CAAP Quarterly Report
June 30, 2024

Project Name: Rhamnolipid: a Bio-based, Ecologically Friendly, Corrosion Inhibitor and SRB Biocide for Crude Pipelines

Contract Number: 693JK32350001CAAP

Prime University: University of Akron

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Reporting Period: April 30, 2024 thru June 30, 2024.

Project Activities for Reporting Period:

Student Hiring.

Again this quarter, a major activity was student hiring. Offers were made to three graduate students in Q2, of which two will be attending in the Fall of '24: Uddipta Mondal, B.S. Chem. Eng., BUET and Tijani Abdul-Gafaru B.S. Petroleum Eng., Kwame Univ. For the summer, one temporary graduate student has been hired (Suria Sultana, UA chemistry) and three undergraduate students have been hired: Jack Darnell (Chem. Eng.), Joseph Botzman (Corr. Eng) and Mikey Markov (Corr. Eng.).

Milestone: RhL Fermentation and Separation.

In Q3, we carried out 3 main tasks: (1) rhamnolipid purification from the broth harvested from a fermentation made during Q2, (2) measurement of rhamnolipid concentrations in two commercial rhamnolipid products (which were used for studying the corrosion inhibition efficiency as described in the next milestone), and (3) rhamnolipid production by a new batch of fermentation. These are described below in more detail.

1. RhL purification. Cells were first removed from the fermentation broth by centrifugation at 5,900 g for 10 minutes. The supernatant collected was added with a 3-fold volume of ethanol to precipitate the extracellular biopolymers produced by the bacterial cells during the fermentation. The precipitate was removed by centrifugation at 2,300 g for 10 minutes. Ethanol in the collected supernatant was removed by vaporization. The remaining aqueous solution was acidified with HCl to pH 2, intended to protonate and precipitate rhamnolipid for collection. However, because excess soybean oil was added in the fermentation, the acidification led to formation of two distinct liquid layers: a darker upper layer and a lighter lower layer. Rhamnolipid concentrations were measured in both layers. The upper layer contained 17.4 ± 3.2 g/L rhamnolipid, and the lower layer contained 6.6 ± 0.2 g/L rhamnolipid. In later fermentations, we will better control the soybean oil addition (and other fermentation process factors) to increase rhamnolipid production and deplete the soybean oil for improved, easier rhamnolipid separation.

2. Quantification of purchased rhamnolipid products. For the liquid rhamnolipid purchased from Merck, 1 mL sample was mixed with 9 mL 0.05M $NaHCO_3$, and the rhamnolipid concentration was measured by the Anthrone method. For the solid rhamnolipid from AGAE, 0.1 g sample was mixed with 10 mL 0.05M $NaHCO_3$, and the rhamnolipid concentration was measured similarly. The liquid rhamnolipid product had 400.4 ± 7.6 g/L rhamnolipid, and the solid product had 0.09 ± 0.01 g rhamnolipid per g of solid.

3. Rhamnolipid production. The fermentation procedure used was almost the same as in the previous fermentation, which was described in the Q2 report. The previous fermentation had some operation issues causing insufficient oxygen supply and lower than expected rhamnolipid production. These issues were corrected in the new fermentation. Another change made was to delay the N source feeding from 15 h to 24 h (after seed culture inoculation). The fermentation was run for 264 hours before harvesting the broth. Rhamnolipid concentrations were measured with periodic samples, which plateaued at 25.5 ± 0.4 g/L after 96 h. The DO and pH profiles indicated negative effects of the delayed N source feeding, which caused premature N source exhaustion, interruption of cell growth, and discontinued rhamnolipid production. These factors will be adjusted in future fermentations. Separation of RhL from this new fermentation broth is ongoing.

<u>Milestone: Corrosion Inhibition Efficiency of RhL in a Produced Water Simulant</u>.
In the FY 24 Q2 report, we described the procedure for evaluating RhL inhibition efficiency in a produced water simulant (1% NaCl solution saturated with CO_2), specifically, corrosion current density measurements from potentiodynamic polarization curves for C1018 carbon steel using a rotating cylinder electrode (1000 rpm). In addition, we reported preliminary results from those experiments.

