End of Fiscal Year Report: January 2017-June 30, 2017
Isolating and Characterizing Microbial Bioreactors of Pesticides and Quantifying Bioremediation Rates in Multichannel Wood Chip Bioreactors

California Department of Pesticide Regulation and CSU Monterey Bay AB20 Agreement

Primary Investigator

Arlene Haffa, PhD, MS, Assistant Professor of Biochemistry and Microbiology,
School of Natural Sciences, California State University, Monterey Bay
100 Campus Center, Seaside, CA 93955
(831) 582-4695, ahaffa@csumb.edu

Key Personnel

John Silveus, MS, Lecturer in Environmental and Biological Sciences,
School of Natural Sciences, California State University, Monterey Bay
100 Campus Center, Seaside, CA 93955
jsilveus@csumb.edu

Project Summary

California is the leading agricultural state with 13% of the US market (CDFA, 2014 Crop Year Report), and this industry is supported by pesticide applications. Woodchip bioreactors are one of the most promising on-farm management practices for mitigation of pesticides, but there is little data in a controlled environment as to conditions that would optimize their functionality. California State University Monterey Bay (CSUMB) has constructed a bioremediation testing facility that currently has 4 four channel bioreactors that can be run in concert to provide a control and triplicate data. Our objective is to isolate microbes capable of pesticide bioremediation, and then seed the bioreactors with these strains. Any strains isolated will be shared with others conducting on-site studies of woodchip bioreactors at the discretion of the California Department of Pesticide Regulation (CDPR). This could be used to help close the knowledge gap and offer guidelines as to how such bioreactors could be best used in tile drains on farms to reduce environmental impacts.

Scope of Work

Our research objectives are to create a library of freezer stocks of microbes that can bioremediate the main insecticides of concern to the CDPR and to obtain preliminary data on removal (adsorption, degradation) of these insecticides in woodchip bioreactors inoculated with these cultures using the multi-channel bioreactor facility. The pesticides we will target are: 1) Organophosphorus insecticides (malathion, chlorpyrifos, dimethoate); 2) A neonicotinoid (imidacloprid); and 3) Pyrethroids (bifenthrin (lambda cyhalothrin, permethrin, cypermethrin)).

Work Completed to Date

Logistical Tasks

The PI (A. Haffa) and key personnel (J. Silveus) met with the Sponsored Program Officer (C. Limesand) shortly after funds were made available on our campus (February 28, 2017). In March a Procard (i.e. corporation credit card) for J. Silveus was requested, in order to for him to make purchases for the bioreactors. The Spectrophotometer was ordered in April, and received in early May. Pesticides were procured in mid-April. Materials needed to repair the bioreactors were obtained.

Monday April 17, 2017 Dr. Xin Deng and 2 co-workers came for a site visit. We toured the laboratories and the site where the multi-channel bioreactors are located.

We have recruited the graduate student who will work on the project (Zane Mortensen), and submitted a request for a 2017-2018 Academic Year Tuition Waiver, which was approved in May. He has been working with Mr. Silveus at the bioreactor site as described below, and was awarded a Northern California Regional Chapter of the Society of Environmental Toxicology and Chemistry (Norcal SETAC) scholarship to supplement his labor. Undergraduate student Alyza Valdez began work on the project in early June alongside the PI in the laboratory. She received some additional funding for her time from the Undergraduate Research and Opportunity Center (UROC). UROC is supporting her through weekly workshops on research methods and ethical conduct throughout the summer. Additionally, another current graduate student, Shawnte' Greenway, has been working with the PI and Ms. Valdez in the laboratory on these and other related projects.

Task 1: Isolation and Characterization of Biodegrading Microbes

Water and sediment samples have been collected in early June from two sites that the CDPR has identified as having pesticide contamination. These are currently being screened for the ability to grow via selective enrichment culture. Samples are being incubated in LeMaster and Richards Medium, a minimal growth media, supplemented with the pesticides as the sole carbon and nitrogen source. The concentration of pesticide with which to supplement the cultures was based on previously published data.[1] This media has both nitrate and phosphate and thus there is not a need for additional supplementation. 1 g of soil was diluted into 9 mL of sterile saline to create a soil suspension. 1.6 mL of pesticide and media and 0.4 mL of the soil suspension were combined. Growth is being monitored via scattered light at 600 nm using a spectrophotometer. Thus far we have only observed growth in the cultures supplemented with cis-Permethrin, and

Imidacloprid, and Malation (Figure 1). These are just preliminary estimates. We have not yet verified the actual CFU/mL using a serial dilution.

