CAAP Quarterly Report, FY '25 Q3
June 30, 2025

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: April 1, 2025 thru June 30, 2025.

Project Activities for Reporting Period:

Students

Current graduate students: Jan. 2025, Elaheh Mozayan, M.S. Biochem., University of Kashan, Iran; Jan. 2025 or May 2025: Kingsford Duah Agyemang, B.S. Petroleum Eng., Kwame Univ. Uddipta Mondal, B.S. Chem. Eng., BUET and Tijani Abdul-Gafaru B.S. Petroleum Eng., Kwame Univ.

Current undergraduate students: none

Previous undergraduate students: Ellie Zimmerer (Chem. Eng.), Rosemary Sterling (Chem. Eng.), Callie Lewis, (Chem. Eng.), Lily Clemente (Chem. Eng.).Joseph Botzman (Corr. Eng) and Mikey Markov (Corr. Eng.), Jack Schulze (Corr. Eng.).

Milestones

Table 1 lists the milestones for this project and their approximate status. As seen in this table, we are nearing completion or our produced water experiments, approximately 90% complete. These were our initial "proof of concept experiments" which were very successful showing that RhL is an effective inhibitor in simulated produced water. There has been a delay on starting two milestones, Flow System Modifications and PW SRB Attachment in Flow. These two milestones are related in that we must first modify our existing system/instrumentation before beginning the attachment investigation. This delay owes to a delay in graduate student hiring which was remedied in the fall and we do not expect any further delays on these milestones.

Table 1. List of milestones (from proposal) and approximate status. Note PW stands for produced water. *Italics* indicate a schedule change and new dates.

	Status	Sched. Begin	Sched. End
1. *RhL Fermentation*	ongoing	10/9/23	5/30/26
2. *Corrosion, %IE and Mechanism*	50% complete	10/9/23	5/25/26
Purchase consumables, cell mods.	complete	10/9/23	12/11/23
Produced Water Exp,	90% complete	12/18/23	8/31/25
Crude Surrogate Exp.	*delayed*	*6/1/25*	*12/1/25*
Actual Crude Exp.		9/22/25	5/25/26
3. *SRB-MIC*		9/1/24	6/1/26
Flow System Mods	*delayed*	1/06/25	*9/1/25*

PW-SRB CorrRate (static)	50% complete	9/1/24	*9/1/25*
PW-SRB Attachment, CorrRate (flow)	*delayed*	*9/1/25*	5/12/26
4. *Di-RhL vs. Mono-RhL*		9/2/25	6/1/26
Separation of Di and Mono		9/2/25	12/31/25
CorrRate IE		1/1/26	5/30/26

Milestone 1, RhL Fermentation

This run achieved a 4.5-fold increase in RhL concentration compared to previous fermentations, reaching a peak of 71 ± 3 g/L. In contrast to earlier quantification methods that relied solely on cell-free supernatants, which likely underestimated total RhL due to precipitation near its pKa, this run employed diluted raw broth for anthrone analysis, capturing both soluble and precipitated fractions. The RhL production trend over time is shown in **Figure 1**.

