# **CAAP Annual Report**

Date of Report:	09/30/2024	
Prepared for:	U.S. DOT Pipeline and Hazardous Materials Safety Administration	
Annual Period:	From October, 1, 2023 to September, 30, 2024	
Contract Number:	693JK32350001CAAP	
<b>Project Title</b> : SRB Biocide for Crud	Rhamnolipid: a Bio-based, Ecologically Friendly, Corrosion Inhibitor and de Pipelines	
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## Section A: Business and Activities

#### (a) Contract Activities

- Contract Modifications:
  - None to report.
- Educational Activities:
  - Student mentoring:
    - Two graduate students were hired in the Spring '24 and began their studies / research in the Fall of '24: Uddipta Mondal, B.S. Chem. Eng., BUET and Tijani Abdul-Gafaru B.S. Petroleum Eng., Kwame Univ. Since the project began, six undergraduate students have been working in the lab carrying out research: Elizabeth Zimmerer, Calli Lewis, Rosemary Sterling, Lily Clemente (all of Chem. Eng.), Joseph Botzman and Mikey Markov (from Corr. Eng.). Zimmerer and Sterling are conducting research for the Sr. Honor's Projects.
  - Student internship:
    - None to report.
  - Educational activities:
    - None to report.
  - Career employed:
    - None to report.
- Dissemination of Project Outcomes:
  - We are planning to present the results to date at the AMPP 2025 Research in Progress (RIP) session New Technologies in Coatings and Inhibitors, in Nashville TN.
- Citations of The Publications:
  - None to report.

#### (b) Financial Summary

- Federal Cost Activities:
  - Total Budget:
    - A total of \$61,350 was budgeted for this project and \$125,457 has been spent leaving \$355,892 remaining.
  - PI/Co-PIs/students involvement:
    - A total of \$234,584 was budget for PI/Co-PIs/students salaries and \$62,982 has been spent leaving \$171,621 remaining.
  - Materials purchased/travel/contractual (consultants/subcontractors):

- A total of \$18,600 was budgeted for Materials and Supplies and \$6,492 has been spent leaving \$12,107 remaining. A total of \$10,000 was budgeted for Travel and \$0 was spent.
- Cost Share Activities:
  - Cost share contribution:
    - Not billed.

## (c) Project Schedule Update

• Project Schedule:

### Task 1: Corrosion Inhibition Efficiency (IE)

- o a) IE in Produced Water Surrogate (PWS): Jan. '24 Sept. '24 (on schedule).
- o b) IE in Crude Surrogate (CS): Jan. '25 Aug '25 (on schedule).
- c) IE in Crude: Oct '25 Aug '26 (on schedule).

### Task 2: Microbial Influenced Corrosion (MIC)

- o a) RhL as an SRB-MIC Biocide in PWS: Jan '24 Sept '24: (started Sept '24).
- o b) RhL as an SRB-MIC Biocide in CS: Sep '24 Mar '25 (delayed to Aug '25).
- o c) Cell Attachment in PWS: Apr '25 Apr '26 (on schedule).

Task 3: RHL Production and Comparison of Di-RhL vs. Mono-RhL in PWS

- a) RhL Production (Fermentation): Jan. '24 June '26 (on schedule).
- b) Initial Separation of Di-RhL and Mono-RhL: Nov. '24 Jan. '25 (delayed to Mar. '25).
- o c) IE in PWS: Jan '25 Dec '25 (delayed to May '25).
- Corrective Actions:
  - While the Corrosion Inhibition Efficiency (IE) and fermentation tasks are on schedule, we experienced several to the MIC and Di-RhL vs. Mono-RhL tasks. The delayed tasks are "higher order" and required the focus of a graduate student while the IE in PWS tasks could be started by undergraduates. Hiring of graduate students was delayed in part due to the timing of the PHMSA funding cycle (Oct. 1) and the primary University hiring date (approx. Sept. 1). Although we were able to hire early, Jan. 1, One of the students had health issues and the other had visa issues. This delay has also lead to us underspending the budget. We now have two graduate students on board and anticipate an increase in research activities though we may need to request a one year no cost extension to finish the project.

## (d) Status Update of the 4<sup>th</sup> Quarter Technical Activities

• In Q4 '24 we have measured the corrosion inhibition efficiency of two commercially available RhL surfactants in our. Potentiodynamic polarization experiments were used to measured corrosion current densities for C1018 carbon steel in a rotating cylinder electrode setup (1000 rpm) in a 1% NaCl solution saturated with CO<sub>2</sub>. For both RhL

surfactants, the corrosion rates were on the order of two orders of magnitude lower than that measured for the uninhibited case and the values of corrosion rate at any given concentration are comparable. In Q4 we have also measured the IE of RhL in long term experiments (8 days) using the same experimental set up described above and, simultaneously recorded mass loss for comparison. Those results are pending. Finally, fermentation of RhL at UA continues and we have also started culturing SRB for our MIC studies.

