Final Report July 14, 2017

**MUS Research Initiative Project 51060-MUSRI2015-01:
Remediation Technology for Chlorinated Pollutants Based on a Natural Product from Soil Bacteria**

T. Lewis, M. Queen, Principal Investigators

This project's objectives addressed the programmatic goal of growing important research sectors that increase the diversity of Montana's economy and lies within the 'Materials' category. Our aim was to derive improved materials for chlorinated pollutant remediation based on an effective process for carbon tetrachloride (CT) destruction in contaminated water. The natural product pyridine-2,6-bis(thiocarboxylic acid)(PDTC) is a natural product discovered by one of the PIs as being responsible for CT destruction when cultures of a bacterium were exposed to that pollutant. Use of PDTC offers the destruction of the pollutant in contaminated groundwater without the expense of removal, for example by pumping from wells (i.e. 'pump and treat' technology) but rather by directly introducing PDTC into the zone of contamination. As that work was published several years before, it exists as 'prior art' in the eyes of patent officials. We have recognized some potential limitations of PDTC for remediation, including its solubility in the active, copper-bound form and its lack of chemical functionality for linking to a solid for applications such as reaching dense plumes that require the material to sink quickly. Given below is a summary of progress toward specific goals elucidated in our proposal and accomplished during the funding period.

Objective 1: Have verified, chemically pure PDTC sulfonate, polymer-linked PDTC, and their copper complexes: December 10, 2017

- **Progress Towards Objective:** The original approach taken for synthesis of the PDTC derivatives involved a route that would allow maximal versatility in the types of derivatives that could be produced in future work. That proposed route involved some novel chemistry, which our collaborators (MSU subcontract) had experience with, but nonetheless offered challenges to achieve the desired payoff in versatility. Attempts at developing that route (lithiation and substitution) have given very poor yields to date. We intend to explore modifications of the methodology, but have also taken a more conventional route more recently (protection, production of acyl chloride, substitution and deprotection). We used that approach on a limited set of starting molecules to obtain a polymer-linked (polyethylene glycol) derivative, and a more soluble (hydroxymethyl) derivative. After purity determinations, tests of those derivatives' CT removal rates and water solubility will be performed in experiments planned for this summer at MSUB.

Objective 2: Have data regarding solubility and dechlorination rates for new derivatives of PDTC: April 1, 2017

- **Progress Towards Objective:** The solubilities of both free PDTC and copper bound PDTC were determined in water at 1M ionic strength and 20°C at a wide range of pHs. Those values were 7mM and 50 μM, respectively with very little pH dependence for either species. We determined a second order rate constant for CT removal by copper PDTC (Cu:PDTC) in phosphate buffer at pH 7.7 under both ambient oxygen and anoxic conditions. Those were 1.1×10^{-5} $s^{-1}mM^{-2}$ and 3.9×10^{-5} $s^{-1}mM^{-2}$ respectively. The utility of those values lies in design of remediation systems (possibly anoxic) by engineers. Those values also represent a deliverable product of the work that we will apply in comparisons with chemically characterized PDTC derivatives in future work.

Objective 3: Have initial toxicology assessment of simulated remediation mixtures, refined dechlorination data to include other solvents, effects of aquifer solids: July 1, 2017

- **Progress Towards Objective:** Toxicology assessment of PDTC or derivatives was not performed as verified derivatives were not obtained during the funding period. Regarding activity toward other solvents (specifically, chlorinated organic pollutants), previous work had shown that Cu:PDTC is not reactive toward the most relevant chlorinated pollutants trichloroethylene (TCE) or tetrachloroethylene (PCE). Assessment of increased reactivity will await production of derivatives with significant differences in chemical properties, aided by rational design using insights gained from X-ray spectroscopy and modeling. Tests using PDTC and aquifer solids will be conducted using instituional funds and methodology developed under Objective 2.

Long-term Impacts

MREDI funding has allowed us to advance our goals of devising materials for remediation technology. This has put us in position to apply for patents for materials not already described in our published work, and for advanced materials research such as X-ray spectroscopy. Those studies are planned and will be conducted at the Stanford Synchrotron Radiation facility. The aim of that work is at elucidating fundamental chemical features that afford carbon-chlorine bond activation for remediation of the largest class of organic pollutant found in U.S. groundwater.

As a result of MREDI funding, we are still pursuing aspects of PDTC-based remediation materials under other funding. Those efforts will include further synthetic chemistry approaches as well as tests of reaction rates under simulated groundwater conditions (e.g. effects of aquifer solids) and toxicology. We intend to use institutional funding to support student-driven toxicology studies with an aquatic invertebrate (*Daphnia* species) using PDTC and available PDTC derivatives and to use that data to leverage further funding for toxicology by a certified laboratory.

Additional grants received "*SSLR Data Collection"* Queen, NIH 5 P20 GM103474 (MT INBRE) subaward: (ID pending). $90,132. 5/1/17-4/30/18. The work proposed under this project builds on the progress made in chemical synthesis under MREDI funding by focusing on the use X-Ray absorption spectroscopy to characterize electronic structure and function relationships for a diverse set of M(PDTC)L derivatives. Ultimately this work aims to develop a computer model for use in computer aided design of novel PDTC derivatives.

Jobs created:

MREDI funding supported one full-time technician throughout most of the funding period. Our initial hire was an MSUB graduate and an experienced researcher who found a career position as a Clinical Research Coordinator at a local hospital. Our second hire was a recent MSU graduate who gained significant experience in analytical chemistry while on the project and is currently seeking employment in a related field. The funding also supported two undergraduate researchers, and expanded to as many as five through institutional and other outside funding.