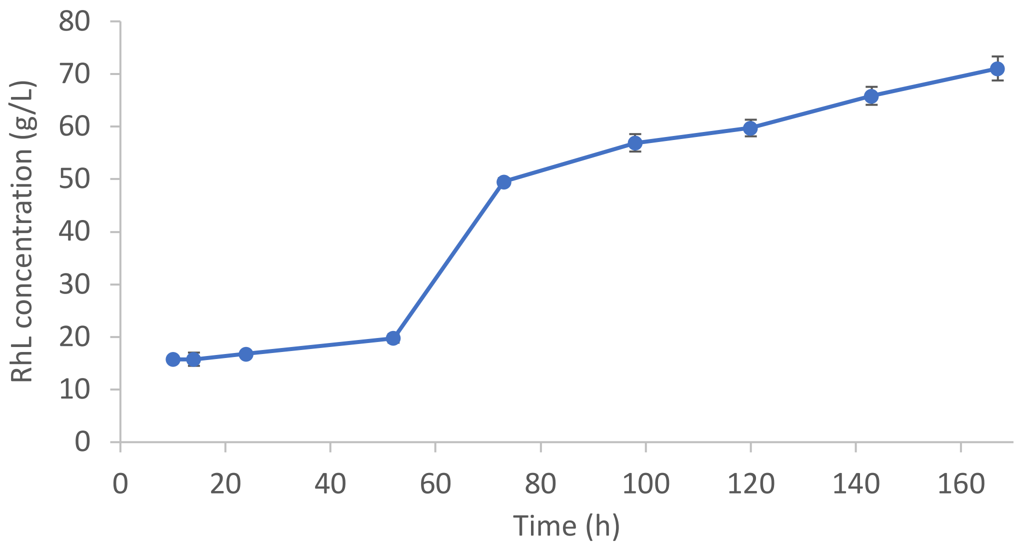

Figure 1. RhL production profile

Cell growth was monitored through intracellular protein (IP) measurements, from which cell dry weight (CDW) was estimated using an established conversion factor. These growth profiles are presented in **Figure 2**. Extended tracking of biomass concentrations beyond the feeding phase was performed to observe the decline in cell viability and biomass once oil addition ceased. This allowed for evaluation of the system's sensitivity to harvest timing.

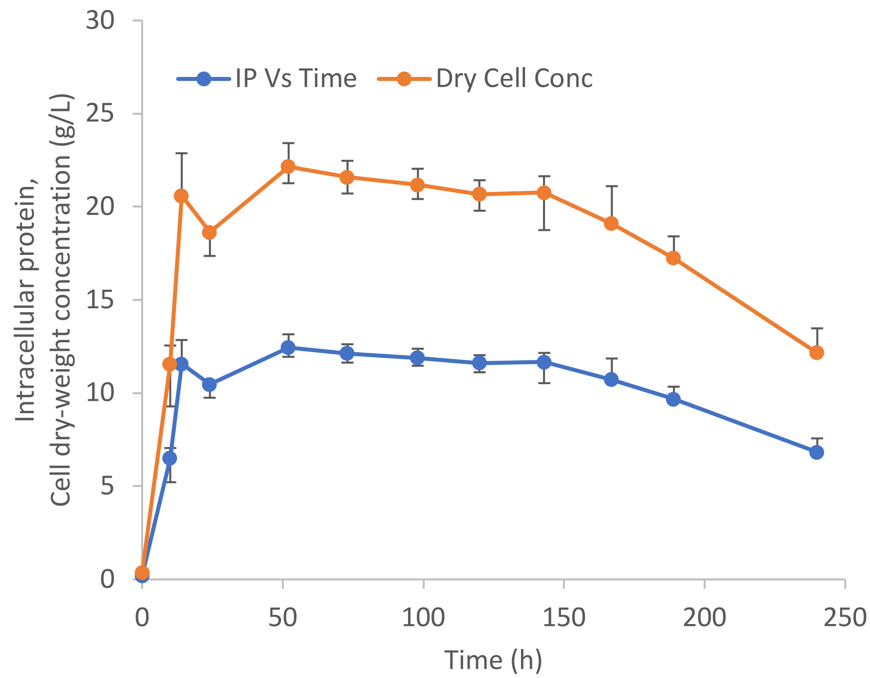

Figure 2. Profiles for intercellular protein (IP) and cell dry-weight (CDW) concentrations

Improved Dissolved Oxygen (DO) control - The fermentation setup was largely unchanged from previous reports, but a major improvement was made in dissolved oxygen (DO) management. In earlier fermentations, the DO control setpoint was fixed at 10%, with an automatic on/off supply of pure oxygen. However, frequent manual adjustments to the O_2 flow rate were required to maintain DO levels between 5% and 100% saturation. This approach often resulted in severe DO fluctuations, including both undershooting and overshooting, especially during the fermentation phases of more dynamic changes.

