This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-96SR18500 with the U.S. Department of Energy.

Elsevier Editorial System(tm) for Journal of Environmental Radioactivity

Manuscript Draft

Manuscript Number:

Title: In-situ Uranium Stabilization by Microbial Metabolites

Article Type: Research Paper

Keywords: Keywords: Uranium, Immobilization, Bacteria, Pyomelanin, Microbial pigment, Goethite, Illite, Fourier transform infrared spectroscopy.

Corresponding Author: Dr Charles Turick, Ph.D.

Corresponding Author's Institution:

First Author: Charles E Turick

Order of Authors: Charles E Turick; Anna S Knox; Chad L Leverette; Yianne G Kritzas

Abstract: Abstract
Soil contaminated with U was the focus of this study in order to develop in-situ, U bio-immobilization technology. We have demonstrated microbial production of a metal chelating biopolymer, pyomelanin, in U contaminated soil from the Tims Branch area of the Department of Energy (DOE) Savannah River Site (SRS) as a result of tyrosine amendments. Bacterial densities of pyomelanin producers were >106 cells/g wet soil. Pyomelanin demonstrated U chelating and mineral binding capacities at pH 4 and 7. In laboratory studies, in the presence of goethite or illite, pyomelanin enhanced U sequestration by these minerals. Tyrosine amended soils in field tests demonstrated increased U sequestration capacity following pyomelanin production up to 13 months after tyrosine treatments.

In-situ Uranium Stabilization by Microbial Metabolites

Charles E. Turick[1*], Anna S. Knox[1], Chad L. Leverette[2], and Yianne G. Kritzas[2]

[1]Biotechnology, Environmental Sciences and Technology, Savannah River National Laboratory, Aiken, SC USA 29808.

[2]Department of Chemistry and Physics, University of South Carolina, Aiken, SC USA 29801.

*Corresponding author. Mailing address: Savannah River National Laboratory, Biotechnology, Environmental Sciences and Technology, Building 999W, Aiken, SC 29808. **Telephone:** (803) 819-8407. **Fax:** (803) 819-8432. **Email:** Charles.Turick@srnl.doe.gov

In-situ Uranium Stabilization by Microbial Metabolites

Abstract

Soil contaminated with U was the focus of this study in order to develop in-situ, U bio-immobilization technology. We have demonstrated microbial production of a metal chelating biopolymer, pyomelanin, in U contaminated soil from the Tims Branch area of the Department of Energy (DOE) Savannah River Site (SRS) as a result of tyrosine amendments. Bacterial densities of pyomelanin producers were $>10^6$ cells/g wet soil. Pyomelanin demonstrated U chelating and mineral binding capacities at pH 4 and 7. In laboratory studies, in the presence of goethite or illite, pyomelanin enhanced U sequestration by these minerals. Tyrosine amended soils in field tests demonstrated increased U sequestration capacity following pyomelanin production up to 13 months after tyrosine treatments.

Keywords: Uranium, Immobilization, Bacteria, Pyomelanin, Microbial pigment, Goethite, Illite, Fourier transform infrared spectroscopy.

1. Introduction

Humics play a significant role in metal immobilization by binding to both metal oxides and soluble metals (Gu et al., 1995; Gu et al., 1996). The presence of hydroxyl groups, carboxyl density, and linear molecular structure of humics contribute to their strong surface complexation to iron oxides (Gu et al., 1995; Gu et al., 1996). Humic compounds form innersphere complexes with metals (Xia et al., 1997) and the mobility of metal/humic-colloid complexes decrease as a result of humic interactions with the soil particles (Artinger et al., 2002).

While naturally occurring soil humics are ubiquitous, their quantities and specific characteristics vary considerably. Consequently the heterogeneity of humics *in-situ* and aging effects decrease their effectiveness and dependability for remedial action. Microbial production of humic-type compounds offers potential for increasing the metal sorption capacities of soils. The class of pigments known as melanins are humic-type compounds and are the most common pigments produced in nature. Hence, microbial melanin has a role to play in bioremediation (McLean et al., 1998) and melanin production offers tremendous untapped potential for immobilization of metals and radionuclides because of its electron shuttling (Turick et al. 2002, 2003) and metal sequestration capacities (McLean et al., 1998). For instance microbial melanin is responsible for enhanced adsorption of numerous metals and tributyltin chloride with the fungus *Aureobasidium pullulans* (Gadd et al., 1990; Gadd and Mowll, 1985; Gadd et al., 1987), Fe(III) reduction and subsequent Fe(II) assimilation by the yeast *Cryptococcus neoformans* (Nyhus et al., 1997) and U accumulation by a melanin-containing fungus associated with the lichen *Trapelia involuta* (McLean et al., 1998).

Melanin is a generic term describing a ubiquitous humic-type metabolite (Ellis and Griffiths, 1974). Microbial melanins account for a measurable portion of naturally occurring soil humic compounds (Scott and Martin 1990). Melanin production is often a function of the enzymes tyrosinase (EC 1.14.18.1) or poly-phenol oxidase. These types are commonly associated with eukaryotes.

Another, less-studied type of melanin is pyomelanin. Pyomelanin production (Yabuuchi and Omyama. 1972) is documented in a number of bacterial species (Yabuuchi and Omyama. 1972; Coon et al., 1994; Kotob et al., 1995; and Ruzafa et al., 1995 and Turick et al. 2002) and originates from bacterial conversion of tyrosine as part of the fumarate pathway. Complete breakdown of tyrosine to acetylacetate and fumarate requires the enzymes 4-hydroxyphenylpyruvic acid dioxygenase (4-HPPD) and homogentisic acid (HGA) oxidase. In the absence of this enzyme (or if HGA production exceeds that of HGA-oxidase), HGA is over-produced and excreted from the cell (Coon et al., 1994). Auto-oxidation and self-polymerization of HGA then results in pyomelanin, an aromatic polymer consisting of numerous quinone moieties (Ruzafa et al., 1995).

