0.0027
82	0	0	0.0018	0.0018
94	0.002	0.001	0.0027	0.0023
118	0.003	0.001	0.0031	0.0023
142	0	0.001	0.0018	0.0023
190	0.002	0	0.0027	0.0018
214	0	0	0.0018	0.0018
242	0.001	0	0.0023	0.0018
290	0	0	0.0018	0.0018
386	0	0	0.0018	0.0018
482	0.001	0	0.0023	0.0018
578	0	0.001	0.0018	0.0023

PIPES + 0.66 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.015	0.021	0.0083	0.0108
8	0.043	0.042	0.0202	0.0198
16	0.065	0.059	0.0297	0.0271
26	0.073	0.072	0.0331	0.0327
35	0.088	0.076	0.0395	0.0344
42	0.103	0.105	0.0459	0.0468
60	0.135	0.133	0.0596	0.0588
70	0.17	0.165	0.0746	0.0725
82	0.195	0.197	0.0853	0.0862
94	0.23	0.215	0.1003	0.0939
118	0.28	0.277	0.1217	0.1204
142	0.15	0.163	0.1321	0.1432
190	0.173	0.178	0.1518	0.1561
214	0.185	0.192	0.1621	0.1680
242	0.193	0.195	0.1689	0.1706
290	0.198	0.204	0.1732	0.1783
386	0.213	0.218	0.1860	0.1903
482	0.219	0.223	0.1912	0.1946
578	0.217	0.224	0.1894	0.1954

PIPES + 0.66 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.018	0.022	0.0095	0.0113
8	0.045	0.044	0.0211	0.0207
16	0.095	0.083	0.0425	0.0374
26	0.135	0.144	0.0596	0.0635
35	0.183	0.162	0.0802	0.0712
42	0.211	0.197	0.0922	0.0862
60	0.304	0.294	0.1320	0.1277
70	0.329	0.333	0.1427	0.1444
82	0.37	0.372	0.1602	0.1611
94	0.415	0.42	0.1795	0.1816
118	0.478	0.482	0.2064	0.2082
142	0.27	0.272	0.2348	0.2365
190	0.297	0.294	0.2579	0.2554
214	0.306	0.304	0.2656	0.2639
242	0.309	0.312	0.2682	0.2708
290	0.312	0.314	0.2708	0.2725
386	0.318	0.319	0.2759	0.2768
482	0.327	0.329	0.2836	0.2853
578	0.329	0.328	0.2853	0.2845

PIPES + 0.66 mg DW cells/ml + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.031	0.025	0.0151	0.0125
8	0.043	0.042	0.0202	0.0198
16	0.076	0.078	0.0344	0.0352
26	0.14	0.133	0.0618	0.0588
35	0.192	0.186	0.0840	0.0815
42	0.21	0.205	0.0917	0.0896
60	0.287	0.283	0.1247	0.1230
70	0.326	0.329	0.1414	0.1427
82	0.37	0.373	0.1602	0.1615
94	0.406	0.404	0.1756	0.1748
118	0.47	0.462	0.2030	0.1996
142	0.258	0.263	0.2245	0.2288
190	0.288	0.285	0.2502	0.2477
214	0.303	0.303	0.2631	0.2631
242	0.308	0.311	0.2674	0.2699
290	0.32	0.319	0.2776	0.2768
386	0.326	0.325	0.2828	0.2819
482	0.33	0.324	0.2862	0.2810
578	0.335	0.33	0.2905	0.2862

PIPES + 0.66 mg DW cells/ml				
Time (Hrs)	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.027	0.021	0.0134	0.0108
8	0.056	0.054	0.0258	0.0250
16	0.088	0.084	0.0395	0.0378
26	0.135	0.127	0.0596	0.0562
35	0.18	0.195	0.0789	0.0853
42	0.204	0.201	0.0892	0.0879
60	0.28	0.29	0.1217	0.1260
70	0.32	0.311	0.1388	0.1350
82	0.367	0.37	0.1589	0.1602
94	0.41	0.398	0.1773	0.1722
118	0.465	0.46	0.2009	0.1987
142	0.255	0.252	0.2220	0.2194
190	0.287	0.29	0.2494	0.2519
214	0.3	0.302	0.2605	0.2622
242	0.308	0.304	0.2674	0.2639
290	0.316	0.315	0.2742	0.2733
386	0.324	0.326	0.2810	0.2828
482	0.329	0.327	0.2853	0.2836
578	0.332	0.334	0.2879	0.2896