To address this, we implemented a dual-line oxygen delivery system. A lean oxygen flow line was introduced to meet basal oxygen demands gradually. When the DO level dropped below the setpoint, a control algorithm activated the main oxygen line by fully opening its valve. This two-tiered approach allowed for more stable DO control, particularly during the exponential growth phase when oxygen demand surged.

However, as broth viscosity rose, the mixing efficiency deteriorated to exacerbate DO fluctuations, particularly in the stationary phase. During this phase, even the highest possible impeller rotation speeds could not sufficiently mix the broth to maintain consistent oxygenation. Moreover, the fermentor's DO sensor began to exhibit feedback delays, further complicating control. To resolve this, we integrated the Bio-Command software with condition-based logic programming. This system dynamically adjusted the lean oxygen flow and DO setpoint in response to changes in cell growth, broth rheology, and oxygen uptake rate. The improved control strategy significantly reduced both undershooting and overshooting. We will implement and further improve this control algorithm in future fermentation. The resulting DO profile in this run is illustrated in **Figure 3**.

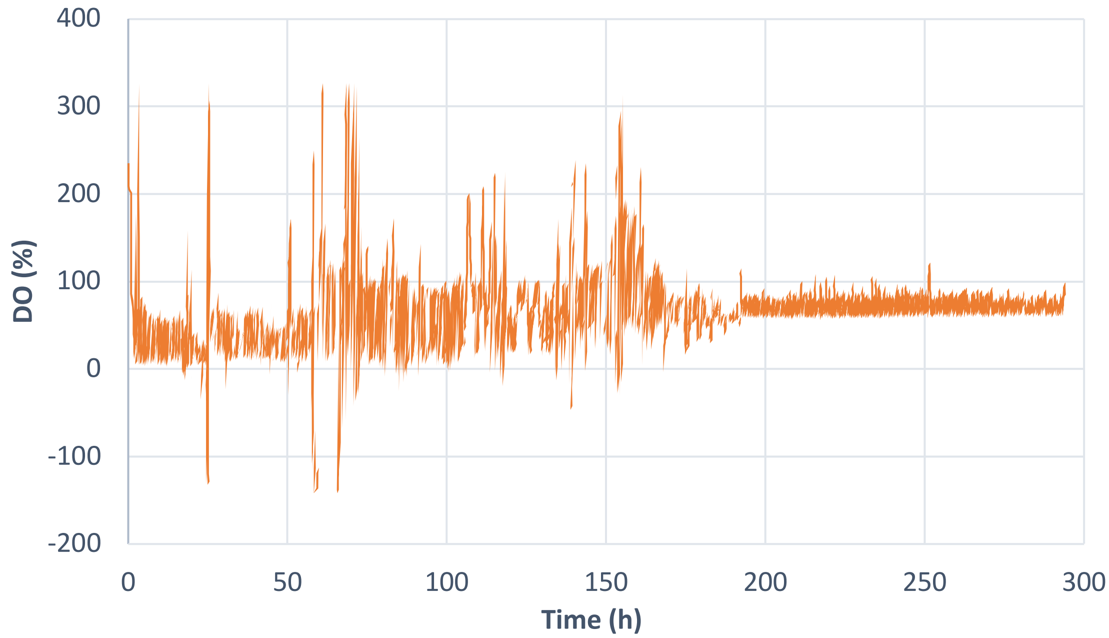

Figure 3. Dissolved oxygen (DO) concentration profile

Improved pH and oil feed control - In the previous run, pH remained beyond the setpoint for a long period due to insufficient addition of soybean oil as the carbon source. In this run, we increased the initial oil feed rate by 50%. But it was still insufficient to support the cells' exponential growth, as indicated by the rapid pH increase after about 22 h. Later we implemented a dynamic control logic to track and adjust oil feed rate based on pH behavior. This change helped maintain the process near the target pH of 5.7, as illustrated in **Figure 4**.