Microbial melanin production by autochthonous bacteria was explored in this study as a means to increase U immobilization in U contaminated soil. This article demonstrates the application of bacterial physiology and soil ecology for enhanced U immobilization.

2 Methods

2.1. Sediment properties and analysis

The Tims Branch watershed makes up a portion of SRS and parts of this watershed have been impacted in the past with U (Pickett et al., 1987, and Evans et al., 1992). Sediments representative of this watershed were selected to study the affects of microbial metabolites on the behavior of U in sediment. Sediment samples were collected aseptically and immediately stored on ice until delivery to the lab. Sediment samples were then refrigerated until processing. These sediments were quantified for melanin producing bacteria and U concentration.

The following sediment properties were analyzed: percent organic matter (OM) by loss-on-ignition at a temperature of 375^{o}Cand pH was determined from a 1:1 mineral/water equilibration solution. Also, total concentration of U in a homogenized sediment sample was

determined by a total microwave digestion of 0.6 g of homogenized sediment material with concentrated acids (10 ml of HNO_3, 4 ml of H_2SO_4, and 2 ml of HCl). The resulting extract solution was analyzed by ICP-MS.

Changes in the organic content of sediments due to pyomelanin production were determined through percent organic matter determination (above), spectrophotometric determination (absorbance, 400nm) of particle free supernatants and Fourier transform infrared spectroscopy (FTIR) spectroscopy. Sediment samples were removed (5-10g/column) 28 days after treatment addition for pore water analysis. Optical density was determined for centrifuged sediment to determine the degree of pigmentation resulting from tyrosine amendments. Dried pigments from sediment effluents (60°C) were then further characterized using FTIR spectroscopy and compared to that of pure tyrosine and dihydroxyphenylalanine (DOPA) melanin (Aldrich Chemicals). Bacterial pyomelanin used for FTIR characterization was obtained as described previously (Turick et al 2002).

2.2. Analysis of Pigment Producing Microorganisms in Sediment

Sediment was diluted (10^{-2} – 10^{-8}) with lactate basal salt medium (LBSM) supplemented with 1g/l tyrosine (Turick et al., 2002). Controls received the same treatment except tyrosine was omitted. Each tube of the 3 tube most probable number (MPN) assay contained 10 ml of growth medium and sediment and was incubated for 8 weeks at 25°C and shaken at 100 rpm. Increased pigmentation as a result of tyrosine amendments was determined spectrophotometrically by scanning the supernatant fluid of each test tube from 600-300 nm. Tubes with increased OD in this range, relative to controls were marked positive for pigment production from tyrosine. The number of positive tubes per dilution was used to calculate the most probable number of pigment producing cells/g sediment.

To isolate pigment-producing microorganisms, samples were removed aseptically from the sediment MPNs above and inoculated onto tryptic soy agar supplemented with tyrosine (2g/l) (TSAT), using the spread plate method. Following incubation at 25°C for 1 week, colonies

demonstrating pigmentation (relative to plates without tyrosine) were transferred from TSAT to LBSM agar with and without 2g/l tyrosine and monitored for pigmentation. Pigment production was determined by comparing coloration from tyrosine amended plates relative to those without tyrosine.

2.3. Pigment Characterization

Initial characterization of pigment included intensity of coloration and diffusion through agar media. Dark black non-diffusive pigments are indicative of DOPA melanin whereas redish brown diffusive pigments are more likely pyomelanin. The chemical sulcotrione (supplied gratis from Zeneca Agrochemicals) was used as a specific competitive inhibitor of 4-HPPD (Secor, 1994); the enzyme required for pyomelanin production. Methods incorporated sulcotrione to determine if pigment production was a result of 4-HPPD. Pure cultures of sediment isolates were grown in LBSM and tyrosine (1g/l) was supplemented to promote pigment production. Sulcotrione (18μM) was added prior to pigmentation in order to differentiate pyomelanin production from other pigments that may be produced. Pigment production was monitored and characterized spectrophotometrically (400 nm) (reference) in cell free culture fluid and visually. Controls for comparison were without tyrosine or without sulcotrione.

2.4. Laboratory studies of pyomelanin complexation with U, goethite and illite.

Bacterial pyomelanin was concentrated and dialyzed from culture as described previously (Turick et al. 2002) stored as a stock solutions in HEPES buffer (pH 7 and pH 4). Various pyomelanin dilutions were incorporated into chelating studies with U and sorption studies with goethite (Alfa Aesar) and illite (Clay Minerals Society) at pH 4 and pH 7. For U chelating studies, desired concentrations of pyomelanin stock were added to 15 ml of HEPES buffer (pH 4 and pH 7) containing 100μg/l U (uranyl nitrate, Sigma Chemicals). The solutions were shaken in sealed polypropylene vials at 150 rpm at 25°C for 13 days prior to analysis. U concentrations unbound to

pyomelanin were measured by removing the pyomelanin through centrifugal concentration (5000 rcf for 1-2 h) with 5 kDa dialysis membranes. Clear, pyomelanin-free liquid was analyzed for U with ICP/MS.

Pyomelanin sorption studies with goethite and illite (10mg/ml) were conducted in 15 ml HEPES buffer (pH 4 and pH 7). Pyomelanin concentrations and incubation were as above. Pyomelanin sorption was determined through optical density measurements (400 nm) of the centrifuged samples (5000 rcf for 30-45 min.) and correlated with known pyomelanin standards.

Interactions with U, pyomelanin and goethite or illite were conducted as above for the mineral studies. U concentrations were determined with ICP/MS.

