Project Title: Field Optimization of Water Flow for the Scaling of a Pesticide-Remediating Bioreactor

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Abstract
The issue of the reducing the off-farm movement of sediment, pest management materials, and nutrients into surface water and groundwater supplies is of principal concern to growers. Some of the solutions to this issue may be either costly, time consuming, or impractical given both grower and consumer concerns and needs. 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 ongoing maintenance and testing of the bioremediation capacity of an in- field bioreactor system. Our previous experimental work showed a significant effect of bioremediation on reducing Neonicotinoid pesticides. This current study indicates that Pyrethroids may also be effectively bioremediated in these systems and that the abundance of dosed-in Pyrethroid remediating bacteria are correlated with this bioremediation activity. Overall, preliminary results from the summer of 2020 showed significant remediation of pesticides at all flow rates, indicating that water residency time may not be a key component to promoting effective remediation and just passing the water through the bioreactor and dosing it regularly with bioremediating microbes is the key step. The COVID-19 pandemic severely limited our sampling capacity last year and we are currently continuing work on this system with the goal of finalizing our assessment of this bioreactor system by September.

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:

Understand the relationship between water residency time, bacterial community dynamics, and pesticide remediation to optimize for bioreactor operations in a field environment through experimental findings in a practical setting.

Procedures:

To accomplish this optimization study of a bioreactor in a field setting, we used an existing ~500 cubic foot bioreactor system integrated into the drainage system at the Odello Ranch (Figure 1). Built in 2019, this system exhibited minimal disruption regular agricultural activities and was subjected to five tests of the system in order to assess operational and experimental guidelines. With our current support from the California Leafy Greens Research Program, we attempted to do a full assessment of the water residency of the current bioreactor system using a fluorometric rhodamine dye test wherein we would add a non-toxic dye to the system and use a specific reader to monitor how long it takes the dye to leave the system and run three replicates of a 3-day tests of bioreactor function at 3 different flow rate settings (10 gallons/minute, 20 gallons/minute and 30 gallons/minute). Due to University and County restrictions related to COVID-19 that inhibited our ability to conduct research and grower watering schedules, we were only able to conduct 4 out of the 9 planned experimental runs. During each experimental run, we sampled 3 times a day to monitor temperature, standard water quality measures, nitrate levels, and collect water and sediment samples at 3 hour intervals at 4 locations in the bioreactor –at in-take, mid-bioreactor, and out-flow areas of the bioreactor – to measure pesticide levels of relevant pesticides and describe microbial community composition using metagenomic methods. Typically, day 1 tested bioreactor remediation activity while water is actually running through the reactor system, whereas days 2 and 3 assessed the ongoing remediation capacity of the system and the post-flow community dynamics of pesticide-remediating bacteria. Bioreactor sampling and the processing of pesticide water samples took place from August through late October. From October through May, all collected water and sediment samples (stored in a -80°C freezer) were processed for 16s rRNA targeted amplicon

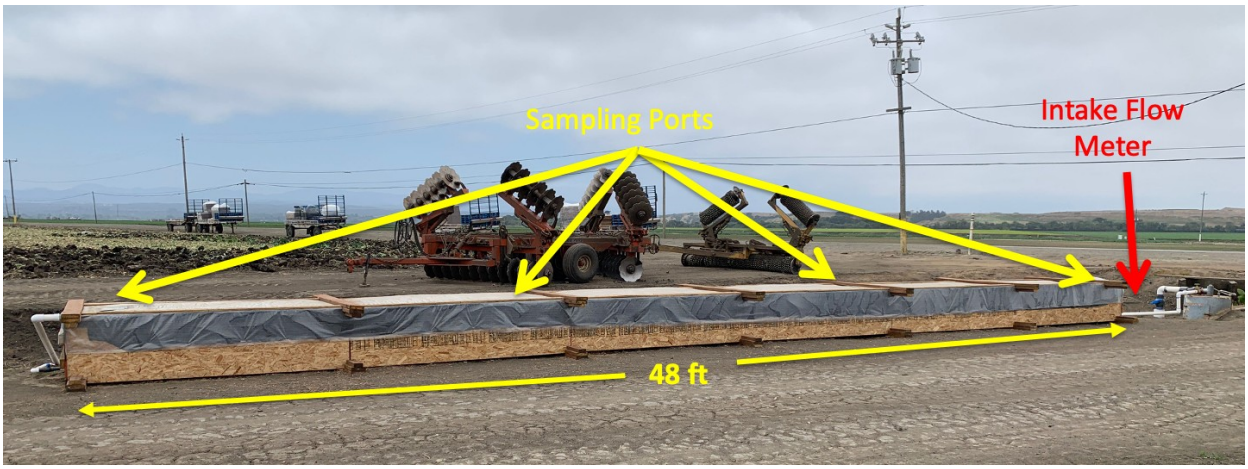

Figure 1. Photo of the bioreactor system installed at the Odello Ranch in Salinas, CA.

sequencing on an Illumina MiSeq to describe dynamics in bacterial community composition. Currently, we are working to analyze this data, assess its relevance to bioreactor design, and complete the rest of our sampling this summer to validate work from last summer. We will compile this work to make optimal operation and develop recommendations for growers on how to scale design to match needs this fall.