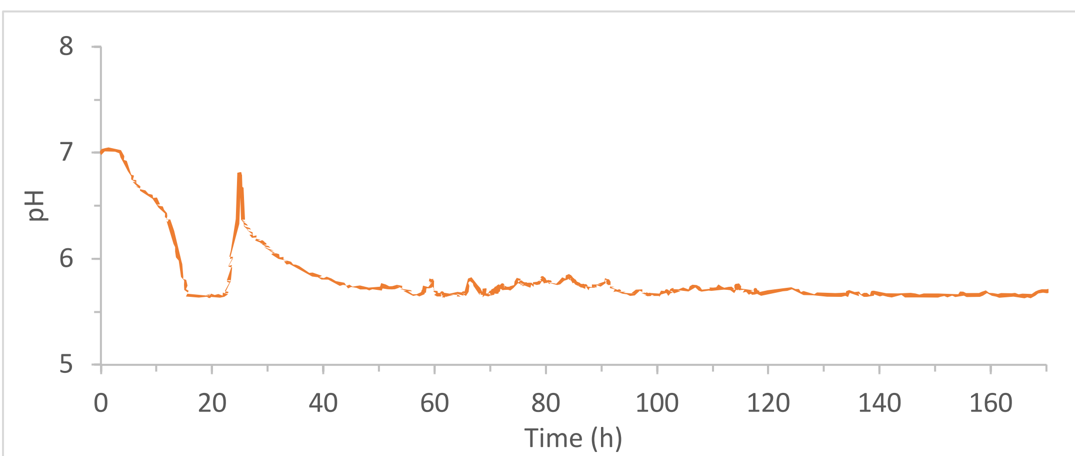

Figure 4. pH profile

Culture purity assessment - For this purpose, we conducted two experiments. Cells were grown in a glycerol-based nutrient medium for 36 hours and then, after dilution, inoculated on agar plates of the same medium added with cetyltrimethylammonium bromide (CTAB) and methylene blue

(MB). The pregrown culture was diluted by a wide range of dilution factors, and then 100 microliters of the diluted cultures were spread on the CTAB-MB agar plates. After 40 hours of incubation at 34° C, the agar plates with proper dilution factors showed clearly isolated colonies. These colonies had uniform morphology as shown in **Figure 5 (left**), indicating high purity of our currently used culture.

RhL productivity screening - Four stored cultures previously prepared by different students were compared for their RhL productivity. The procedures were similar to the above for purity assessment, but shallow wells were made on the CTAB-MB agar plates, and different wells were added with 10 microliters of different inocula. After 50 hours of incubation, all strains formed consistent halos surrounding the wells, suggesting equivalent RhL production capabilities. This is depicted in **Figure 5 (right)**. These results from the purity and RhL productivity assessments confirmed the suitability of using them for the subsequent RhL production studies.