Table B 2: Soluble U(VI) data in PIPES buffer

The following tables contain data for soluble U(VI) precipitated by inorganic phosphate released by *Cellulomonas* sp. ES6 in PIPES buffer under non-growth conditions. Soluble U(VI) concentrations were measured using unfiltered samples from serum bottle by KPA

PIPES + 0.1 mM U(VI) [Cell free control]				
Time (Hrs)	U(VI) concentration (ppm)		U(VI) concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.876	23.173	0.1045	0.0974
8	23.673	23.383	0.0995	0.0982
16	23.437	23.403	0.0985	0.0983
26	23.283	23.494	0.0978	0.0987
35	23.293	23.489	0.0979	0.0987
42	24.312	23.589	0.1022	0.0991
60	23.437	23.124	0.0985	0.0972
70	24.121	23.428	0.1013	0.0984
82	23.479	23.487	0.0987	0.0987
94	23.137	24.366	0.0972	0.1024
118	23.954	23.498	0.1006	0.0987

PIPES + 0.66 mg DW cells/ml + 0.1 mM U(VI)				
Time (Hrs)	U(VI) concentration (ppm)		U(VI) concentration (mM)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	22.439	23.45	0.0943	0.0985
8	23.138	23.447	0.0972	0.0985
16	20.498	19.739	0.0861	0.0829
26	11.438	9.347	0.0481	0.0393
35	7.389	6.489	0.0310	0.0273
42	4.297	3.958	0.0181	0.0166
60	3.28	2.843	0.0138	0.0119
70	2.876	2.428	0.0121	0.0102
82	1.938	2.138	0.0081	0.0090
94	1.382	1.29	0.0058	0.0054
118	1.329	1.965	0.0056	0.0083

PIPES + 0.66 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	22.498	23.984	0.0945	0.1008
8	17.398	16.302	0.0731	0.0685
16	13.38	12.48	0.0562	0.0524
26	8.367	7.489	0.0352	0.0315
35	3.587	2.547	0.0151	0.0107
42	3.182	2.129	0.0134	0.0089
60	2.468	2.538	0.0104	0.0107
70	1.738	1.287	0.0073	0.0054
82	1.479	1.782	0.0062	0.0075
94	1.549	1.498	0.0065	0.0063
118	N/A	N/A	N/A	N/A

Table B 3: Inorganic phosphate data in bicarbonate buffer

The following tables contain data for inorganic phosphate released by *Cellulomonas* sp. ES6 in bicarbonate buffer under non-growth conditions. Inorganic phosphate concentrations were measured by reading the absorbance at 880 nm with time.

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0.001	0.0018	0.0023
11	0	0.002	0.0018	0.0027
20	0.004	0.005	0.0036	0.0040
35	0.002	0	0.0027	0.0018
60	0.005	0	0.0040	0.0018
76	0	0	0.0018	0.0018
88	0.001	0.002	0.0023	0.0027
106	0.002	0.001	0.0027	0.0023
140	0.002	0.001	0.0027	0.0023
160	0.001	0	0.0023	0.0018
184	0	0	0.0018	0.0018
232	0.001	0.004	0.0023	0.0036
280	0.001	0.003	0.0023	0.0031
326	0.001	0	0.0023	0.0018
432	0	0.002	0.0018	0.0027
504	0	0	0.0018	0.0018
600	0.001	0	0.0023	0.0018
672	0.002	0	0.0027	0.0018
720	0.001	0.002	0.0023	0.0027

Bicarbonate + 1.8 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.036	0.033	0.0172	0.0160
11	0.048	0.052	0.0224	0.0241
20	0.059	0.061	0.0271	0.0280
35	0.113	0.117	0.0502	0.0519
60	0.181	0.189	0.0793	0.0827
76	0.228	0.221	0.0994	0.0964
88	0.268	0.261	0.1166	0.1136
106	0.301	0.309	0.1307	0.1341
140	0.397	0.386	0.1718	0.1671
160	0.415	0.427	0.1795	0.1846
184	0.22	0.222	0.1920	0.1937
232	0.268	0.264	0.2331	0.2297
280	0.307	0.323	0.2665	0.2802
326	0.345	0.361	0.2990	0.3127
432	0.432	0.441	0.3735	0.3812
504	0.222	0.228	0.3875	0.3977
600	0.248	0.251	0.4320	0.4371
672	0.251	0.255	0.4371	0.4440
720	0.255	0.256	0.4440	0.4457