As mentioned above, COVID-19 interrupted our work at a key juncture, and we were forced to shut down all lab activities at various time for significant periods of time or operated at a much lower level of personnel capacity, which interrupted our sampling effort and significantly slowed down all of our subsequent lab work. Currently, our lab scheduled to complete sampling and genetic data acquisition and analyze data assessing its relevance to bioreactor design this summer, so that we can make recommendations for further implementation in September when our 6-month no-cost extension ends.

Results and Discussion:

In total, we conducted 4 3-day experimental runs of the bioreactor system (a 5^{th} run was attempted, but due to lack of water flow was not completed): two at 20 gal/min and, one at 10 gal/min and one at 15 gal/min. During those trials, we collected 144 data points that included measurements of water quality (nitrates, phosphates, temperature, dissolved oxygen (DO), turbidity, and pH, N=144), pesticide concentrations for Imidacloprid and Pyrethroid pesticides (N=67), and bacterial community data (N=131). 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 Zampro (ametoctradin, dimethomorph), Endura (boscalid), Forum(dimethomorph), Sivanto (flupyradifurone), DuPont Lannate SP (Methomyl), Previcur Flex (Propamocarb Hydrochloride), Admire Pro (imidacloprid), Warrior II (lambda-cyhalothrin), Sithane (mancozeb), Radiant (spinetoram), Revus (mandipropamid), and Sequoia (sulfoxaflor). On average across all trials, conductivity was 927 ± 74 mg/L, temperature was 18.2 ± 0.16 °C, turbidity was 9.175 ± 1.27 mg/L, phosphates were 3.37 ± 1.31 mg/L, nitrates were 62.3 ± 1.54 mg/L, DO was 1.18 ± 0.21 ppm, salinity was 1.66 ± 0.047 ppm, pH was 7.09 ± 0.012, and imidacloprid and pyrethroid concentrations were 0.0027 ± 0.00096 ppb and 0.0922 ± 0.056 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 nitrates. It is likely that this system could be further optimized for this type of function in the future. We have not added 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.

Our ability to assess the relationship between water residency time in the bioreactor and the amount of pesticides that are remediated by the bioreactor system was significantly affected by COVID-19 which limited by our ability to sample as previously mentioned. However, for the runs that were completed successfully there was little effect of water residency time on bioremediation potential. Instead, pesticides became virtually undetectable with the regular addition of pesticide remediators (Figure 2). In other words, based on a limited samples, it appeared that the bioreactor was functioning very well at all of the flow speeds examined on

these runs (10, 15, and 20 gal/min). This matched results from 2019 on Imidacloprid, but with much more noticeable effects. Additionally, while our previous work had detected an inconsistent effect on pyrethroid pesticides, samples from 2020 showed almost no detectable pyrethroids in our samples. While a very promising result, the abbreviated sampling we were able to do, underscores the need for further replication to confirm this pattern. This work is currently underway this summer.

