

FINAL REPORT
U.S. Department of Energy

**"Green" Biopolymers for Improved Decontamination of Metals from Surfaces: Sorptive Characterization
and Coating Properties**

Principal Investigator: Dr. Brian H. Davison
Oak Ridge National Laboratory
PO Box 2008, MS 6226, Oak Ridge, TN 37831, (865) 576-8522; davisonbh@ornl.gov
Collaborators: Dr. Tanya Kuritz,
Oak Ridge National Laboratory, (865) 241-6013; ztk@ornl.gov
Dr. Koen P. Vercruyse, Asst. Professor, Dept. of Chemistry
Tennessee State University: (615)-963-5340; KVercruyse@tnstate.edu
Catherine K. McKeown, Oak Ridge National Laboratory

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2. Table of Contents

3. Executive Summary	2
4. Research Objectives.....	2
5. Methods and Results	3
6. Relevance, Impact and Technology Transfer	6
7. Project Productivity	6
8. Personnel Supported	7
9. Publications	7
10. Interactions	8
11. Transitions	8
12. Patents	8
13. Future Work	8
14. Literature Cited	9
15. Feedback	10
16. Appendices	na

3. Executive Summary

“Green” biopolymers were studied to develop a decontamination method that is efficient, safe, and environmentally friendly. We have successfully confirmed the core hypothesis of that proposal: to use aqueous biopolymer solutions to coat a contaminated metal surface (i.e., steel), solubilize the heavy metals (uranium) from the surface, bind the heavy metals into the biopolymer, and remove the biopolymer-radionuclide complex. This “apply, wait, and remove” procedure should reduce the amount of worker time spent in decontamination activities.

A semipurified algal biopolymer system was developed in our research that, at present, removes >80% of the *fixed* uranium (VI) from contaminated steel coupons. These results required us to screen and separate the appropriate fraction of several promising biopolymers, develop a technique to quantitatively construct contaminated coupons, and then apply and remove coats of contaminated biopolymer (Davison and Kuritz, 2000).

These experiments strongly support the hypothesis that physical removal can be enhanced by sorption. StripCoat and other commercial coatings do not sorb the metals; they have a purely physical method of removal. The charged biopolymer was known to have higher metal capacities, and thus would have the greatest enhancing effect compared to the uncharged biopolymer. Other possible explanations for the extent of binding that require further study include purely chemical effects -- such as aqueous oxidation of the steel surface or low pH.

4. Research Objectives:

The proposed research aimed to develop a fundamental understanding of important biological and physical chemical parameters for effective decontamination of metal surfaces using environmentally benign aqueous-based biopolymer solutions. Understanding how heavy metal–chelating biopolymers coat and interact with contaminated surfaces will benefit the development of novel, safe, easy-to-apply decontamination methodologies for removal of radionuclides and heavy metals. The benefits of these methodologies include the following: decreased exposure hazards for workers; decreased secondary waste generation; increased efficiency of decontamination; positive public appeal and development of novel, nature-friendly business opportunities; and lower cost of cleanup to the government.

We proposed to use aqueous biopolymer solutions to coat a contaminated metal surface (i.e., steel), solubilize the heavy metals (e.g., uranium) from the surface, and bind the heavy metals into the biopolymer. The biopolymer coating (containing the immobilized hazardous metal contaminants) was to be removed as a viscous film, as a dry powder, or by washing. This “apply, wait, and remove” procedure will reduce the amount of worker time spent in decontamination activities.

5. Methods and Results

Biopolymer testing. We have measured and confirmed the metal sorptive capabilities of several biopolymers at radionuclide concentrations of up to 0.2 g U/g dry biomass and have selected a promising cyanobacterium, *Nostoc muscorum* strain HPDP22 (gift of L. Sirenko, Institute of Hydrobiology, Academy of Sciences, Ukraine). We have also isolated and analyzed the effectiveness of metal-binding and rheological properties of whole biomass and of different biopolymer fractions (different types of biopolymers from the same microorganism) from several sheath-producing algal species. As a result, we have selected the fraction that is most promising for metal chelation on surfaces from *Nostoc*-produced biopolymers. This charged soluble fraction (SPS) had a viscosity of 20 cp.

