Project Title: Field Implementation and Assessment of a Pesticide-Remediating Bioreactor

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Abstract

The ability of natural and constructed wetlands to remediate agricultural effluent through soil absorption, solar degradation, floral uptake, and bacterial degradation has been shown in an abundance of studies[1–3]. However, these approaches present an array of costs that could deter a grower from implementing a constructed wetland. Woodchip bioreactors have a smaller footprint, cost and can be used to reduce wildlife intrusions. This more efficient and effective method of treating agricultural effluent could provide less of an economic hurdle or burden to the local agricultural community while offering impactful solutions to environmental and human health issues. Our project directly addresses this issue by contributing to the development of a pesticide and nitrogenous waste remediating bioreactor that would provide a low-cost, small-footprint solution for mitigating concerns on the impacts of agricultural practices on water resources. Funding provided by the California Leafy Greens Research Program has supported the construction and testing of the bioremediation capacity of an in-field bioreactor system. During our experimental work, we were able to specifically show a significant effect of bioremediation on reducing Neonicitinoid pesticides.

Objectives:
Long-term objective: *Develop a low-cost, small-footprint bioreactor that uses microbial communities to decrease the environmental impacts of agricultural activities through the bioremediation of agricultural chemicals such as pesticides and nitrogenous waste.*
Grant objective:
Install a bioreactor in a field environment and assess the feasibility of current design and implementation of experimental findings in a practical setting.

Procedures:

To accomplish this feasibility study of implementing a bioreactor in a field setting, we built an ~500 cubic foot experimental bioreactor system integrated into a local grower's drainage system (Figure 1) and assessed its capacity for bioremediation of neonicotinoid and pyrethroid pesticides, both with and without the addition of bioremediating bacteria. We conducted five 5-day tests of the bioreactor over the 2019 growing season. Water intake for the bioreactor was set to 25 gallons/minute. During each experimental run, we collected water samples four times a day – sampling at the in-take, two mid-bioreactor positions, and the out-flow of the bioreactor – to track temperature, standard water quality measures, nitrates and phosphates, the levels of relevant pesticides and describe microbial community composition (collecting both sediment and water samples) using amplified 16s rRNA methods. All Abraxis Imidacloprid/ Clothianidin and Pyrethroid ELISA assays to measure each pesticide-type concentration in water samples were done within two weeks of each sampling events. To test the effect of artificially manipulating bacterial communities, the first two experimental runs did not include seeding the bioreactor with any pre-identified pesticide-remediating bacteria. The third, fourth and fifth experimental runs involved testing for a bioremediation effect by adding a slurry (~10 L) of 18 different lab-cultured, naturally-occurring bioremediating bacteria to the bioreactor on Day 1 of the test period. After completing sampling, DNA was isolated from all collected water and soil samples (stored in a -80°C freezer) using Qiagen Power Soil and Power Water Sterivex kits, amplified for 16s rRNA genes, and sequenced on an Illumina MiSeq to describe bacterial community composition of each sample.