In Q3 we have completed the evaluation of one of the commercially available RhL products (liquid, 95% pure). The average corrosion current densities from the potentiodynamic polarization tests is presented in **Figure 1**. As seen in this figure, the measured corrosion current densities for all RhL concentrations are on the order of two orders of magnitude lower than that measured for the uninhibited case. Corresponding corrosion inhibition efficiencies calculated from the data in **Figure 1** are presented in **Figure 2**. Given that the reduction in corrosion current density is on the order of two magnitudes for all concentrations of RhL tested, it is not surprising that the corrosion inhibition efficiencies are all greater than 98%. The results from our initial tests of the second commercially available product at a concentration of 31.25 ppm ae also shown in these plots. As seen, the corrosion current density and inhibition efficiency measured for the dry product are similar to the that measured for the liquid.

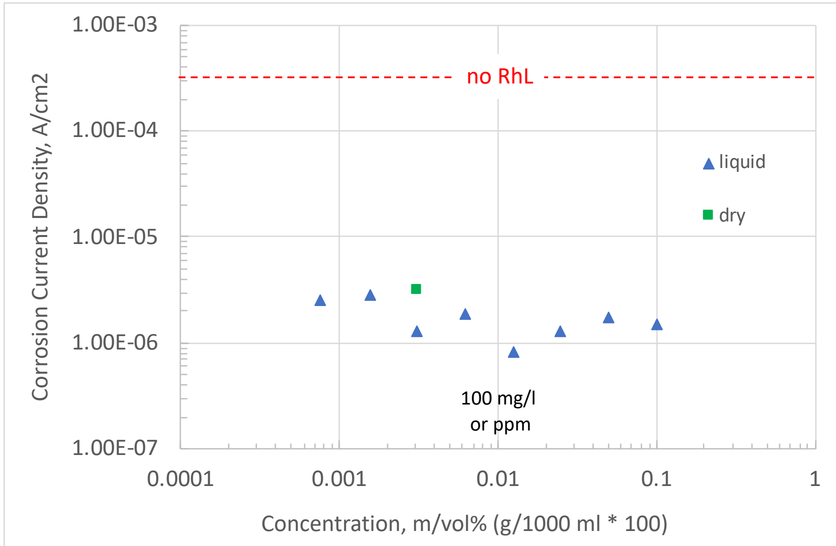

Figure 1 Average corrosion current density (2 measurements), measured using potentiodynamic polarization curves, as a function of RhL concentration for two commercial products: liquid (95% pure) and dry (90% pure). The uninhibited case is shown for comparison. Evaluation of the dry product is ongoing. Standard deviations were on the order of 15%-23% and not visible on a logarithmic scale.

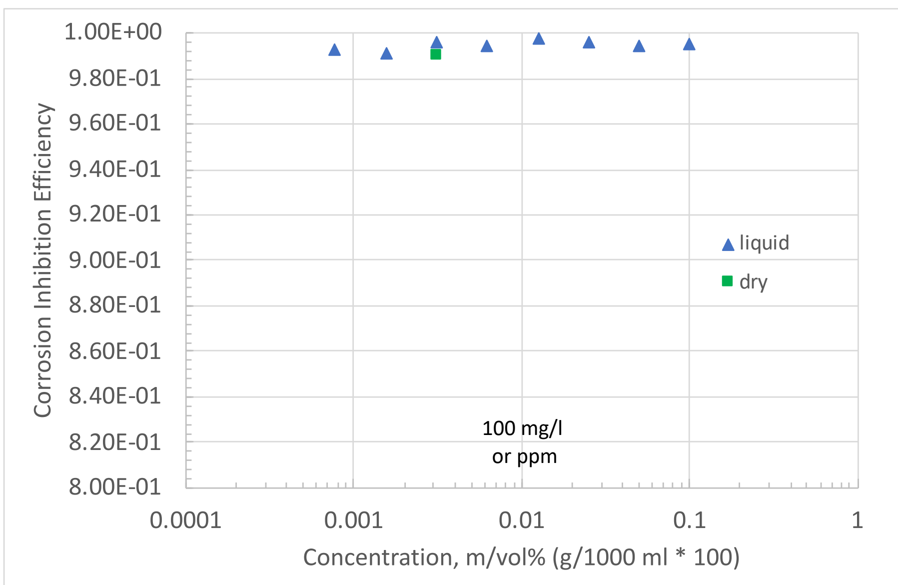

Figure 2 Corrosion inhibition efficiencies calculated from the data in Figure 1.