Different microalgal biomasses and biopolymer fractions showed widely varying sorption properties with regard to barium, cadmium, iron, copper, and uranium as measured by inductively-coupled plasma emission spectroscopy (ICP). Comparative isotherms for uranium (VI) and cadmium shown in **Figure 1** indicate the greater specificity and capacity for uranyl by the *Nostoc* sp. High uranyl capacity and specificity is desired to allow for competition with other metal ions that may be present. The capacity greater than 0.05 g/g is greater than ion exchange resins at low concentration.

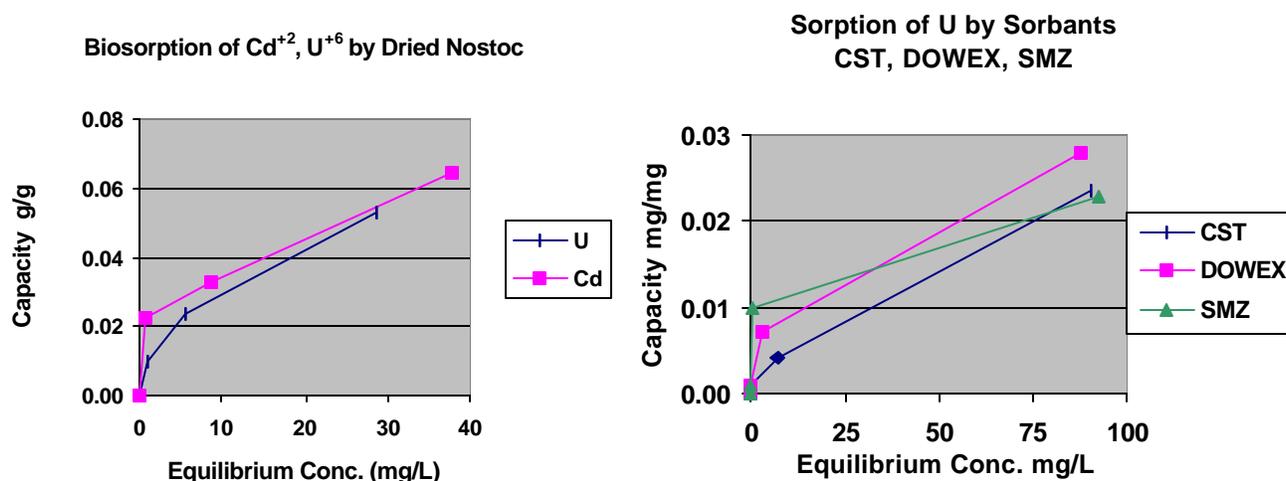


Figure 1. Sorption isotherms for uranium and cadmium onto biomass from *Nostoc*. **Figure 2.** Uranyl sorption isotherms for several resins. By comparing **Figure 1** with **Figure 2**, one can observe that the dried alga has a more than 10X higher capacity for uranyl than any of the commercially available ion exchange resins tested.

Numerous chromatographic resins have been designed and tested for their ability to remove metals from solution. Three of these were chosen for comparative testing to our dried *Nostoc* algae: Surfactant Modified Zeolite (SMZ), Crystalline Silico-Titanate (CST) and DOWEX 21K (**Figure 2**). The protocol followed for testing these sorbants was virtually identical to the one used for dried algae, differing only in the amounts tested, 10 mg for sorbants vs 4 mg for algae. (Due to particle size, the sorbants could not be accurately weighed below the 10 mg amount.)