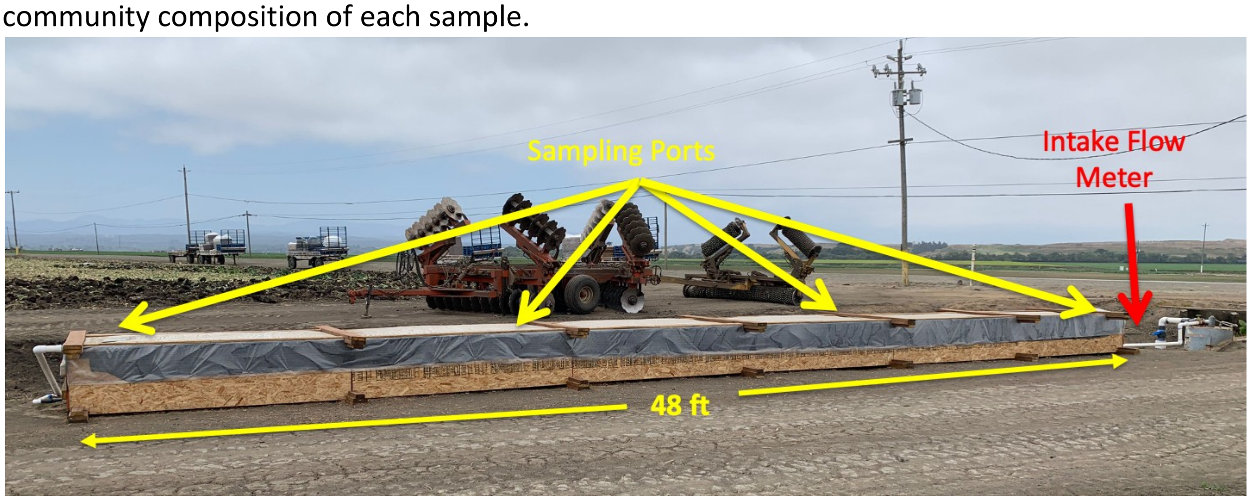

Figure 1. Photo of the bioreactor system installed at the Odello Ranch in Salinas, CA.

COVID-19 interrupted our work at a key juncture, and we were forced to shut down all lab work, which arrested finishing sequencing and, thus, final data analysis. Currently, our lab is still in the process of finalizing this data, analyzing it, assessing its relevance to bioreactor design, and recommendations for further implementation.

Results and Discussion:

In total, we conducted five 5-day experimental runs of the bioreactor system: two pre-addition of pesticide-remediating bacteria and three post-addition of pesticide remediating bacteria. During those trials, we collected 468 data points that included measurements of water quality (nitrates, phosphates, temperature, dissolved oxygen (DO), turbidity, and pH), pesticide concentrations for Imidacloprid and Pyrethroid pesticides, and bacterial community data.
During this period of time, crops planted on the fields contributing to runoff waters included head lettuce, romaine lettuce, and cauliflower. Pesticides treatments on the ranch during this period included agri-mek (abamectin), zampro (ametoctradin, dimethomorph), endura (boscalid), bravo (chlorothalonil), forum(dimethomorph), reason (fenamidone), sivanto (flupyradifurone), aliette (fosetyl-al), admire (imidacloprid), warrior (lambda-cyhalothrin), dithane (mancozeb), perm-up (permethrin), radiant (spinetoram), movento (spirotetramat), and sequoia (sulfoxaflor). On average across all trials, conductivity was 1844 $\pm$ 657 mg/L, temperature was 20.4 $\pm$ 3.1 °C, turbidity was 47.1 $\pm$ 56.5 mg/L, phosphates were 1.16 $\pm$ 0.27 mg/L, nitrates were 33.4 $\pm$ 18.1 mg/L, DO was 5.2 $\pm$ 2.6 mg/L, salinity was 1.5 $\pm$ 1 ppm, pH was 7.5 $\pm$ 0.1, and imidacloprid and pyrethroid concentrations were 0.132 $\pm$ 0.209 ppb and 0.874 $\pm$ 1.728 ppb, respectively. The bioreactor system had a modest effect on reducing nitrate concentrations as evidence by an ~10% difference between intake and outflow measurements of

