CAAP Quarterly Report

March 29, 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

Prepared By: Scott Lillard, rsl@uakron.edu, 330-972-7463

Reporting Period: January 1, 2024 thru March 29, 2024.

Project Activities for Reporting Period:

Student Hiring.

A major activity this period was graduate student hiring. The two graduate students (Mondal/Iqbal) hired in Nov. '23 did not come to UA for separate reasons (health, visa). The PIs have extended offers to three additional graduate students: 1. Niamul Hassan, B.S Chem. Eng, BUET, 2. Abdur Rakib, B.S. Chem. Eng., BUET and 3. Tijani Abdul-Gafaru B.S. Petroleum Eng., Kwame Univ. All three students accepted their offers and have an anticipated start date of Aug, 2024. For the interim, the PIs have used the help of an existing PhD student: Md Fauzul Kabir, and hired two undergraduate students in the Chem. Eng program: Elizabeth Zimmerer and Callie Lewis. The latter two are juniors in the program. These students along with input from the co-PIs, preformed the work discussed below.

Milestone: RhL Fermentation and Separation.

A batch of *Pseudomonas aeruginosa* fermentation was carried out for RhL production. This bacterium produces rhamnolipids actively in the stationary phase of fermentation, when at least one essential nutrient for growth becomes limiting. Following the procedure established in our previous research, the fermentation was made with the stationary phase induced by limiting the non-nitrogen source. Soybean oil was used as the carbon source; a mixture of NH_4Cl, yeast extract and peptone was used as the initial nitrogen source; and KH_2PO_4, NaCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCL_2.2H_2O$, $MnCl_2.4H_2O$ and trace elements were used for non-nitrogen nutrients.

Pseudomonas aeruginosa culture was activated by transferring 1.5 mL of a glycerol-preserved culture to 10 mL of 30 g/L tryptic soy broth (TSB) and incubating it at 32°C for 12 h in an orbital shaker operating at 150 rpm. The activated culture was added to 90 mL TSB and grown at the same condition for 20 h before being used as the seed culture for fermentation. The fermentation was carried out in a 3 L fermentor with 1 L initial medium containing 100 g soybean oil, 2.51 g nitrogen (from NH_4Cl, yeast extract and peptone) and other nutrients in deionized water. The fermentor was equipped with probes, pumps, and other equipment to control the temperature (32°C), pH, foaming, and dissolved oxygen concentration. The whole system was autoclaved at 250°F for 20 minutes and then cooled to room temperature. The fermentor was then inoculated with 100 mL seed culture. The dissolved oxygen concentration was controlled with a set point of 10% air saturation (about 0.8 mg O_2/L) by addition of pure O_2 as needed. pH was allowed to drop initially from 7 to 5.7 and was then controlled at that level by addition of 1N NaOH and H_2SO_4. The medium was agitated at 800 rpm with two sets of 6-blade Rushton turbines. In addition to the nutrients in the initial medium, soybean oil was continuously added at the rate

of 0.83 g/(L-h) throughout the fermentation, and a 300 g/L NH_4NO_3 solution was added at 2.00 g/(L-h) during 15-41 h and then at 0.15 g/(L-h) till the end of fermentation. The fermentation was stopped after 9 days. Analysis and separation of RhL from the harvested fermentation broth are ongoing. Results will be reported later.

Milestone: Corrosion Inhibition Efficiency of RhL in a Produced Water Simulant.

Procedure: Potentiodynamic polarization curves for C1018 carbon steel in a rotating cylinder electrode setup (1000 rpm) were generated to assess the corrosion current density (proportional to corrosion rate) as a function of rhamnolipid (RhL) concentration. The base solution was a 1% NaCl solution saturated with CO_2 and was chosen as it is the most aggressive produced water simulant known as well as having system properties that are well characterize. Once prepared, the solution was placed in a water-jacketed cell to maintain temperature (30 °C) and subsequently purged with CO_2 for up to 12 hrs. This cell also contained a graphite counter electrode and SCE reference electrode. The C1018 specimen was ground to silicon carbide 600 grit to provide a reproducible surface finish. After grinding, the specimen was ultrasonically cleaned in successive baths acetone, ethanol and DI water followed by drying with CO_2. The initial RhL concentration test was 0.1 mass/volume % (1000 ppm). All experiments were run in duplicate to insure reproducibility.

Results: Typical polarization curves for uninhibited and inhibited solutions are presented in Figure 1. As seen in this figure, a dramatic reduction in current density at equivalent potentials was observed in the inhibited solution. To quantify these changes and obtain the corrosion rates in the two solutions, the data were fitted to the Wagner-Traud equation. A typical fit is presented in Figure 2 for the uninhibited case. The results from the fits are summarized in Table 1. As seen in this table, the reduction in corrosion rate (CR) for the 0.1% RhL case as compared to the uninhibited case is a factor of 215. That is, CR(base) = 966 mils per year, CR(RhL) = 4.5 mils per year where 1 mil = 0.001 inches.