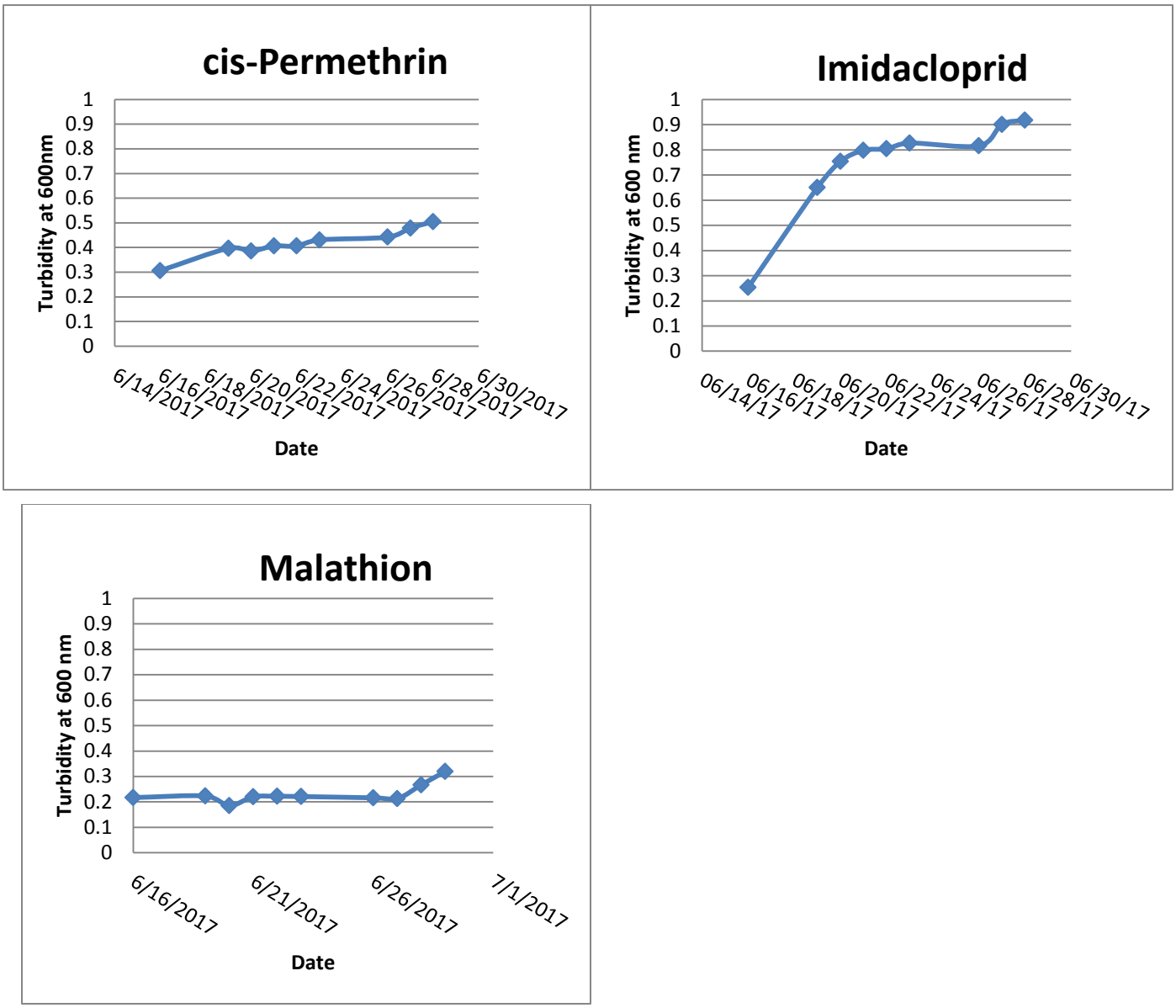

Figure 1. Growth of sediment/water samples taken from drainage ditches near Salinas, CA on 06/12/17 in LeMaster and Richards Minimal Media supplemented with cis-Permethrin (0.123 g/dL), Imidacloprid (0.1875 g/dL) or Malathion (0.218 g/dL). These concentrations provide a constant carbon concentration of 0.08 g/dL.

Task 2: Testing Biodegradation Ability in Woodchip Bioreactors

The CSUMB bioreactor team has been working on the bioreactor facility to repair damage from the 2016-2017 winter storms and prepare the reactors to perform experiments utilizing the woodchip bioreactors to decrease pesticide levels within simulated agricultural runoff effluent.

The bioreactor greenhouses have been repaired and updates on the bioreactor channels and circulation systems are currently underway. All reconfigurations and updates to the system are expected to be completed before August of 2017 in anticipation of beginning experiments in the fall and winter of 2017.

Future Work

Then cultures will be grown to confluence and spread plated onto minimal agar plates supplemented with the pesticides. Individual colony forming units will be selected and streaked to axenic culture, and then re-suspended in the liquid cultures to continue selecting for the ability to digest pesticides (microbes may be able to utilize the agar directly). Freezer stocks will be made using glycerol stock solution. Immunoassays will be used to confirm the biodegradation of pesticides.

The isolates with the most optimal growth will be identified by the amplification of the 16 rRNA gene using the primers 27F and 1492R[2]. Products will be screened on an agarose gel prepared with a DNA marker, GelGreen and a molecular marker with a low range plus DNA ladder (EXACTGene). QIAquick PCR purification kits will be used to clean the amplified DNA before sending for sequencing (Mc Lab, San Francisco). Results will be analyzed using BLAST against the 16S Bacterial and Archeal database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) DNA using MicroLysis, and amplify and send it to McLabs for 16S sequencing to provide genera identity. Further morphological and biochemical testing will be performed (e.g. Gram staining, oxidase, catalase, etc).

Once preliminary studies in the laboratory have been completed, single-strain and mixed bacterial cultures will be tested for bioremediation efficacy (including controls to test for adsorption/absorption by the wood chip media) the full-scale multi-channel wood chip bioreactors that have been dosed with the pesticides. We anticipate that this work will commence in the fall.

1. Gunasekera, T. S. & Paliy, O. Growth of E. coli BL21 in minimal media with different gluconeogenic carbon sources and salt contents. *Appl. Microbiol. Biotechnol.* **73,** 968–968 (2006).

2. Weisberg, W.G., Barns S. M., Pelletier D.A., & Lane D. J. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*, 173(2): 697–703 (1990)..