Biopolymer production. Algal cultures were grown in photobioreactors for 10-12 days in mineral media, then left to mature for an additional 10-12 days (**Figure 3**). “Maturation” of cyanobacteria is a poorly understood process in which these microorganisms produce copious quantities of biopolymers, predominantly polysaccharides. In our experiments, we tested the following biopolymers under different application conditions: (1) dry; whole-fraction biomass, (2) the insoluble polysaccharide fraction (IPS); and (3) the soluble polysaccharide fraction (SPS). We

examined polymer abundance, process cost, and rheological properties of the fractions, and selected the SPS fraction of biopolymers produced by *Nostoc muscorum* HPDP22 for all second- and third-year work. This organism grows easily on minerals in the presence of light and produces large amounts of chelating biopolymers upon “maturation”.

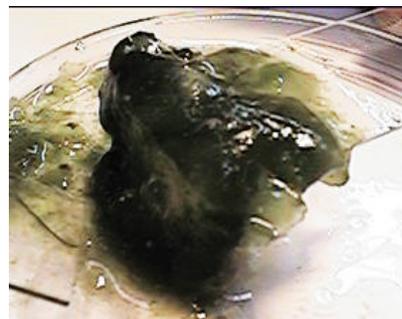


Figure 3. The alga *Nostoc* sp. and its biopolymer.

Biopolymer characterization As part of the general project, this specific segment dealt with the soluble exocellular polysaccharide (SPS 485) produced by a strain of cyanobacterium capable of binding uranyl cations (UO_2^{2+}). The specific goals were to study the chemical composition of this polysaccharide and to determine the binding constant of the interaction between the polysaccharide and uranyl cations. Equilibrium dialysis experiments were set up to obtain the binding isotherms for the chelation of uranyl cations to SPS 485. The chemical composition of the polysaccharide was evaluated using the carbazole assay for uronic acids and HPLC analysis of the monosaccharides generated following acid hydrolysis of the polysaccharide. These analyses indicated the presence of glucose (10%) and fucose (13%) monomeric units in the polysaccharide. Mannose, galactose and/or xylose (25% total) were present also, but as these monosaccharides could not be resolved in this analysis, no conclusions could be made about the relative abundance or absence of these monosaccharides. Other carbohydrates were present that could not be identified with the standards used so far. In addition, no glucuronic or galacturonic monomers could be observed in appreciable quantities (1%). The presence of signals at lower retention times might indicate that there is a core oligosaccharide structure that resists the acid hydrolysis. This TFA-resistant core might still contain uronic acids. More drastic hydrolysis conditions need to be applied to obtain further details regarding the chemical structure of the repeating unit of this polysaccharide.

Biosorption of soluble biopolymer fraction (SPS).

We measured the binding capacity of the soluble fraction, which had shown the best properties for sorption and for film formation. Conventional sorption isotherm experiments were not possible for the soluble biopolymer. A novel way to directly measure the amount of uranyl sorbed by biopolymer was carried out by using commercially available small volume (1.0 mL) dialysis bags. Biopolymer, in buffer, at a known concentration was introduced into a series of bags, each immersed in tubes of uranyl solution. Buffer alone in bags and immersed served as controls. Tubes and bags of each were sacrificed at various time points and the uranyl concentrations determined by ICP analysis. Figure 4 shows that equilibrium was reached in 2-3 days. The resulting isotherm is shown in Figure 5.

Other heavy metals. During the first year of the project, copper (a model heavy metal) was used to screen for biopolymer sorption and for testing of decontamination procedures on surfaces. This was discontinued due to poor quantification of surface copper concentrations; both spectroscopy and indirect measurements from mass balances on the prepared coupons and removed solutions failed to produce reliable results. Despite the higher regulatory and safety requirements, we began using depleted uranium (in the form of uranyl); the actual radionuclides give