Bicarbonate + 1.8 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.045	0.04	0.0211	0.0190
11	0.053	0.056	0.0245	0.0258
20	0.071	0.071	0.0322	0.0322
35	0.122	0.115	0.0541	0.0511
60	0.198	0.202	0.0866	0.0883
76	0.245	0.247	0.1067	0.1076
88	0.285	0.288	0.1238	0.1251
106	0.339	0.345	0.1469	0.1495
140	0.426	0.433	0.1842	0.1872
160	0.486	0.479	0.2099	0.2069
184	0.266	0.267	0.2314	0.2323
232	0.347	0.354	0.3007	0.3067
280	0.388	0.391	0.3358	0.3384
326	0.436	0.438	0.3769	0.3786
432	0.539	0.541	0.4651	0.4668
504	0.298	0.275	0.5176	0.4782
600	0.306	0.297	0.5313	0.5159
672	0.313	0.31	0.5433	0.5381
720	0.316	0.315	0.5484	0.5467

Bicarbonate + 1.8 mg DW cells/ml + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.033	0.036	0.0160	0.0172
11	0.044	0.046	0.0207	0.0215
20	0.07	0.063	0.0318	0.0288
35	0.12	0.118	0.0532	0.0523
60	0.177	0.197	0.0776	0.0862
76	0.242	0.241	0.1054	0.1050
88	0.278	0.285	0.1208	0.1238
106	0.327	0.321	0.1418	0.1392
140	0.446	0.439	0.1927	0.1897
160	0.48	0.495	0.2073	0.2137
184	0.254	0.263	0.2211	0.2288
232	0.335	0.334	0.2905	0.2896
280	0.395	0.397	0.3418	0.3435
326	0.449	0.441	0.3881	0.3812
432	0.545	0.539	0.4702	0.4651
504	0.288	0.284	0.5005	0.4936
600	0.298	0.296	0.5176	0.5142
672	0.316	0.315	0.5484	0.5467
720	0.32	0.318	0.5552	0.5518

Bicarbonate + 1.8 mg DW cells/ml				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.036	0.042	0.0172	0.0198
11	0.049	0.052	0.0228	0.0241
20	0.066	0.062	0.0301	0.0284
35	0.108	0.121	0.0481	0.0536
60	0.185	0.203	0.0810	0.0887
76	0.249	0.255	0.1084	0.1110
88	0.286	0.281	0.1243	0.1221
106	0.33	0.335	0.1431	0.1452
140	0.44	0.448	0.1902	0.1936
160	0.475	0.484	0.2052	0.2090
184	0.26	0.272	0.2263	0.2365
232	0.344	0.352	0.2982	0.3050
280	0.403	0.392	0.3487	0.3393
326	0.455	0.454	0.3932	0.3923
432	0.558	0.552	0.4814	0.4762
504	0.287	0.285	0.4987	0.4953
600	0.303	0.307	0.5261	0.5330
672	0.318	0.315	0.5518	0.5467
720	0.32	0.32	0.5552	0.5552

Table B 4: Soluble U(VI) data in bicarbonate buffer

The following tables contain data for soluble U(VI) precipitated by inorganic phosphate released by *Cellulomonas* sp. ES6 in bicarbonate buffer under non-growth conditions. Soluble U(VI) concentrations were measured using unfiltered samples from serum bottle by KPA

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.897	24.176	0.1004	0.1016
11	22.156	24.234	0.0931	0.1018
20	24.856	23.564	0.1044	0.0990
35	23.546	23.879	0.0989	0.1003
60	23.471	23.712	0.0986	0.0996
76	22.196	23.167	0.0933	0.0973
88	24.187	23.618	0.1016	0.0992
106	23.794	23.987	0.1000	0.1008
140	23.657	24.157	0.0994	0.1015
160	23.823	23.538	0.1001	0.0989
184	22.951	23.268	0.0964	0.0978
232	23.545	23.861	0.0989	0.1003
280	23.227	23.47	0.0976	0.0986
326	23.928	23.174	0.1005	0.0974
432	23.187	23.189	0.0974	0.0974