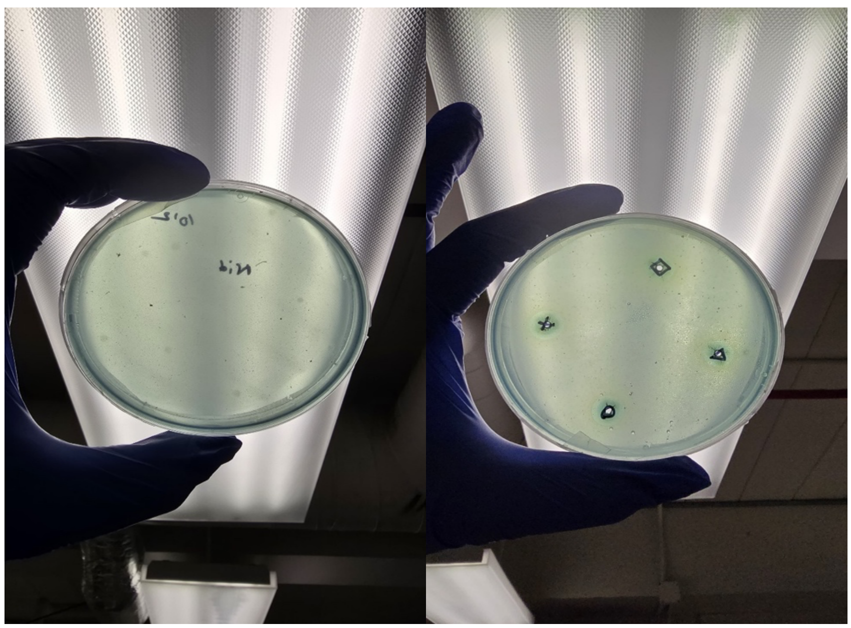

Figure 5. Assessment of culture purity check (left) and RhL productivity (right)

Milestone 2, Corrosion in PW simulant.

This quarter work was performed on characterizing the effect of CO_2 purging on the pH and dissolved oxygen (DO) concentration of the produced water (PW) simulant. One of the goals of this work was to optimize the solution chemistry for the microbial corrosion investigation, that is, the solution pH for SRB survivability is on the order of pH 5-8 and the current PW simulant is likely outside that range. As such, the pH and DO was measured for three different PW simulants as a function of CO_2 purging: 1% (mass) NaCl, 1% NaCl + 88 ppm RhL and 1% NaCl + 0.01 M $NaHCO_3$ (buffer). the experimental volume was on the order of 350 ml. For comparison, the open circuit potential (OCP, also referred to as E_{corr}) and the linear polarization resistance (inversely proportional to corrosion rate) data were also collected for C1018 specimens immersed in these solutions.

Figure 6a presents the pH and DO changes over a 20 hr. period for a 1% (mass) NaCl solution as a function of purging. For the first 90 mins (approx.) of the experiment, the solution was bubbled with lab air (aquarium pump). As seen this figure, the DO changes from approximately 3 ppm to 5.8 ppm during this period. Correspondingly, the solution pH increases from approximately 5.5 to 6.2. The change in OCP during this initial 90 mins. for the C1018 specimen is seen in **Figure 6b** along with the initial LPR measurement. After approximately 90 mins. of bubbling with lab air, the purge gas was changed to CO_2. Correspondingly, a rapid drop in DO was observed, from 5.8 ppm to a steady state value of 0.4 ppm after approximately 1 hour of purging. As expected, a precipitous drop in solution pH (pH 6.2 to pH 4) is observed during this time owing to the CO_2 / HCO_3 equilibria reactions:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO^{-3}$$

Over the next 18 hrs. of purging with CO_2, the DO remains constant while the solution pH increases to a steady state value of approximately pH 4.3. The OCP during this time rapidly decreased to -0.72 VSCE as the cathodic reaction changed from O_2 reduction to H^+ reduction in the CO_2 environment. As might be expected, the polarization resistance decreased from 700 Ω·cm2 to 320 $\Omega \cdot cm^2$ reflecting higher corrosion rates in the CO_2 environment.

Figure 7a presents the pH and DO changes over a 20 hr. period for a 1% (mass) NaCl solution with 88 ppm RhL as a function of purging. As before, for the first 90 mins (approx.) of the experiment, the solution was bubbled with lab air (aquarium pump). With respect to the effect of RhL, initial DO and pH values are slight lower than those observed in the non-RhL solution and changes with time appear to lag those recorded in the non-RhL (**Fig. 6a**.). However, the final values are similar to those in the non-RhL case, with a pH of 4.4. The polarization resistance in the RhL solution (**Fig. 7b**) is a factor of x40 greater (corrosion rate x40 lower) than the solution without RhL consistent with previous reports.