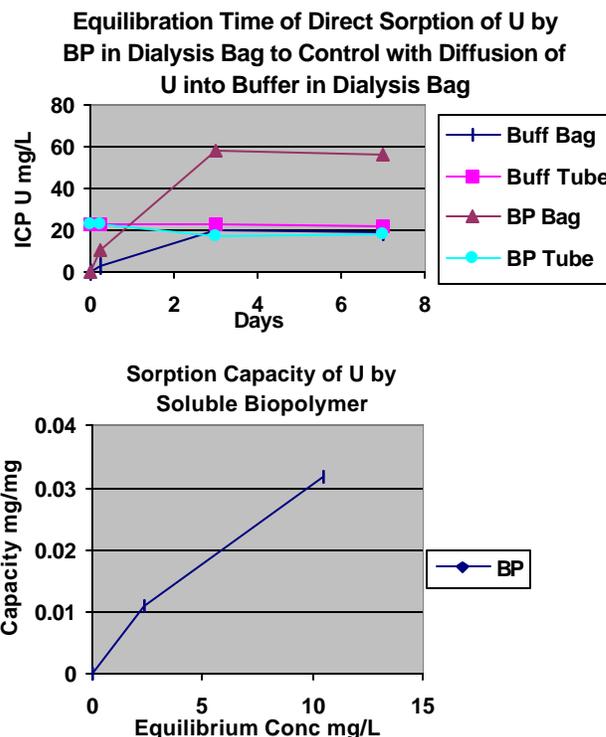


Figure 4. Concentration vs. time in typical soluble biopolymer experiment showing equilibration.

Figure 5. Uranyl isotherm with soluble biopolymer at 2 d.

higher analytical accuracy and reproducibility both in direct surface measurements by alpha disintegrations and by solution analysis using ICP of radionuclides than the use of nonradioactive surrogate metals. Other sorption tests were performed with Cd and Ba.

Development of quantified contaminated sample coupons. A critical ancillary achievement of the project was the development and validation of a method to quantitatively apply and “fix” uranyl contamination onto a metal surface (steel) and measure the resulting alpha radiation; this was essential to quantify contaminant removal. “Fixed” contamination is tightly bound to a surface where “loose” or “transferable” contamination can be dislodged by simple physical rubbing. “Fixed” contamination removal is the challenge of decon. Coupon preparation was aided by the prior work of Pierce (1960) and Demmer (1994) for development of simulated contaminated surfaces. To prepare a ‘hot’ coupon, silicone epoxy was used to create a dike at the coupon edge; this allowed a uniform layer of contaminated liquid to remain on the surface. The coupon was then oven-baked (100°C) until the liquid evaporated and the salts were baked onto the surface (**Figure 6**). A simple, quantifiable rinse was then used to remove “easily removed” contamination (<5%); a subsequent aggressive smear only removed 2% more. This was presumed to be the “loose” or transferable contamination, with the remainder presumed to be “fixed.” Several methods were used to quantify contaminant surface concentrations; we primarily relied on direct alpha disintegration counts using a sample counter. Multiple readings on single coupons varied by about 1%; coupon to coupon variability was 5%. The reproducible use of this instrument required construction of the coupons as 3.8-cm-diam. unpolished A36 carbon steel discs. The steel was chosen to simulate ductwork. The actual mass of uranium contamination on the coupon was estimated to be 0.93 mg U per coupon or 0.08 mg U/cm².



Figure 6. Quantitatively Uranyl contaminated coupons used in tests.

Tests of biopolymer decontamination of contaminated steel coupons. An example of a representative experiment follows. Two polymer fractions (IPS and SPS) were isolated from mature *N. muscorum* HPDP22 by centrifugation. The cell pellet was used for the isolation of another, cell-bound, insoluble biopolymer fraction (IPS). The two formulations (and a whole dried biomass control) were applied individually to steel coupons and allowed to dry.

The whole biomass and the insoluble biopolymer fractions were more difficult to remove than the soluble fraction from the surface after treatment; the latter of these formed easily-removable flakes. In a procedure similar to those performed on ion-exchange resins, the soluble biopolymer was pre-treated in a low ionic strength buffer to render it uncharged (near neutral pH) or was positively charged by adjusting to pH 3. **Figure 7** shows results from six individually tracked contaminated coupons – in each case, the biopolymer was applied as a coat, dried, and then removed from the coupons. Comparing the “before” and “after” alpha counts, the biopolymer successfully removed much (~60%) of the radioactive contamination (Davison, et al., 1999) after a single application. After a second application of the charged biopolymer, over 80% of the contamination was removed. In our experimental