2.5. Sediment Column studies – field deployment

2.5.1. Sediment Sampling

Sediment samples taken from each sediment column were analyzed for moisture, organic matter content and metal concentrations just prior to tyrosine supplementation. Percent organic matter (OM), pH, and the concentration of U were determined as described above.

2.5.2. Sediment Column Construction

Sediment columns were constructed to facilitate field testing of the methodology. These columns were constructed to allow rainfall to leach through the columns and be collected for analysis at different depths and at the discharge. The design consisted of an inner and outer housing with the inner housing constructed of 10cm diameter Schedule 40 PVC and the outer housing constructed of 20cm diameter Schedule 40 PVC. Construction details for the sediment columns are shown in Figure 1.

At the field site, a pit was dug by hand to facilitate installation of the sediment columns. Sediment from the pit was homogenized and cleaned of any roots or other debris. This homogenized, native sediment was then placed in the inner housing of each sediment column.

After the inner housings were filled with the native sediment, lysimeters (Sediment Moisture Equipment Corp. Rhizon lysimeters) were installed at three different depths (10, 30 and 50 cm) by inserting them through predrilled holes perpendicular to the long dimension of the housing. Figure 1 shows the general orientation for the lysimeters. Each lysimeter was connected to the top of the sediment column using color coded nylon tubing. For each sediment column, the inner housing was then placed inside the outer housing.

After the lysimeters were installed, the annulus between the inner and outer housing was filled with clean sand. Nylon tubing was connected to a predrilled hole in the bottom of the outer housing and extended to the top of the sediment column. This allowed for the collection of the leachate exiting the inner housing. A protective cap with holes to allow for rainfall infiltration was fitted to the top of the inner housing of each sediment column (Fig. 1). A cover was placed over the annulus between the inner and outer housing to prevent rainfall infiltration (Fig. 1).

After assembly, the sediment columns were placed in the pit and void space between the sediment columns was then filled with the remaining sediment from excavation. Metal sheeting was used to protect the sediment columns from animal damage.

2.5.3. Treatment conditions

Sediment columns remained untreated for 2 months to allow for settling and periodic lysimeter checks. Sterile amendments were added to numbered columns chosen randomly for each treatment on August 3, 2005. Twenty four hours prior to sampling, excess water was removed from the sediment columns with a peristaltic pump attached to the tubing connected to the bottom of the outer housing of the sediment columns. Triplicate treatments included tyrosine

(2 g/kg sediment) (10mM), and 100 ml sterile DI water. Sterile DI water alone served as the control. One kg of sediment constituted approximately 30% of the volume of each field column.

2.5.4. Leachate Sampling

Throughout the 28 days of incubation the top 5 cm of sediment of each column was mixed with sterile plastic spatulas on day 7 and 14, to assist tyrosine mixing and solubility. During field incubation rainfall was measured at 12.1 cm. After incubation sediment pore water was obtained from each lysimeter (three depths per column). Samples were removed from sediment by fitting each lysimeter with a 18 gauge hypodermic needle and inserting the needle into a negative-pressure, gas-tight test-tubes. The negative pressure in each 10 ml test tube pulled pore water from the sediment at the lysimeters sediment depth. Pore water samples were stored immediately on ice until overnight storage in the lab at 4°C. Samples were processed the next day and analyzed for metals and pH.

3. Results

3.1. Analysis of Pigment Producing Microorganisms in Sediment

Bacterial densities of pigment producers from sediment samples of the study site demonstrated MPN values of 1.1×10^6 cells /g wet wt of soil. Pigment production was evident with tyrosine treatments but not controls. Dilutions (10^{-1}) of sediment-free pore water (from 5 cm cores) effluents demonstrated a significant increase in pigmentation one month after tyrosine addition, with an average absorbance (OD_{400}) of 1.78 (SD = 0.6) for tyrosine treated sediments compared to an absorbance of 0.01 for controls.

3.2. Pigment Characterization

Extracellular bacterial pigmentation was evident on TSAT and LBSM plates with tyrosine 48-72 h after inoculation and increased in intensity for several weeks. The pigment was redish-brown in color and diffused throughout the agar. DOPA melanin would be expected to be dark back in color and diffuse poorly in agar. Pigment production was halted in bacterial cultures with sulcotrione, relative to pigment production without the inhibitor (data not shown), indicating that pyomelanin was the pigment produced.

Effluents from soils incubated for 30 days with tyrosine were analyzed to determine if differences existed in FTIR response relative to treatments. Tyrosine amended soils demonstrated a characteristic response similar to that of pyomelanin (Fig. 2) but dissimilar to pure tyrosine or DOPA melanin (Fig. 3). In particular, similarities between bacterial pyomelanin and tyrosine amended soils were observed at wavenumbers corresponding to C=O, aromatic rings, phenolic OH groups, acetate, benzene rings, and C-O bonds associated with alcohols (Conley, 1966; MacCarthy and Rice, 1985; Turick, et al. 2002; van der Mei, et al. 1989). FTIR scans of pyomelanin were different from the other compounds used as standards (Figs. 2 and 3), demonstrating the potential to discriminate between pyomelanin and other related compounds.

3.3. Pore water analysis.

Pore water samples taken 30 days after treatments, from 10, 30 and 50 cm depths demonstrated significantly decreased U in tyrosine treated sediments compared to controls (Fig. 4). U concentrations were only slightly higher one year after tyrosine amendments occurred, indicating a capacity for U immobilization for an extended time. Pore water from any depth sampled of tyrosine amended sediment was not pigmented. This is in contrast to surface sediments of the same treatments one month after tyrosine was applied.