Bicarbonate + 1.8 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.595	24.143	0.0991	0.1014
11	23.879	23.783	0.1003	0.0999
20	24.187	24.378	0.1016	0.1024
35	23.871	23.649	0.1003	0.0994
60	22.498	22.413	0.0945	0.0942
76	22.389	22.982	0.0941	0.0966
88	21.978	21.79	0.0923	0.0916
106	21.278	20.989	0.0894	0.0882
140	19.627	18.927	0.0825	0.0795
160	16.29	16.568	0.0684	0.0696
184	13.987	13.729	0.0588	0.0577
232	10.568	9.679	0.0444	0.0407
280	8.129	8.367	0.0342	0.0352
326	5.293	4.279	0.0222	0.0180
432	2.489	1.749	0.0105	0.0073

Bicarbonate + 1.8 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.457	24.172	0.1028	0.1016
11	19.126	20.367	0.0804	0.0856
20	15.728	14.679	0.0661	0.0617
35	10.956	11.278	0.0460	0.0474
60	4.896	4.152	0.0206	0.0174
76	3.978	4.568	0.0167	0.0192
88	3.526	3.478	0.0148	0.0146
106	N/A	N/A	N/A	N/A
140	2.167	1.978	0.0091	0.0083
160	N/A	N/A	N/A	N/A
184	1.475	1.942	0.0062	0.0082
232	N/A	N/A	N/A	N/A
280	N/A	N/A	N/A	N/A
326	N/A	N/A	N/A	N/A
432	1.782	1.559	0.0075	0.0066

APPENDIX C

The data in this appendix is the raw data of experiments performed to analyze samples for U oxidation state using XANES

Table C 1: Inorganic phosphate data

The following tables contain data for inorganic phosphate released by *Cellulomonas* sp. ES6 in either bicarbonate or PIPES buffer under non-growth conditions. Inorganic phosphate concentrations were measured by reading the absorbance at 880 nm with time.

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0	0.001	0.0018	0.0023
12	0	0	0.0018	0.0018
28	0.001	0	0.0023	0.0018
45	0.001	0.002	0.0023	0.0027
63	0.002	0.002	0.0027	0.0027
80	0	0.001	0.0018	0.0023
104	0.001	0	0.0023	0.0018
128	0	0.001	0.0018	0.0023
170	0	0.001	0.0018	0.0023
218	0	0	0.0018	0.0018
266	0	0.001	0.0018	0.0023

Bicarbonate + 2.07 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.026	0.023	0.0130	0.0117
12	0.034	0.038	0.0164	0.0181
28	0.077	0.083	0.0348	0.0374
45	0.12	0.118	0.0532	0.0523
63	0.175	0.177	0.0767	0.0776
80	0.201	0.196	0.0879	0.0857
104	0.26	0.255	0.1131	0.1110
128	0.303	0.296	0.1315	0.1285
170	0.18	0.174	0.1578	0.1526
218	0.23	0.235	0.2006	0.2049
266	0.27	0.265	0.2348	0.2305

Bicarbonate + 2.07 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.021	0.018	0.0108	0.0095
12	0.032	0.043	0.0155	0.0202
28	0.08	0.07	0.0361	0.0318
45	0.135	0.13	0.0596	0.0575
63	0.19	0.197	0.0832	0.0862
80	0.24	0.238	0.1046	0.1037
104	0.312	0.314	0.1354	0.1362
128	0.37	0.38	0.1602	0.1645
170	0.246	0.258	0.2143	0.2245
218	0.318	0.327	0.2759	0.2836
266	0.389	0.372	0.3367	0.3221

Bicarbonate + 2.07 mg DW cells/ml				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.024	0.022	0.0121	0.0113
12	0.036	0.039	0.0172	0.0185
28	0.077	0.072	0.0348	0.0327
45	0.136	0.139	0.0601	0.0613
63	0.201	0.194	0.0879	0.0849
80	0.246	0.241	0.1071	0.1050
104	0.324	0.321	0.1405	0.1392
128	0.381	0.378	0.1649	0.1636
170	0.252	0.238	0.2194	0.2074
218	0.311	0.308	0.2699	0.2674
266	0.38	0.382	0.3290	0.3307