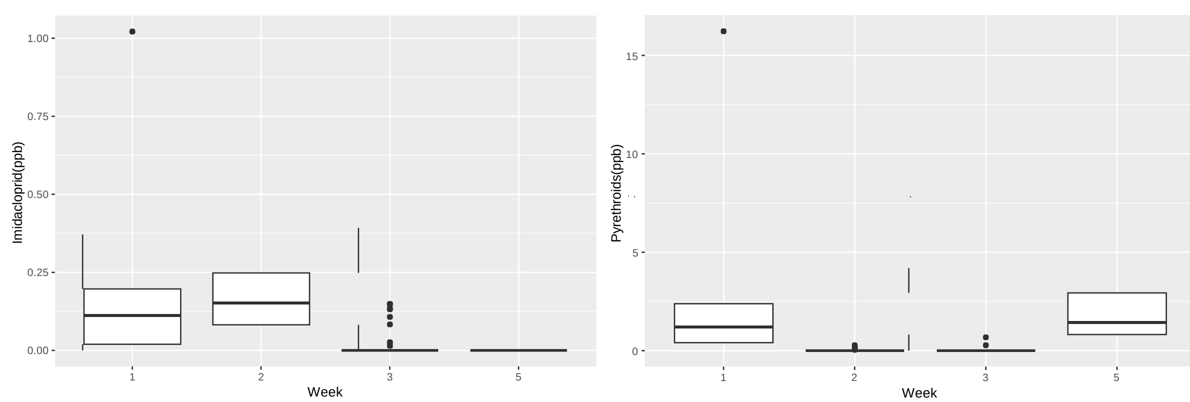

Figure 2. Box plots of Imidacloprid (left) and pyrethroid (right) concentrations taken from the bioreactor. Weeks 1 and 2 had no remediating bacteria added to the bioreactor compared to weeks 3, 4, and 5, which had pesticide-remediating bacteria added to the bioreactor at the beginning of each experimental week.

nitrates. It is likely that this system could be further optimized for this type of function in the future. We did not add any nitrate-specific bioremediators to this system, and it might be fruitful to do so in the future in order to develop a bioreactor system that could improve the simultaneous targeting of nitrogenous and pesticide wastes.

To describe the effects of adding pesticide-remediating bacteria to the bioreactor's ability to process pesticides, we compared the concentrations of imidacloprid and pyrethroid pesticides, both pre- and post-addition of pesticide remediator. Using a nested-ANOVA approach where the effects of the difference between weeks were nested within the treatment effect of microbial additions to the bioreactor, we were able to show a significant effect of adding bioremediators on the remediation of Imidacloprid ($F_{1,2}$=55.17, p-value = 0.0176), but a non-significant effect on Pyrethroid pesticides ($F_{1,2}$= 0.027, p-value = 0.885) (Figure 2). There was a 24.0% and 9.1% reduction from intake to outflow of the reactor for Imidacloprid and Pyrethroid pesticides, respectively. This indicates that the bioreactor is being successful in, at least, reducing water soluble pesticides, which correlate well with the volume of water flow (Pearson's Ranked Correlation between water flow and Imidacloprid concentrations = 0.66). The lack of effect of pesticide remediators on Pyrethroid concentrations is likely due to the nature of the pesticide having low water solubility and, thus, being difficult to track well with water flow through from the bioreactor (Pearson's Ranked Correlation between water flow and Pyrethroid concentrations = -0.277).

Patterns of diversity in bacterial communities of the bioreactor system during this experiment were described using 16s rRNA sequencing results that can identify and quantify the microbial communities in each sample. To accomplish this task, we amplified and sequenced 236 and 204 microbial community DNA samples from the bioreactor water and sediment sampling efforts, respectively. 138 water samples and 104 sediment samples produced at least 1000 sequencing reads that passed all pre-analysis filters for each sample for the 16s rRNA gene. In examining these results, the taxonomic diversity of communities was relatively consistent across samples and treatments (Table 1). However, sediment samples, in particular, indicated an increase in bacterial community diversity after remediating bacteria were added to the bioreactor (Figure 3). Additionally, we observed a shift in both water and soil microbial communities after the addition of microbial remediators to the bioreactor (Figure 4).

Table 1. Diversity indices the various treatment and sample groups from each week.