3.4. Sediment analysis

Following tyrosine additions, organic matter content of tyrosine amended sediment was 1.76% compared to 1.14% for untreated sediment ($P<0.1$). pH values did not change significantly over time nor with treatment conditions. For soil depths of 10 and 30cm, pH was 4.2($\pm$0.3) and for 50cm depth, pH values were 5.0($\pm$0.3).

3.5. Laboratory studies of pyomelanin complexation with U, goethite and illite

Pyomelanin demonstrated the ability to complex U as a function of pH and pyomelanin concentration (Fig. 5). Nearly complete complexation of the 100µg/l spike at pH 4 took place with all pyomelanin concentrations analyzed. Pyomelanin demonstrated complete adsorption to geothite at both pH 4 and pH7 and illite at pH4 (Fig. 6). Adsorption also occurred with illite at pH 7, albeit to a lesser degree than that of pH4. U adsorption to goethite and illite at pH4 was enhanced in the presence of pyomelanin compared to samples without pyomelanin (Fig. 7). At pH7, U sorption to goethite and illite was complete in the absence of pyomelanin (Fig. 7). The amount of U sorbed at pH7 did not decrease as a function of increasing pyomelanin concentrations, indicating that pyomelanin was not detrimental to U-mineral sorption at this pH.

4. Discussion

Melanin producing microorganisms are common in sediments and melanin pigments are capable of binding metals and actinides as well as sorbing to clays and iron minerals. This project incorporated the physiological potential of bacteria to retard U mobility through the production of the recalcitrant, humic-type pigment, pyomelanin.

The quantity of pigment producing bacteria determined through MPN assays from sediment at the study site demonstrated potential for melanin production in-situ. Tyrosine amended soils and pure cultures from the study site confirmed this, and resulted in pyomelanin

production as the most abundant pigment produced, as determined by growth observations, enzyme inhibitor studies and FTIR analysis.

Pyomelanin was abundant in surface sediments of tyrosine treatments, but absent in pore water at 10-50 cm depths from the same treatments throughout the study. The lack of pigmentation at depth indicated pyomelanin sorption to soil. Laboratory studies with pyomelanin confirmed complete pyomelanin sorption to iron minerals at pH4 and pH7 as well as complete sorption to clay at pH4. The laboratory studies demonstrated the high probability of pyomelanin sorption to iron and clay minerals in the pH 4 sediments of the study site.

U complexation with pyomelanin was also demonstrated in the laboratory with nearly 100% of U associated with pyomelanin in all concentrations studied at pH4. The ability of pyomelanin to complex with U and also sorb well to clay and iron minerals at pH4 was further demonstrated in laboratory studies, with significant increases of U associated with goethite and illite in the presence of pyomelanin. One possible scenario is that pyomelanin served to "tether" U to goethite and illite and thereby enhance U immobilization.

Field studies corroborate the laboratory results with significant U immobilization in pore water from tyrosine amended sediments. The low U concentrations in pore water more than one year after tyrosine addition to the sediment indicates that pyomelanin, a humic-type compound, is recalcitrant and thereby contributes to U immobilization for an extended time.

This study demonstrated the physiological potential of subsurface bacteria to produce metabolites capable of U sequestration for extended periods. The one-time addition of tyrosine to sediment exploited the ability of indigenous microbes to produce pyomelanin, resulting in U immobilization for at least 13 months. To our knowledge, this is the first demonstration of the stimulation of in-situ melanin-type pigment production for contaminant immobilization.

Acknowledgements

This research was supported in part through funding by the Department of Energy Natural and Accelerated Bioremediation Research (NABIR) program; The Savannah River National Laboratory Independent Research and Development Program and Soil and groundwater Closure Projects of the Savannah River Site. The authors thank Denis Jackson, Ken Dixon and Raymond Roseberry for assistance with design and implementation of field studies. The authors also thank Thomas H. Cromartie of Zenica Agrichemicals for furnishing the sulcotrione.

References

Artinger, R., Schuessler, W., Schaefer, T., Kim, J-I., 2002. A Kinetic Study of Am(III)/Humic Colloid Interactions. Environ. Sci. Technol. 36: 4358–4363.

Conley, R.T., 1966. Quantitative analysis. In. Infrared spectroscopy. Boston, MA. USA. Allyn and Bacon Inc. p. 87-175.

Coon S.L., Kotob, S., Jarvis, B.V., Wang, S., Fuqua, W.C.,Weiner, R.M., 1994. Homogentisic acid is the product of MelA, which mediated melanogenesis in the marine bacterium *Shewanella colwelliana* D. Appl. Env. Microb.60:3006-3010.

Ellis, D.H., Griffiths, D.A., 1974. The location and analysis of melanins in cell walls of some soil fungi. Can. J. Microbiol. 20:1379-1386.

Evans, A.G., Bauer,L.R., Haslow, J.S., Hayes, D.W., Martin, H.L., McDowell, W.L., Pickett, J.B., 1992. Uranium in the Savannah River Site environment. Rep. WSRC-RP-92-315. Westinghouse Savannah River Company. Aiken, SC.

Gadd, G.M., Gray D.J, Newby, P.J., 1990. Role of melanin in fungal biosorption of tributyltin chloride. Appl. Microbiol. Biotechnol.34:116-121.

Gadd G.M. Mowll, J.L., 1985. Copper uptake by yeast-like cells, hyphae, and chlamydospores of *Aureobasidium pullulans*. Exp. Mycol. 9:230-240.

Gadd G.M., White C., Mowll J.L., 1987. Heavy metal uptake by intact cells and protoplasts of *Aureobasidium pullulans*.FEMS Microbiol. Lett.,. 45:261-267.