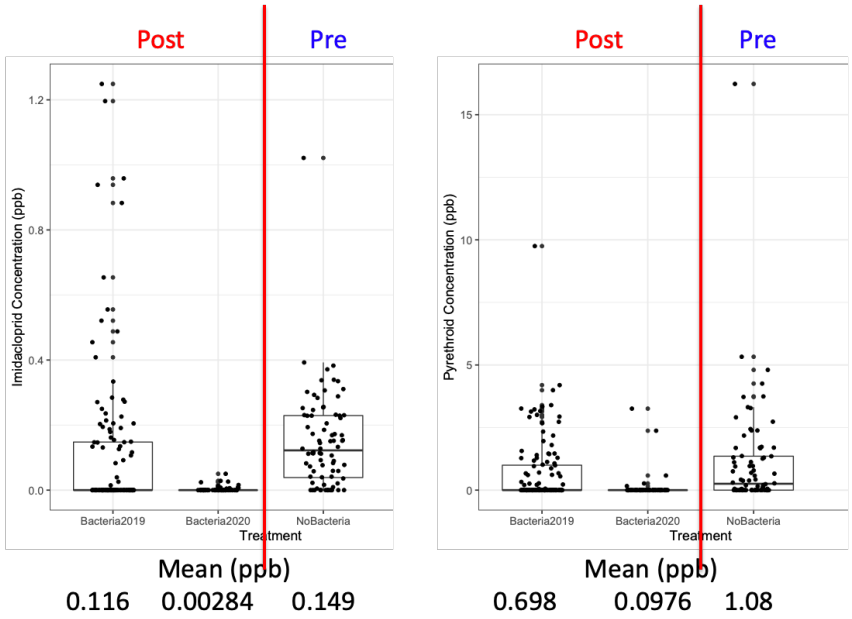

Figure 2. Box plots of Imidacloprid (A) and Pyrethroid (B) concentrations for before the additions of microbial bioremediators and after the addition of microbial bioremediators.

Patterns of diversity in bacterial communities of the bioreactor system during this experiment presented an interesting results. Throughout the experiment, we were dosing the bioreactor each week with a suite of up to 19 different bioremediating bacteria. During this time, we took sediment and water samples to assess microbial community dynamics. These dynamics 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 144 microbial community DNA samples from the bioreactor water and sediment sampling efforts, respectively. 138 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 alongside our previous year's data, the taxonomic diversity of communities revealed that the proportion of bacterial genuses which we have

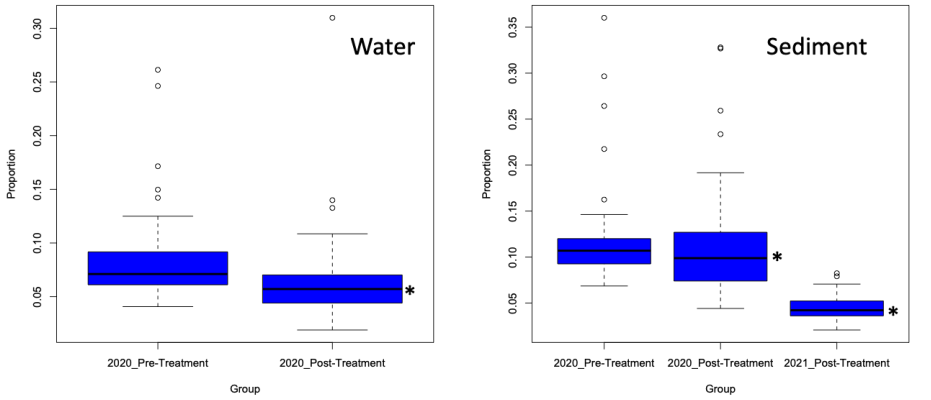

Figure 3. Comparisons of the proportion of bacteria identified from 16s rRNA sequencing that had been added to the bioreactor as confirmed pesticide remediators for both water (left) and sediment (right) samples. * indicates a significant difference in the proportion of identified pesticide remediator in the bacterial community between pre- and post-addition of the bioreactor with pesticide remediating bacteria via T-test (t=2.9812, df = 129.62, p-value=0.00343) and One-Way ANOVA with a post hoc Tukey ($F_{2,224}$ = 87.35, p-value < 2e-16).

associated with bioremediation capacity was actually a lower proportion of the total after we dosed the bioreactor with them. This held for both water and sediment data (Figure 3). These difference were significant for both 2020 water sample pre- and post-remediator dosing (T-test: t=2.9812, df=129.62, p-value=0.00343) and 2020 and 2021 sediment samples (One-Way ANOVA: F2,224=87.35, p-value<2e-16, Tukey post hoc shows post-remediator dosing significantly different for both 2020 and 2021 vs. 2020 pre-dosing proportion remediator). This could be interpreted as a paradox in which the total percentage of remediating taxa in the community is decreasing while the remediation capacity of the bioreactor system is increasing. Despite the overall pattern, however, we did observe an increase in the representation of some specific pesticide remediating bacteria, especially genuses associated with Pyrethroid remediation (*Klebsiella, Citrobacter, Lelliottia, Raoultella* and *Burkholderia*) (Figure 4). Thus, it may be that while not all pesticide remediators are thriving, some specific taxa are. Additionally, there is another possibility that the bacteria we are adding to the bioreactor are causing a change in the genetic environment of the bioreactor and affecting the genetic make-up of other species. Bacteria are known to regularly exchange genetic information via horizontal gene transfer and it could that our regular dosing of pesticide remediating bacteria is leading to the transformation of pesticide remediating genes into other bacteria better adapted to the bioreactor environment. Our current sampling methods of target 16s rRNA genes to characterize the identity of a bacterium would not detect this type of change. To do so, we would need to generate whole metagenome sequencing. Luckily, we still have the DNA samples from this study and have the ability to assess this possibility in the future if funding allows.