Week	Sample Type	Treatment	Shannon	Simpson
1	Soil	Pre	4.55	0.982
1	Water	Pre	4.86	0.989
2	Soil	Pre	4.16	0.976
2	Water	Pre	4.75	0.987
3	Soil	Post	4.67	0.982
3	Water	Post	4.50	0.985
4	Soil	Post	4.34	0.984
5	Soil	Post	4.51	0.984
5	Water	Post	5.04	0.990

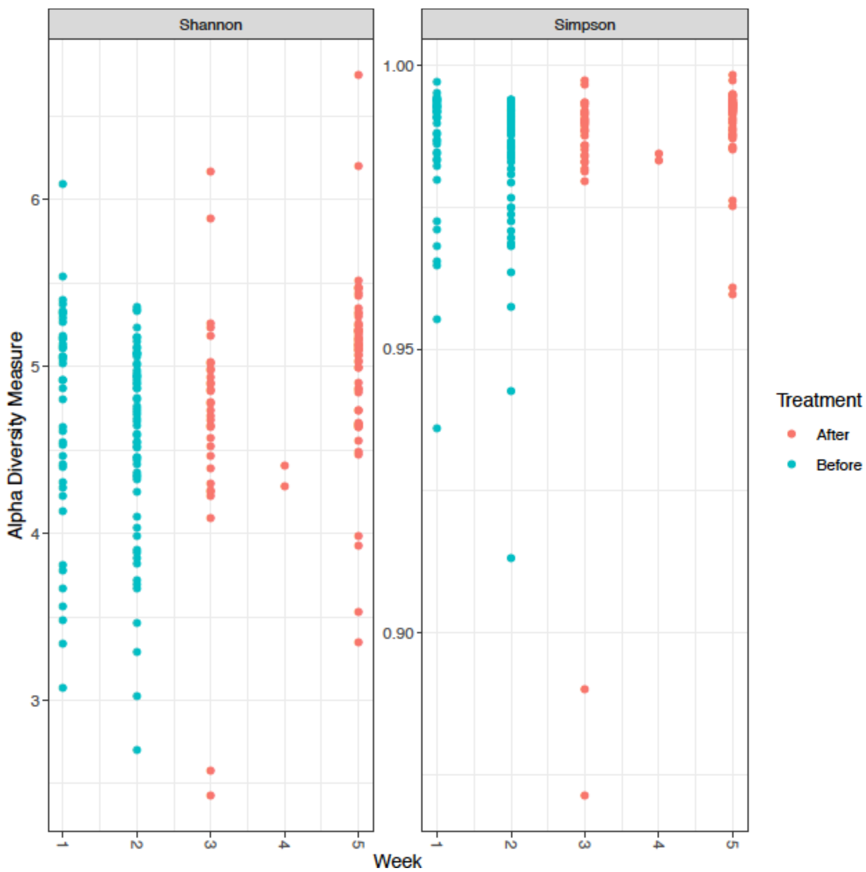

Figure 3. Distribution of Shannon-Weiner and Simpson diversity indices both before (blue) and after (orange) the addition of pesticide-remediating bacteria to the bioreactor.

These shifts in bacterial community are also seen in the significant increase of the proportion of pesticide remediating bacterial genera in sediment sample microbial communities($F_{1,73}$ = 5.911, p-value = 0.0175; mean proportion before = 0.164; mean proportion after = 0.194); no significant increase was observed in water microbial samples ($F_{1,122}$ = 1.535, p-value=0.218; mean proportion before = 0.0842; mean proportion after = 0.0756). These genera included the following: *Bacillus, Brevibacterium, Burkholderia, Citrobacter, Klebsiella, Lelliottia, Microbacterium, Paenarthrobacter, Pectobacterium, Pseudomonas, Raoultella, Serratia, and Stenotrophomonas.* These results indicate that spiking in pesticide remediators will likely allow for some retention of a certain amount of sustained remediation activity.

There is still a significant amount of work to be done on this project. As mentioned previously, the finalization of sequencing results was on-going when the COVID-19 pandemic hit. This has arrested our lab work since the University has shut down all on-campus activities. We are now just restarting this work and will soon finish the sequencing for 198 samples that need additional sequencing. Once completed, we will have much more power to describe community shifts responding to the introduction of bioremediatory microbes.