Gu, B., Mehlhorn, T.L., Liang, L., McCarthy, J.F., 1996. Competitive adsorption, displacement, and transport of organic matter on iron oxide: II. Displacement and transport. Geochim. Cosmochim Acta. 60:2977–2992.

Gu, B., Schmitt, J., Chen, Z., Liang, L., McCarthy, J.F., 1995. Adsorption and desorption of different organic matter fractions on iron oxide. Geochim. Cosmochim Acta. 59:219–229.

Kotob S.I., Coon, S.L., Quintero E.J., Weiner, R.M., 1995. Homogentisic acid is the primary precursor of melanin synthesis in *Vibrio cholera*, a *Hyphomonas* strain, and *Shewanella colwelliana*. Appl. Environ. Microbiol. 61:1620-1622.

MacCarthy, P., Rice, J.A., 1985. Spectroscopic methods (other than NMR) for determining functionality in humic substances. In: Humic substances in soil, sediment, and Water. (Eds.) G.R. Aiken, D.M. McKnight, R.L. Wershaw, and P. MacCarthy. New York, NY. USA. Wiley-Interscience. p. 527-560.

McLean, J., Purvis, O.W., Williamson, B.J., Bailey E.H., 1998. Role for lichen melanins in uranium remediation. Nature. 391:649-650.

Nyhus, K.J., Wilborn, A.T. & Jacobson E.S., 1997. Ferric iron reduction by *Crypotococcus neoformans*. Infection and Immunity. 65:434-438.

Pickett, J.B., 1990. Heavy metal contamination in Tims Branch sediments. Westinghouse Savannah River Co., Aiken, SC.

Ruzafa, C., Sanchez-Amat A., Solano F., 1995. Characterization of the melanogenic system in *Vibrio cholerae* ATCC 14035.Pigment Cell Research. 8:147-152.

Scott, D.E., Martin J.P., 1990. In Humic substances in soil and crop sciences. pp. 44-72. (Eds) P. MacCarthy, C.E. Clapp, R.L. Malcolm and P.R. Bloom. American Society of Agronomy Inc., Madison WI. USA.

Secor, J., 1994. Inhibition of barnygrass 4-hydroxyphenylpyruvate dioxygenase by sulcotrione. Plant Physiol.106:1429–1433.

Turick, C.E., Tisa, L.S., Caccavo, F.Jr., Melanin production and use as a soluble electron shuttle for Fe(III) oxide reduction and as a terminal electron acceptor by *Shewanella algae* BrY. Appl. Environ. Microbiol. 68:2436–2444, 2002.

Yabuuchi, E., Omyama A., 1972. Characterization of "pyomelanin"-producing strains of *Pseudomonas aeruginosa.* Internat. J. Syst. Bacteriol. 22:53:64.

van der Mei, H.C., Noordmans, J., Busscher, H., 1989. Molecular surface characterization of oral streptococci by Fourier transform infrared spectroscopy. J. Biochim. Biophys. Acta. 991:395-401.

Xia, K., Bleam, W., Helmke, P.A., 1997. Studies of the nature of binding sites of first row transition elements bound to aquatic and soil humic substances using X-ray absorption spectroscopy Geochim. Cosmochim ACTA. 61:2223–2235.

Figure legends

Figure 1. Construction details for the sediment columns used for field studies.

Figure 2. FTIR spectra from pore water one month after addition of tyrosine. Comparison of pore water pigment to pyomelanin produced from a pure bacterial culture demonstrate similarities between peaks as indicated on graph.

Figure 3. FTIR spectra of tyrosine and DOPA-melanin. FTIR data of soil effluents following 30 days incubation with tyrosine (Fig. 2) were not similar to pure tyrosine or DOPA-melanin.

Figure 4. Pore water uranium concentrations. Uranium concentrations were consistently lower in tyrosine treated soil (TY-U) compared to controls (DI-U) measured at 3 depths, 1month (2005) and 13 months (2006) after treatments were added.

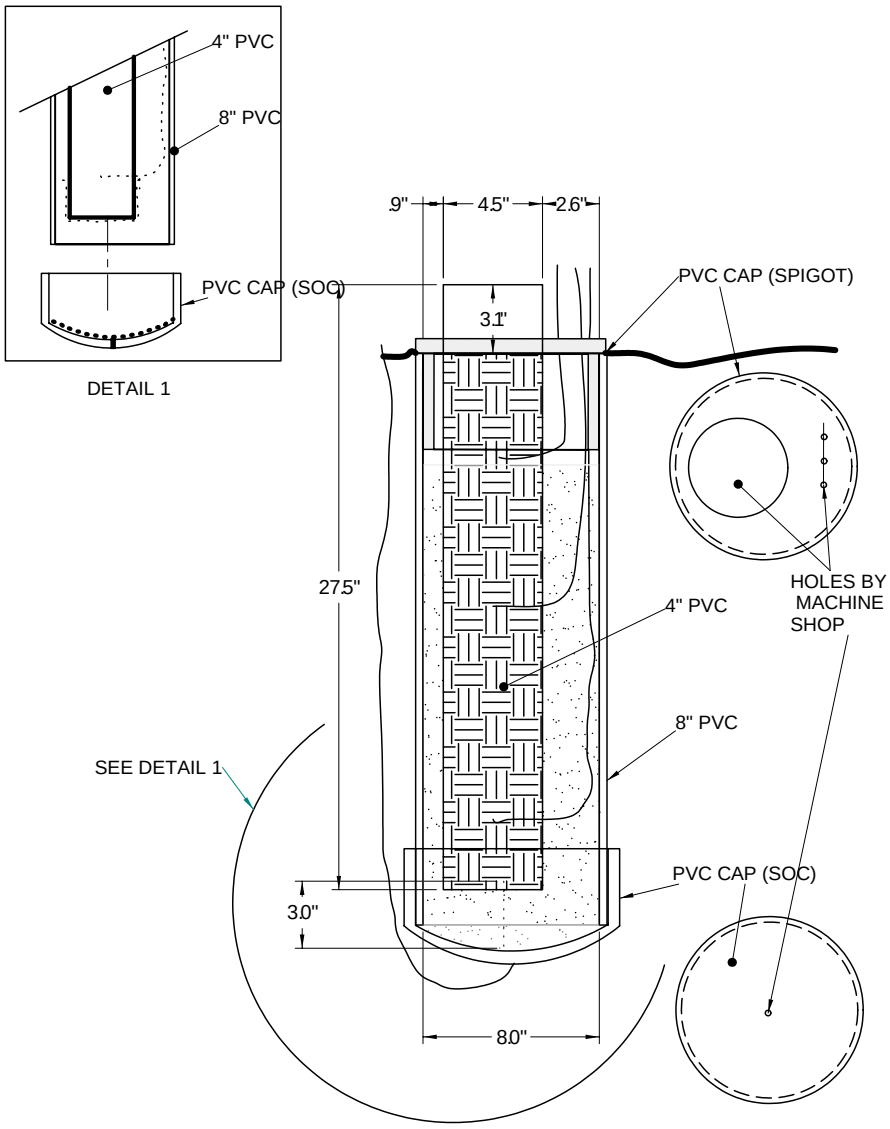
Figure 5. U complexation with pyomelanin. U complexed with various pyomelanin concentrations, demonstrating U complexation, with the greatest degree occurring at pH 4.