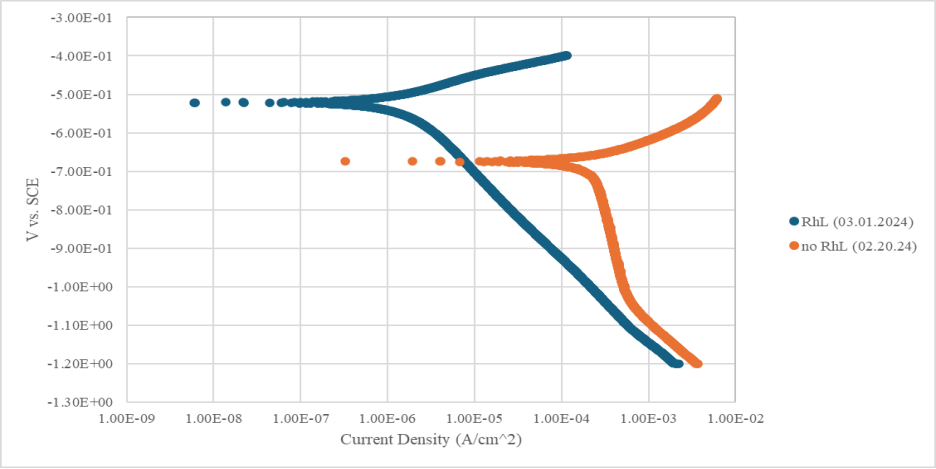

Figure 1 - Typical potentiodynamic polarization curves in a produced water simulant with and without RhL.

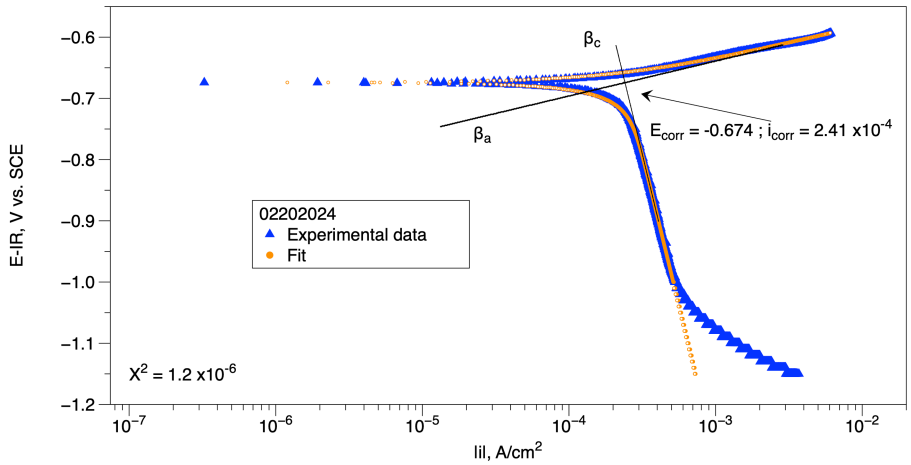

Figure 2 - Typical results from fitting the potentiodynamic polarization curves in the produced water simulant (no inhibitor) to the Wagner-Traud equation to obtain the corrosion current density.

Table 1 - Averages from fitting the potentiodynamic polarization curves.

	avrg. value, base solution	avrg. value, inhibited
i_{corr}, A/cm^2	3.28×10^{-4}	1.52×10^{-6}
E_{corr}, V_{SCE}	-0.676	-0.513
β_a V	0.050	0.067
β_c V	-1.72	-0.227
CR, mpy	966	4.5

Project Financial Activities Incurred during the Reporting Period:

During FY ’24 Q2 we have spent $11,391.33 (with encumbrances): $5,217.55 salary, $1,702.72 fringe, $574.02 supplies $3,897.04 indirect cost. The project break down is shown below.

Role Principal Investigator (Grant) **Period** 2024 - Mar

Worktags Grant: GR 000072 Lillard AWD-000044 USDOT

8 items

Ledger Account	Grant	Budget	Pre-Encumbrance	Encumbrance	LTD Actuals	Current Period Actuals	Total Spend (with Encumbrances)	Remaining Balance
Salary		$182,515.00	0.00	$0.00	$5,217.55	$5,108.79	$5,217.55	$177,297.45
Fringe Benefits		$9,260.00	0.00	$0.00	$1,702.72	$1,700.22	$1,702.72	$7,557.28
Supplies & Services		$7,600.00	$0.00	$0.00	$574.02	0.00	$574.02	$7,025.98
Student Aid		$30,000.00	0.00	0.00	0.00	0.00	0.00	$30,000.00
Travel		$10,000.00	0.00	0.00	0.00	0.00	0.00	$10,000.00
Total Direct Costs		$239,375.00	$0.00	$0.00	$7,494.29	$6,809.01	$7,494.29	$231,880.71
Indirect Cost		$108,875.00	0.00	0.00	$3,897.04	$1,221.34	$3,897.04	$104,977.96
Total Direct & Indirect Costs		$348,250.00	$0.00	$0.00	$11,391.33	$8,030.35	$11,391.33	$336,858.67

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 Q3 of FY ’24, the PIs anticipate continuing the rotating cylinder experiments in produced water simulants as a function of RhL concentration. The goal of this work is to have completed the measurement of corrosion inhibition efficiency as a function of RhL concentration by the end of the summer. Completing this work will allow us to focus on a specific set of exposure conditions (RhL conc., temperature, salinity ...) to use for testing in the 2-phase system (produced water / crude simulants).

Potential Impacts to Pipeline Safety:

FY ’24, Q2 – The results, thus far, are extremely encouraging. We have demonstrated proof of concept experiments that show RhL may be an effective inhibitor crude pipelines.