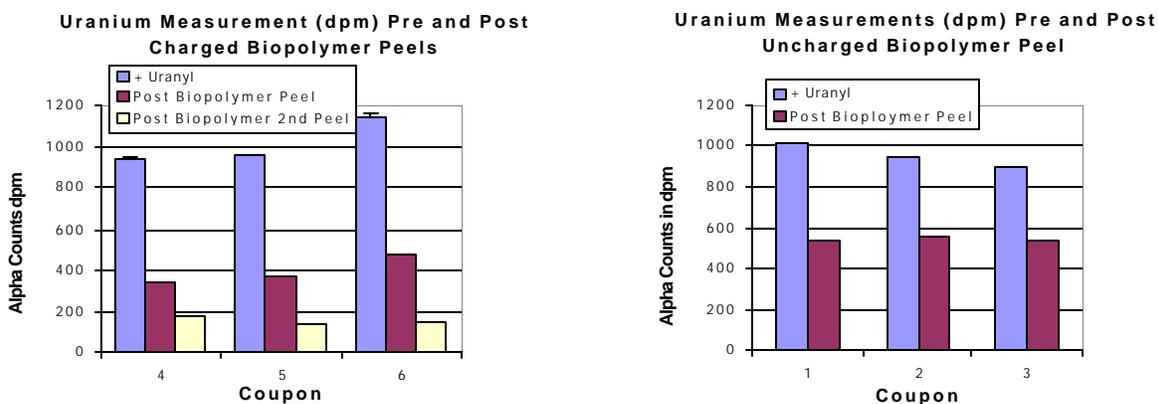


Figure 7. The charged RPS biopolymer fraction removed > 80% of surface-bound U⁺⁶, as assessed by both radioactive alpha disintegrations per min (dpm) and inductively coupled plasma spectroscopy.

estimates, 0.02 mL containing 5 mg of dry biopolymer was used to treat 1 cm² and was removed as the dry biopolymer (< 0.01 mL final volume). We have shown that the dried biopolymer is removable without secondary washing. In addition, the secondary rad waste generated in two applications was 10 mg of biopolymer containing 80% of the surface contamination - estimated at 0.7 mg U. This is a substantial volume reduction for secondary waste compared to washing.

In an important experiment (Figure 8), the charged biopolymer solution was compared with a commercial strippable coating (“StripCoat”, Sanchem, Inc.). In these tests, StripCoat removed ~30% of the contamination, while the biopolymer solution removed 45% of surface-bound uranium after 24 h. The error bars from the replicate tests on triplicate individual contaminated coupons indicate that the difference is statistically significant. In Florida International University’s field tests on actual surfaces, StripCoat removed about 80% of the contamination (Madaris, 1999). The discrepancy between the absolute removal by StripCoat may relate to the amount of loose contamination on the actual SRS surfaces and to the nonoptimized nature of the biopolymer tests. Nevertheless, in direct comparison, our biopolymer is clearly competitive with StripCoat and potentially more effective than other non-biological strategies.

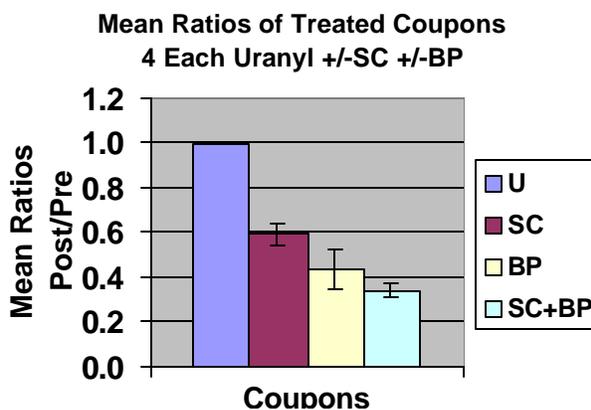


Figure 8. Comparison of Physical removal with combined Physical and Sorptive removal. U (initial contaminated coupon), SC (Stripcoat removal), BP (soluble biopolymer removal).