## Section B: Detailed Technical Results in the Report Period

#### 1. Background and Objectives in the 1<sup>st</sup> Annual Report Period

#### 1.1. Background

This project is focused on a novel biobased surfactant, rhamnolipid, as a corrosion inhibitor and sulfate reducing bacteria (SRB) biocide for crude pipelines. Rhamnolipids (RhL) are a group of glycolipids produced by bacteria, here by *Pseudomonas aeruginosa*, that are biodegradable, ecologically acceptable, and renewable. From a corrosion engineering standpoint, there is evidence from our past work that RhLs inhibit corrosion and reduce SRB attachment. To evaluate the application of RhLs to crude pipelines we propose to: 1-Determine the critical concentration of RhL necessary for corrosion inhibition / SRB mitigation in an oil field produced water simulant, 2- Evaluate whether or not one RhL structure (mono vs. di) is a better inhibitor or if a mixture of the two is better, 3- Evaluate how the presence of an organic phase that simulates the crude affects RhL partitioning between the organic and aqueous phases and, ultimately, corrosion inhibition, and 4 – Validate our results by making measurements in actual crude samples.

#### 1.2. Objectives in the 1st Annual Report Period

This proposal will answer the question: Can RhLs be used as a corrosion inhibitor and SRB biocide for crude oil pipelines? To accomplish this goal, we will undertake four main tasks: 1- Determine the critical concentration of RhL necessary for corrosion inhibition / SRB mitigation in an oil field produced water simulant, 2- Evaluate whether or not one RhL structure (mono vs. di) is a better inhibitor or if a mixture of the two is better, 3- Evaluate how the presence of an organic phase that simulates the crude affects RhL partitioning between the organic and aqueous phase and, ultimately, corrosion inhibition, and 4 – Validate our results by making measurements in actual crude samples to determine if variables such as mass% water, sulfur content, total acid number and API gravity, etc. affect the inhibition efficiency of RhL. The goals of FY '24 were to a) quantify the corrosion inhibition efficiency of RhL in a produced water simulant (Task 1) and b) ferment *Pseudomonas aeruginosa* at UA and harvest RhL and quantify the purity concentration of commercially available RhL (Task 3).

#### 2. Experimental Program in the 1<sup>st</sup> Annual Report Period

#### 2.1. Task 1, Corrosion Inhibition Efficiency

2.1.1. 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>. The initial RhL concentration test was 0.1 mass/volume % (1000 ppm). All experiments were run in duplicate to insure reproducibility. A diagram of this set up is presented in **Figure 1**.



#### 2.2. Task 3, Fermentation of Pseudomonas aeruginosa

2.2.1. 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<sub>4</sub>Cl, 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<sub>2</sub>/L) by addition of pure O<sub>2</sub> 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<sub>2</sub>SO<sub>4</sub>. 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.

#### 3. Results and Discussions

#### 3.1. Task 1: Corrosion Inhibition Efficiency

Typical polarization curves for uninhibited and inhibited solutions are presented in **Figure 2**. 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 3** 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 2** - Typical potentiodynamic polarization curves in a produced water simulant with and without RhL.



**Figure 3** - 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.

	avrg. value, base solution	avrg. value, inhibited
$i_{corr}$ , A/cm <sup>2</sup>	$3.28 \times 10^{-4}$	$1.52 \text{ x10}^{-6}$
$E_{corr}$ , $V_{SCE}$	-0.676	-0.513
$\beta_a V$	0.050	0.067
$\beta_{c} V$	-1.72	-0.227
CR, mpy	966	4.5

**Table 1** - Averages from fitting the potentiodynamic polarization curves.

The evaluation of the commercially available RhL products (liquid, 95% pure and dry, 90% pure). The average corrosion current densities from the potentiodynamic polarization tests is presented in **Figure 3**. The results shown are averages of duplicate runs and the error bars were too small to be seen in the plot. 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 3** are presented in **Figure 4**. 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%.

Comparison of the two commercial RhL products finds that both the corrosion current densities (**Figure 3**) and %IE (**Figure 4**) are similar across all concentrations. this has two implications as it relates to the implementation of RHL in crude pipelines: 1) commercially available RhL products that can be used as inhibitors are relatively uniform in the efficacy and 2) given the high %IE at low RhL concentrations this inhibitor is an affordable choice to mitigate steel corrosion.



**Figure 3** 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 4 Corrosion inhibition efficiencies calculated from the data in Figure 3.

#### **3.2. Task 3: Fermentation**

- 3.2.1. *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  $17.4 \pm 3.2$  g/L rhamnolipid, and the lower layer contained  $6.6 \pm 0.2$  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.
- 3.2.2. Quantification of commercial rhamnolipid products. For the liquid rhamnolipid purchased from Merck, 1 mL sample was mixed with 9 mL 0.05M NaHCO<sub>3</sub>, 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<sub>3</sub>, and the rhamnolipid concentration was measured similarly. The liquid rhamnolipid product had  $400.4 \pm 7.6$  g/L rhamnolipid, and the solid product had  $0.09 \pm 0.01$  g rhamnolipid per g of solid.

#### 4. Future work

While we will continue to finish the tasks 1a and 3a as described above, work in the next six months will focus on MIC Task 2a and 2b as well as Task 1b developing a crude simulant for test corrosion inhibition efficiency. In the first two quarters of FY '25, we also anticipate progress on Task 2c, Cell Attachment in PWS and Task 3b Initial separation of Di-RhL and Mono-RhL.