Project Financial Activities Incurred during the Reporting Period:
As of June 11 2024, during FY '24 we have spent $78,115.34 (including cost share): $37,963.86 salary, $10,479.92 fringe, $3,308.73 supplies $26,723.67 indirect cost. The spending break down is shown below in Table 1. Shown in Figure 3 is a comparison between expenditures and linearized budget. As can be seen in this figure, the project is underspent by approximately 30% at this point. We anticipate this will change by the end of the 24' fiscal year with additional purchases, summer salaries and graduate students start full time.

Table 1 Expenditures, to date, without cost share.

Ledger Account	Budget	LTD Actuals	Current Period Actuals	Total Spend	Remaining Balance	% Remaining
> Salary	$234,584.00	$37,963.86	$14,918.75	$37,963.86	$196,620.14	83.82%
Fringe Benefits	$20,599.00	$10,119.08	$2,435.44	$10,119.08	$10,479.92	50.88%
Supplies & Services	$18,600.00	$3,308.73	$23.01	$3,308.73	$15,291.27	82.21%
> Student Aid	$30,000.00	0	0	0	$30,000.00	100.00%
Travel	$10,000.00	0	0	0	$10,000.00	100.00%
Total Direct Costs	$313,783.00	$51,391.67	$17,377.20	$51,391.67	$262,391.33	83.62%
Indirect Cost	$147,567.00	$26,723.67	$9,036.15	$26,723.67	$120,843.33	81.89%
Total Direct & Indirect Costs	$461,350.00	$78,115.34	$26,413.35	$78,115.34	$383,234.66	83.07%

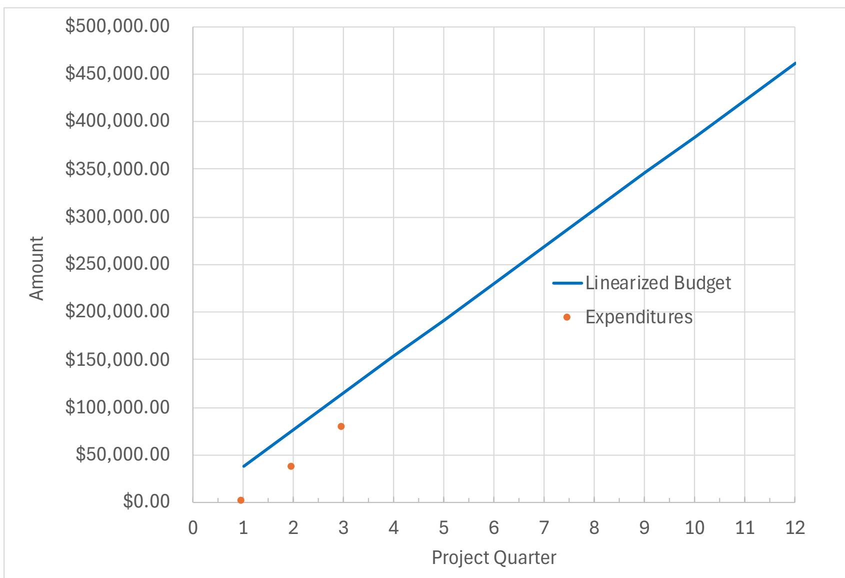

Figure 3 Total expenditures without cost share in comparison with linearized budget.

Project Activities with Cost Share Partners:
None.

Project Activities with External Partners:
None.

Potential Project Risks:
One potential risk to the project in the near term is a delay in the US embassy granting the students visas, thus, delaying graduate student arrival in the Summer 2024. To help lessen the impact of this if it does occur we are hiring undergraduate students in parallel as discussed above.

Future Project Work:
During Q4 of FY ’24, the PIs anticipate completing the comparison of commercially available RhL products in produced water simulants. In addition, we anticipate completing a third fermentation of *Pseudomonas aeruginosa* fermentation for testing in FY 2025 Q1. New tasks that will be started in Q4 of FY ’24 include: long-term polarization resistance / mass loss tests to determine inhibition efficiency with time, electrochemical impedance spectroscopy and quartz crystal microbalance studies in produced water simulant to better understand the mechanism of inhibition and preliminary testing of biocide efficacy for mild steel exposed to sulfate reducing bacteria.

Potential Impacts to Pipeline Safety:
FY ’24, Q2 – The results, thus far, are extremely encouraging. We have completed proof of concept experiments that show RhL may be an effective inhibitor crude pipelines.