An enhancement of uranyl removal from metal coupons was obtained by the combination of the sorptive properties of the biopolymer with the physical removal obtained from StripCoat. The actual mixing of charged biopolymer with StripCoat before application to the “contaminated” coupon resulted in better removal than additive layers of biopolymer followed by StripCoat. Biopolymer plus StripCoat removed 10% more uranyl than biopolymer alone.

6. Relevance, Impact and Technology Transfer

The core hypothesis was demonstrated - a biopolymer which combines sorption and physical removal has improved removal properties both in absolute and when compared to either physical only or sorptive removal. However, this system has not been directly applied at a contaminated site. We have tested a layered application of biopolymer and StripCoat for easier removal; and we are preparing several manuscripts and presentations. An EMSP decon renewal proposal was submitted in 2001 to address additional critical questions and hypotheses which have arisen during this project: 1] how to enhance the impact of biosorption in addition to the physical removal of contaminants by the biopolymer? 2] What are the critical characteristics and treatments of biopolymer that make it a good metal chelator and coating? and 3] How can biopolymer production be stimulated in cyanobacteria? This renewal was not selected, determined as too applied for the EMSP.

The use of “strippable” coatings. The concept of easy physical removal of contamination on surfaces is being explored and tested in the DOE-EM. In one of the best studies (Madaris, et al. 1999), Florida International University tested six commercial strippable coatings at Savannah River. The coatings were applied and allowed to dry and polymerize in place, entrapping the contaminant particles. The commercial coatings were removed either as a film or as flakes to be vacuumed. Five of the six could be removed successfully from surfaces at SRS having both fixed and transferable contamination. The strippable coatings removed about 60 to 80% of the transferable and fixed contamination. Other variables judged included the curing time and the cost per unit decontaminated. All were organic polymers and generally part of an aqueous solution or mixture. The ALARA 1146 (a water-based vinyl) coating was judged to be the most effective method. While not tested, these polymers did not generally have metal sorption properties. The key utility of these coatings was to contain and remove a significant portion of loose contamination from surfaces. Further demonstration tests as part of an LSDDP confirmed these results with overall contamination removal of up to 85% (DOE-EM-OST, 2000). This demonstration also showed that a strippable

coating could produce three orders of magnitude less waste mass – and this did not include the evaporative loss of water from the coating as it dried into a solid.

The above nonbiological demonstration helps to identify steps needed for full-scale deployment *after further laboratory work is completed*. Key parameters are costs/sq.ft. of the biopolymer coating, ease of application or removal as well as maintenance of a humid environment for the coating. In this project's plans, we will perform tests on coupons from actual contaminated surfaces. We hope to test combinations of biopolymer with other polymeric coatings to gain improved removal properties. We will also make initial estimates on the possible application methods (e.g., spraying or painting) and the costs to produce the biopolymer (e.g., algal growth). Further steps will be: a more complete cost estimate of biopolymer production, production and formulation of biopolymer coating in a quantity suitable for testing, small tests on surfaces at a DOE contamination site, tech transfer to firms that produce algal products, and a larger side-by-side demonstration.

This project needs one additional year of targeted applied research to prepare for a demonstration since the basic fundamentals were proven. See section on **Future Work** below.

7. Project Productivity

Did the project accomplish all of the proposed goals? Yes.

Was the project on schedule? Yes, with a 7-month no-cost extension.