PIPES + 1.24 mg DW cells/ml + 0.1 mM U(VI)				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.033	0.031	0.0160	0.0151
12	0.058	0.055	0.0267	0.0254
28	0.11	0.09	0.0489	0.0404
45	0.242	0.228	0.1054	0.0994
63	0.3	0.287	0.1303	0.1247
80	0.375	0.385	0.1624	0.1666
104	0.23	0.22	0.2006	0.1920
128	0.28	0.291	0.2434	0.2528
170	0.355	0.36	0.3076	0.3119
218	0.43	0.428	0.3718	0.3701
266	0.45	0.456	0.3889	0.3940

PIPES + 1.24 mg DW cells/ml				
	Absorbance at 880 nm		PO_4^{3-} concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	0.023	0.037	0.0117	0.0177
12	0.083	0.087	0.0374	0.0391
28	0.19	0.195	0.0832	0.0853
45	0.32	0.305	0.1388	0.1324
63	0.42	0.425	0.1816	0.1838
80	0.528	0.533	0.2278	0.2300
104	0.32	0.333	0.2776	0.2888
128	0.395	0.398	0.3418	0.3444
170	0.466	0.478	0.4026	0.4129
218	0.539	0.548	0.4651	0.4728
266	0.56	0.558	0.4831	0.4814

Table C 2: Soluble U(VI) data

The following tables contain data for soluble U(VI) precipitated by inorganic phosphate released by *Cellulomonas* sp. ES6 in either PIPES or bicarbonate buffer under non-growth conditions. Soluble U(VI) concentrations were measured using unfiltered samples from serum bottle by KPA

Bicarbonate + 0.1 mM U(VI) [Cell free control]				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.178	23.027	0.1016	0.0968
12	23.615	22.188	0.0992	0.0932
28	24.487	23.057	0.1029	0.0969
45	25.076	23.117	0.1054	0.0971
63	23.886	23.312	0.1004	0.0979
80	23.252	23.961	0.0977	0.1007
104	23.733	23.305	0.0997	0.0979
128	23.433	23.601	0.0985	0.0992
170	22.623	23.818	0.0951	0.1001
218	22.865	23.148	0.0961	0.0973
266	23.547	23.589	0.0989	0.0991

Bicarbonate + 2.07 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.48	22.983	0.0987	0.0966
12	22.39	22.328	0.0941	0.0938
28	21.349	22.92	0.0897	0.0963
45	20.329	21.587	0.0854	0.0907
63	11.323	11.32	0.0476	0.0476
80	7.328	6.928	0.0308	0.0291
104	4.302	4.401	0.0181	0.0185
128	4.501	5.489	0.0189	0.0231
170	5.298	5.228	0.0223	0.0220
218	3.497	3.492	0.0147	0.0147
266	2.187	2.482	0.0092	0.0104

Bicarbonate + 2.07 mg DW cells/ml + 0.1 mM U(VI) + 0.1 mM AQDS				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	23.498	22.439	0.0987	0.0943
12	24.375	22.368	0.1024	0.0940
28	23.479	23.493	0.0987	0.0987
45	22.347	22.478	0.0939	0.0944
63	22.308	22.193	0.0937	0.0932
80	21.392	21.328	0.0899	0.0896
104	20.397	19.329	0.0857	0.0812
128	17.38	16.329	0.0730	0.0686
170	14.297	15.287	0.0601	0.0642
218	8.362	7.217	0.0351	0.0303
266	3.492	2.541	0.0147	0.0107

PIPES + 1.24 mg DW cells/ml + 0.1 mM U(VI)				
	U(VI) concentration (ppm)		U(VI) concentration (mM)	
Time (Hrs)	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	24.576	23.943	0.1033	0.1006
12	18.298	16.283	0.0769	0.0684
28	9.265	7.267	0.0389	0.0305
45	3.429	3.394	0.0144	0.0143
63	1.379	1.323	0.0058	0.0056
80	1.324	1.425	0.0056	0.0060
104	1.534	1.624	0.0064	0.0068
128	1.478	1.984	0.0062	0.0083
170	N/A	N/A	N/A	N/A
218	N/A	N/A	N/A	N/A
266	1.437	1.526	0.0060	0.0064