Figure 6. Pyomelanin sorption to goethite and illite. Pyomelanin sorbed completely to 10 mg/ml goethite at pH 4 and 7, and illite at pH 4. Sorption decreased with illite at pH 7.

Figure 7. U sorption to minerals as a function of pyomelanin concentration. U sorption to 10 mg/ml of goethite and illite was significantly enhanced in by pyomelanin at pH 4.

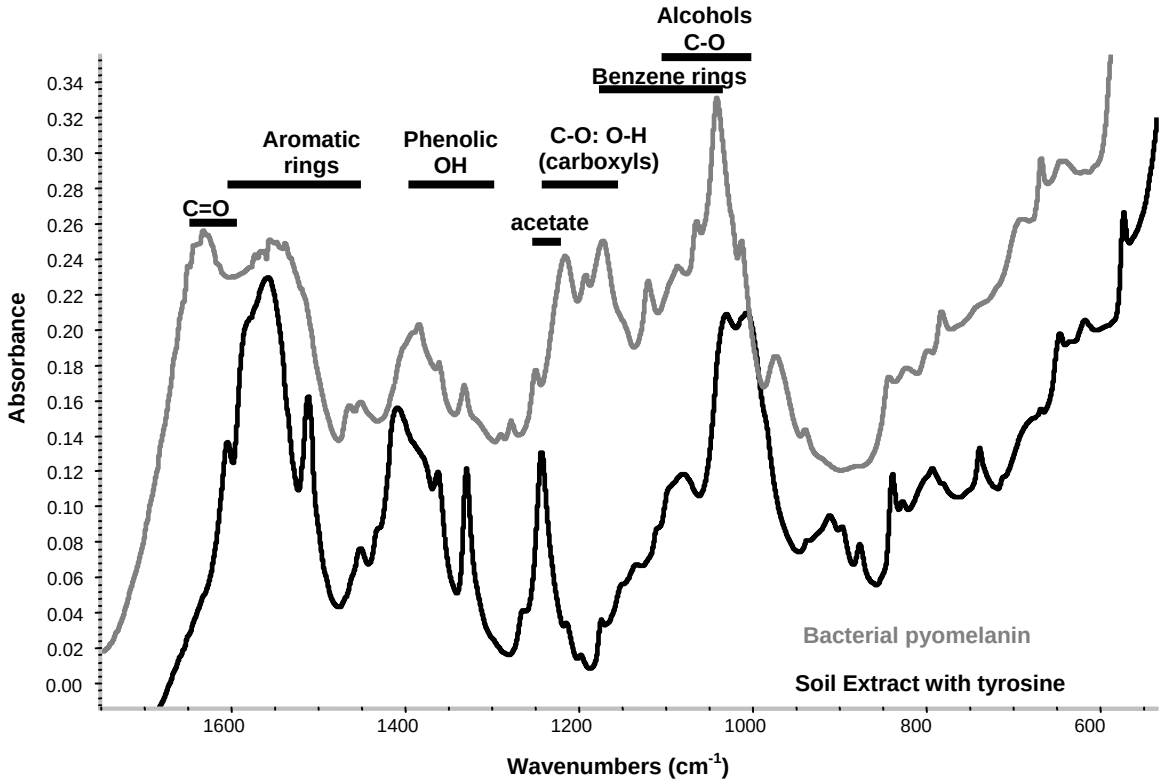
Figure(s)