Was the work plan revised? No

Successes:

- We have confirmed and measured the metal sorptive capabilities (up to 0.2 g uranyl/g dried biopolymer) and selected an algal biopolymer from *Nostoc* sp. for further studies. The research team isolated different biopolymer fractions from several sheath-producing algal species (algal “slime”) and tested for polymer abundance, process cost, and rheological properties, eventually choosing *Nostoc muscorum*, which grows easily on minerals in the presence of light. Different polymeric fractions have different sorption properties with regard to cadmium, iron, copper, and uranium. Metal sorption was the highest if polymer fractions were “charged” by different treatments.
- One of the polysaccharide fractions removed up to 80 percent of surface-bound U⁺⁶ without optimization as assessed by radioactive disintegration and inductively coupled plasma spectroscopy. This uranium stayed immobilized in a peelable film. Effective contact was critical. Washing was not needed to remove the biopolymer.
- A critical ancillary achievement of the project has been the development and validation of a simpler method to quantitatively apply and “fix” uranyl contamination to a metal surface (steel) and measure the alpha radiation, which was essential to quantify contaminant removal. The development of these techniques delayed the project.

Disappointments:

- Use of copper as an “easier” screen for biosorption and for decon of surfaces was discontinued due to poor quantification of surface concentrations using spectroscopy or indirect measurements. Despite regulatory and safety requirements, the use of depleted uranyl has given higher measurement accuracy and consistent reproducibility. The additional costs of handling radioactivity required the experiments to be more tightly focused.
- The reproducible testing of actual legacy contamination was not accomplished.

8. Personnel Supported

Brian H. Davison, Sr. Scientist, ORNL
Tanya Kuritz, Scientist, ORNL

Catherine K. McKeown, Research Assistant, ORNL
Catherine Mattus, Research Assistant, ORNL
John W. Barton, Scientist, ORNL
Frank Barrera, Health Physics, ORNL

9. Publications

Davison, BH. "Green Biopolymers for Decontamination" – poster presentation at “Workshop on integration of end user needs with research projects for EMSP: Focus on Deactivation and Decommissioning” at Savannah River Site on Nov. 17-18, 1998.

Davison, BH, T Kuritz, CK McKeown, 1999. “Green Biopolymer for Decon of Contaminated Surfaces,” *Proceedings: 2nd Topical Meeting on Decontamination, Demolition and Restoration (DD&R) Topical Meeting on Site Restoration of Government and Commercial Facilities*, Sep. 12-16, 1999, Knoxville, TN.

Davison, BH, and T Kuritz, 2000 “Peeling Off Contamination” *Initiatives in Environmental Technology Investment* 7 (Fall):12.

Davison, B.H., et al. 2000. "Green Biopolymers for Decon of Contaminated Surfaces," EMSP National Workshop, Atlanta, GA USA, DOE-EMSP, 04/25/2000-04/27/2000.

Davison, BH; McKeown, CK; Kuritz, T; Barton, JW, 2001. "Green" Biopolymer Coatings for Improved Decontamination of Metals from Surfaces. 13th Annual Technical Information Exchange Conference, Albuquerque, NM USA 11/13/2001-11/15/2001.

Davison, BH, and T Kuritz 2001. ""Green" Biopolymers for Improved Surface Contamination: Strippable Coating Properties and Sorptive Characteristics," National AIChE Convention, Reno, NV USA, American Institute of Chemical Engineers, 11/04/2001-11/09-2001.

Kuritz, T. 1999. “Remediation by Cyanobacteria”, T. Kuritz, International Meeting on Applied Algology, Monte Cantini Terme, Italy (9/99)

Papers in preparation

Davison, BH; Verduyck, K.. McKeown, CK; Kuritz, T. “Uranyl binding by soluble algal biopolymers”. *Biotech Lett.* (2001).

McKeown, CK, Davison, BH. “A simplified technique for quantitative radioactive contamination of metal surfaces,” *Hydrometallurgy*.

Davison, BH, McKeown, CK, Barton, JW, Kuritz, T. “Decontamination of uranium contaminated metal surfaces with a removable sorptive biopolymer” *Appl Environ. Microbiol.*

10. Interactions

We established contacts with several manufacturers of coating for Decon (Florida International University, Pentek, Barlett Services).

We contacted Rick Dehmer of INEEL for information and procedures on “Simcon”; a reproducible fixed contaminated metal surface.

Algal biomass producers: Elisha Ter-Or, Hebrew University, Israel; Shosham Arad, Ben Gurion University, Israel.