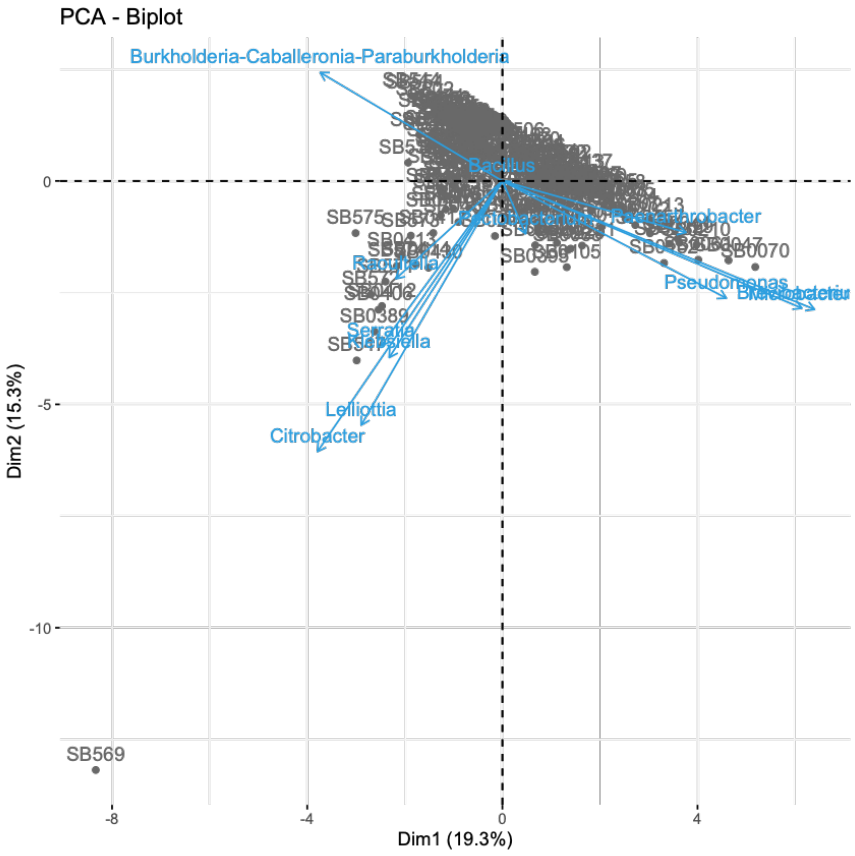

Figure 4. PCA Biplot of bacterial community proportions for pesticide remediating microbes dosed into the bioreactor system. Point grouping indicates the correlations of various taxonomic groups, specifically, those related to Pyrethroid remediation and may indicate correlations of individual pesticide remediator with an increased remediation capacity for the bioreactor system.

There is still a significant amount of work to be done on this project, but it is currently underway and is on track to be accomplished by the end of September. We have now restarted

this work and working on finishing sequencing for 198 samples that need additional sequencing as well as completing replication of our field trials this summer. Once completed, we will have much more power to assess and validate the impacts of water residency time and pesticide remediator additions on the remediation capacity of our bioreactor system.

In summary, trial runs of our experimental bioreactor system in the Salinas Valley have shown great potential in removing pesticide waste from agricultural wastewaters. Combining results from our past two years of experimentation, we have observed a significant effect of introducing naturally-occurring bioremediators on pesticide concentrations in agricultural wastewater, dropping them to virtually undetectable levels. Additionally, we observed a modest and consistent slight reduction in nitrogenous waste in the bioreactor (~10%). This effect on pesticide detectability interestingly coincides with an overall reduction the proportion of bioremediating bacteria in the bioreactor microbial community; however, there is an increase in some specific species that were among the dosed bioremediatory. Many of these species are identified Pyrethroid bioremediators. The goal of this project was to assess the relationship between water residency time and the amount of pesticides that could be remediated from the water. The current results would indicate that the amount of time the water is exposed to the bioreactor does not have a strong influence on how much pesticide is remediated. Instead, simply passing the water through the bioreactor at any of the speeds we examined (10-20 gal/min) was sufficient to make pesticide concentrations virtually undetectable in our samples. Due to COVID-19, these results are largely preliminary as much of our proposed work was not completed and is currently underway.

If these results hold from this summer's work, they would indicate that the bioreactor system that we have developed is much more effective than we have previously projected and systems of such modest size would be appropriate for implementation in other field systems of similar size. Scaling to large scale implementation would thus be very straightforward and require a very limited footprint. 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.