Click here to download Figure(s): Fig 1-PDF.pdf



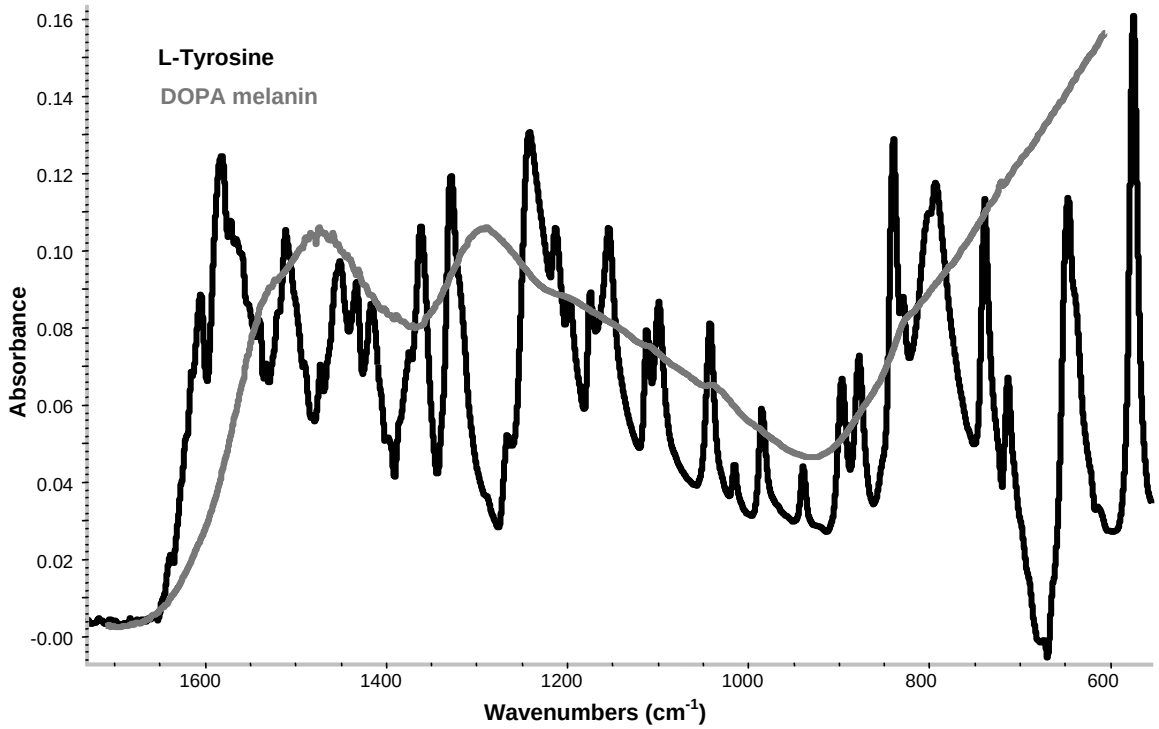
Figure(s)

Click here to download Figure(s): Fig 2 - PDF.pdf



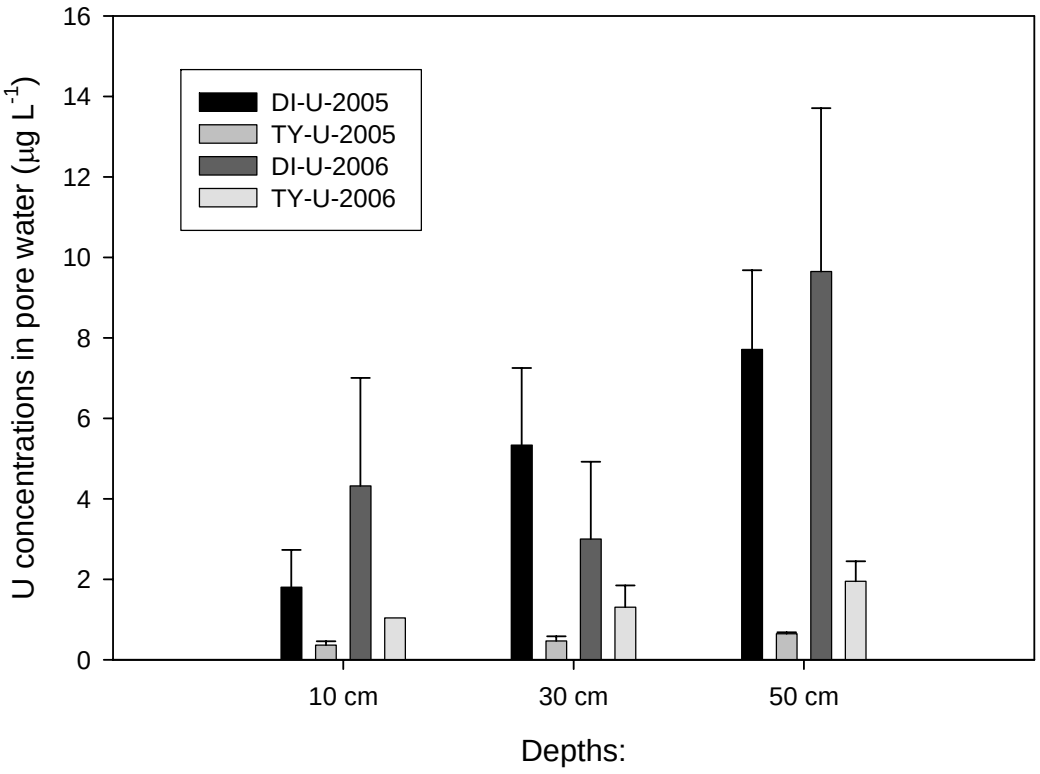
Figure(s)