Participation/presentations at meetings, workshops, conferences, seminars, etc. We have presented platform and poster presentations at several national conferences including AIChE, TIE, EMSP, and an international algal meeting.

Collaborations. We collaborated with professors at Tennessee State University (an HBCU).

11. Transitions

12. Patents

none.

13. Future Work

Critical questions have arisen in this project that are unanswered. We need to identify why charged soluble biopolymers have improved sorption and removal characteristics, as well as gain a better fundamental understanding of biological, interfacial, transport, and chemical processes that govern effective decontamination using aqueous biopolymer solutions for decontamination of surfaces. In particular, we must separate and quantify physical removal due to the biopolymer from the sorptive and solubilization chemical enhancements. This is needed in order to understand and prepare a proper deployment formulation.

Particular scientific goals include elucidation of:

1. Effect and enhancement of sorption in the “strippable” biopolymer – In particular, we must clearly separate and quantify physical removal due to the biopolymer from the sorption and solubilization enhancements. This is needed in order to understand and prepare a proper deployment formulation and for possible combination with other nonsorptive commercial polymer products. This includes measurement of dynamics and capacities of heavy metal removal by biopolymers on surfaces.
2. Characterization of algal biopolymers – We have identified a biopolymer fraction from blue-green alga (cyanobacterium) *Nostoc muscorum* as a very effective candidate and shown that “pH charging” can enhance its chelating properties. How does the state of the biopolymer relate to the increased metal-chelating, rheological and surfactant properties? For example, we must identify why charged biopolymers have improved sorption and removal characteristics. We expect this to relate to ion exchange theory and the charge of uranyl groups on the polysaccharides. Can this be accomplished by pretreatments other than lowered pH? Other properties that may affect metal sorption and need to be factored in include the interfacial and rheological properties for effective application to surfaces.
3. Basic knowledge of stimulation of algal production of metal-chelating biopolymers – Current empirical practices for cyanobacteria (blue-green algae) involve growing batch cultures photosynthetically until late log-phase and then allowing a “maturation” phase where copious biopolymer is produced. Better methods of biopolymer production are needed. We believe that there should be molecular genetic regulatory systems which trigger maturation (e.g., quorum sensing) and that there are environmental methods to get growth enhancement and improved metal-chelating properties (e.g., light delivery and cation depletion). This will utilize other fundamental DOE data generated through the Microbial Genome Initiative.
4. Biosolubilization and contamination – How does solubilization occur on the metal surface? We expect that this can only occur at the metal-biopolymer interface while the biopolymer remains as an aqueous film. Will concurrent aqueous oxidation or surfactant solubilization enhance or detract from the coating’s function? We propose that some initial oxidation may help provide access for solubilization of the contaminants that may be just below the steel surface layer.

14. Literature Cited

Anon., Innovative Technology Summary DOE/EM-0533, “ALARA 1146 Strippable Coating”, 4/2000.

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Demmer, R.L., 1994. "Development of Simulated Contaminants (SIMCON) and Miscellaneous SIMCON Scoping Tests." Idaho National Engineering Laboratory, Westinghouse Idaho Nuclear Company, Inc., Idaho Falls, ID.

Madaris, SC, BM Castro, M Ahlen. 1999. Proceedings: 2nd Topical Meeting on Decommissioning, Decontamination and Reutilization, Sept 12-16, Knoxville, TN

Pierce, EE, 1960. Oak Ridge National Laboratory Report #CF-60-6-54; NSA Accession #NSA-14-018160.

15. Feedback

This project was technically successful. It would have greatly benefited by more interaction with the EM focus areas (we did present at one of their meetings, but did not receive much targeted advice). More interaction with other decon EMSP teams should have been encouraged. The project also ended at a difficult transition point. It had proven the core hypothesis but had not "reduced to field practice". Realistically it needed more R&D over about a year to confirm the promise, before showing enough potential for a field test. However, there was no obvious funding mechanism for this - it was premature for demonstration, and it had moved beyond fundamental (as evidenced by the EMSP renewal proposal).