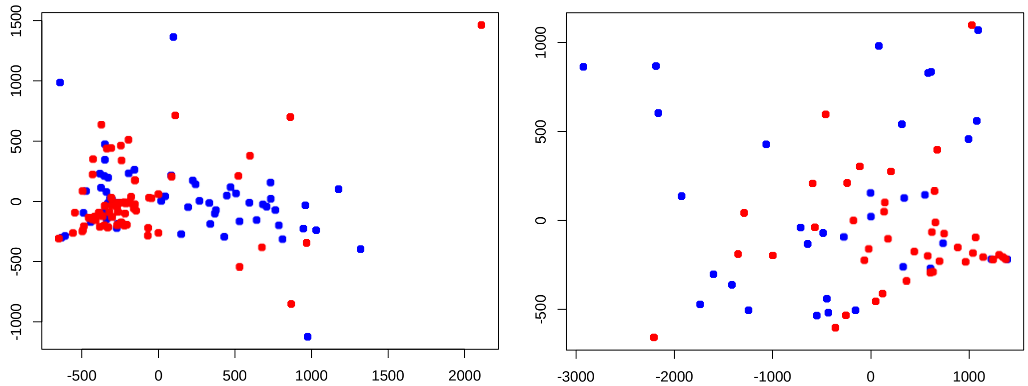

Figure 4. Multi-dimensional scaling plots presenting relative community distances based on taxon representation from water (right) and sediment (left) bioreactor 16s sequencing samples. Blue dots indicate samples taken pre-treatment. Red dots indicate samples taken post-treatment

In summary, in order to develop new solutions for address concerns over pesticide levels in surface and ground waters, we conducted a field test of an experimental bioreactor system in the Salinas Valley. In this experiment, we observed a significant effect of introducing naturally-occurring bioremediators on pesticide concentrations in agricultural wastewater. Under a single set of conditions, the bioreactors system demonstrated variable success on either water-soluble or non-water-soluble pesticides but showed a ~25% reduction in the commonly used pesticide, Imidacloprid. The introduction of pesticide-remediating bacteria to the system had the positive effect of altering the microbial community and supporting a sustained increased proportion of pesticide remediators in the bioreactor. Additionally, we observed a modest reduction in nitrogenous waste in the bioreactor (~10%).

This study was meant to be the first in a series of feasibility/optimization studies necessary for the development of an efficient, small-footprint, low-cost bioreactor system that could be easily installed in agricultural areas throughout the central coast and Salinas valley. Our next steps will be to better understand the role of water residency times in the bioreactor to better predict and optimize the scale of bioreactor necessary to achieve specific target reductions. Also, we plan to further explore the role that microbial community dynamics play in the metabolism of pesticides. In the end, our goal remains to provide explicit guidance, plans, support, and recommendations to growers on the scale and operation of pesticide-remediating bioreactors in order to address concerns over water quality while having as little impact and/or cost as possible on agricultural operations.

SUMMARY:

In order to address concerns over pesticide usage, we have developed a low-cost, small-footprint bioreactor system that is targeted to break down pesticides before they migrate into either surface or groundwater. Funds from the California Leafy Greens Research Program supported the construction and feasibility testing of our bioreactor concept in a field setting. We ran five 5-day experiments, two without adding pesticide-remediating bacteria, and three experiments where we added pesticide-remediating bacteria on day 1 of each experiment and collected data on bioreactor water quality, pesticide levels, and bacterial communities. We were able to show a significant effect of adding bioremediating microbes to the bioreactor on pesticide concentrations, specifically the water-soluble, commonly-used neonicotinoid pesticide Imidacloprid (~24% reduction). Additionally, a spike of pesticide-remediating bacteria to this system led to a persistent shift in the community of bacteria. Overall, our results are very promising, and with additional work, we are confident we will be able to create recommendations and guidelines for using these types of bioreactors wherever they are appropriate.