Click here to download Figure(s): Fig 3 - PDF.pdf



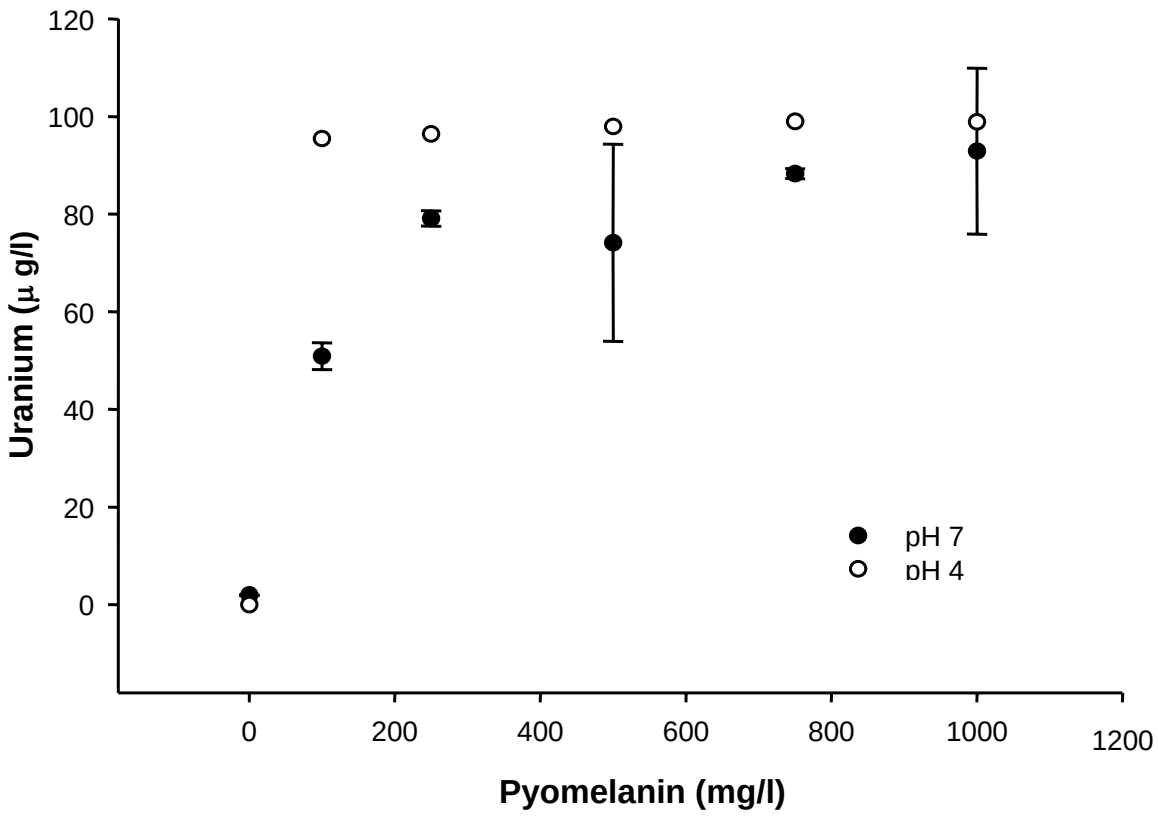
Figure(s)

Click here to download Figure(s): Fig 4 - PDF.pdf



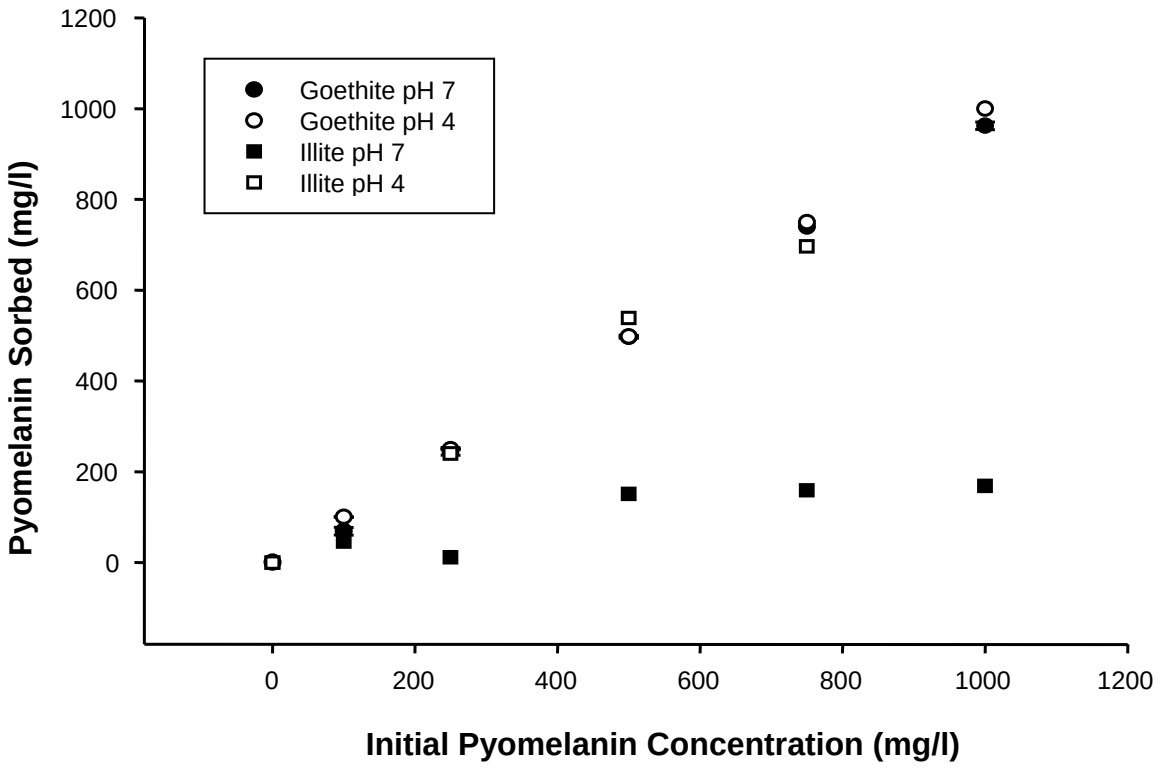
Figure(s)

Click here to download Figure(s): Fig 5 - PDF.pdf



Figure(s)

Click here to download Figure(s): Fig 6 - PDF.pdf



Figure(s)

Click here to download Figure(s): Fig 7 - PDF.pdf