Figure 8 presents the effect of adding sodium bi-carbonate buffer to the 1% (mass) NaCl solution on DO, pH, OCP and LPR. As it relates to DO and LPR, trends and steady state values are similar to those observed in NaCl without $NaHCO_3$, thought the corrosion rate in this solution is a factor of x2 lower than the case without $NaHCO_3$ (e.g. LPR is x2 higher, **Fig. 8b.**) With respect to the effect of 0.01 M $NaHCO_3$ to buffer the PW simulant (forcing the above equilibria to the left) in a range that is hospitable to SRB growth, pH 5 to 8. Given that 0.01 M $NaHCO_3$ buffers the solution pH of the PW simulant into a range that is hospitable to SRB growth, this buffer will be added to the solutions (modified Baar's, modified Postgate C) that are used for MIC testing.

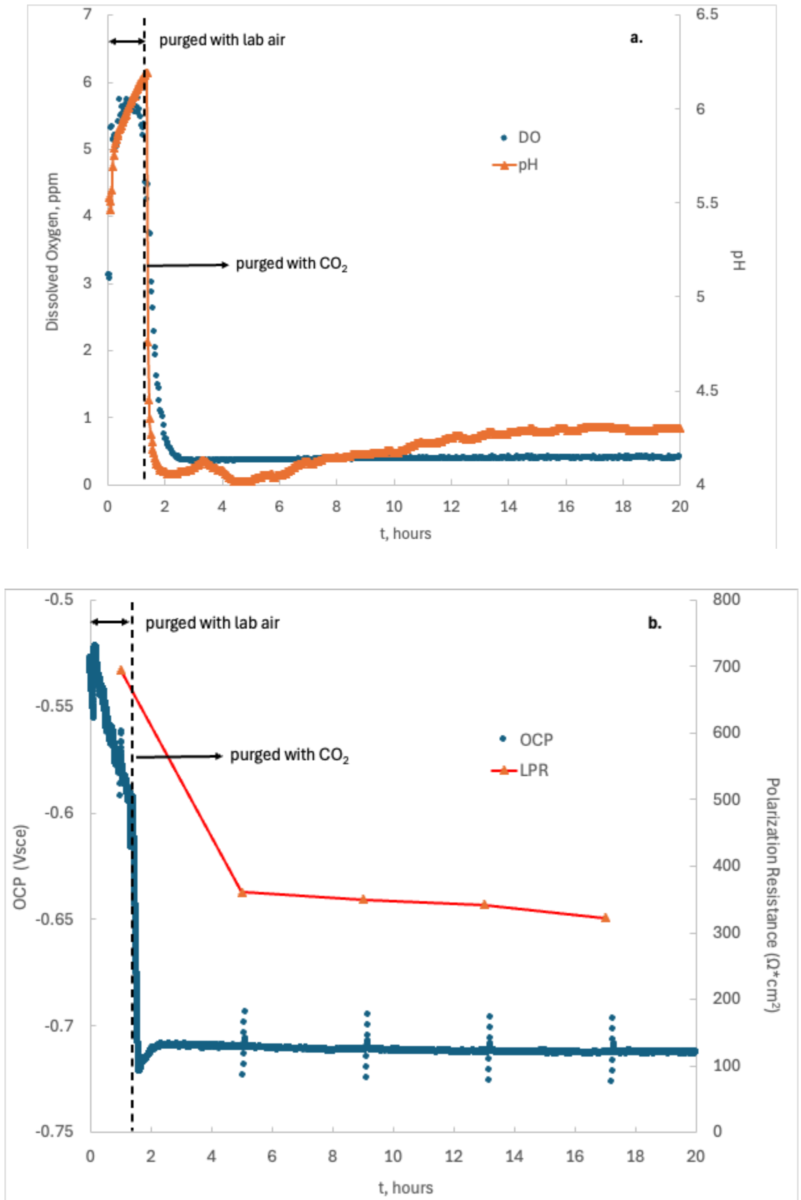

Figure 6. 1% (mass) NaCl solution: a) solution dissolved O_2 and pH with time and b) corresponding C1018 open circuit potential and polarization resistance with time.

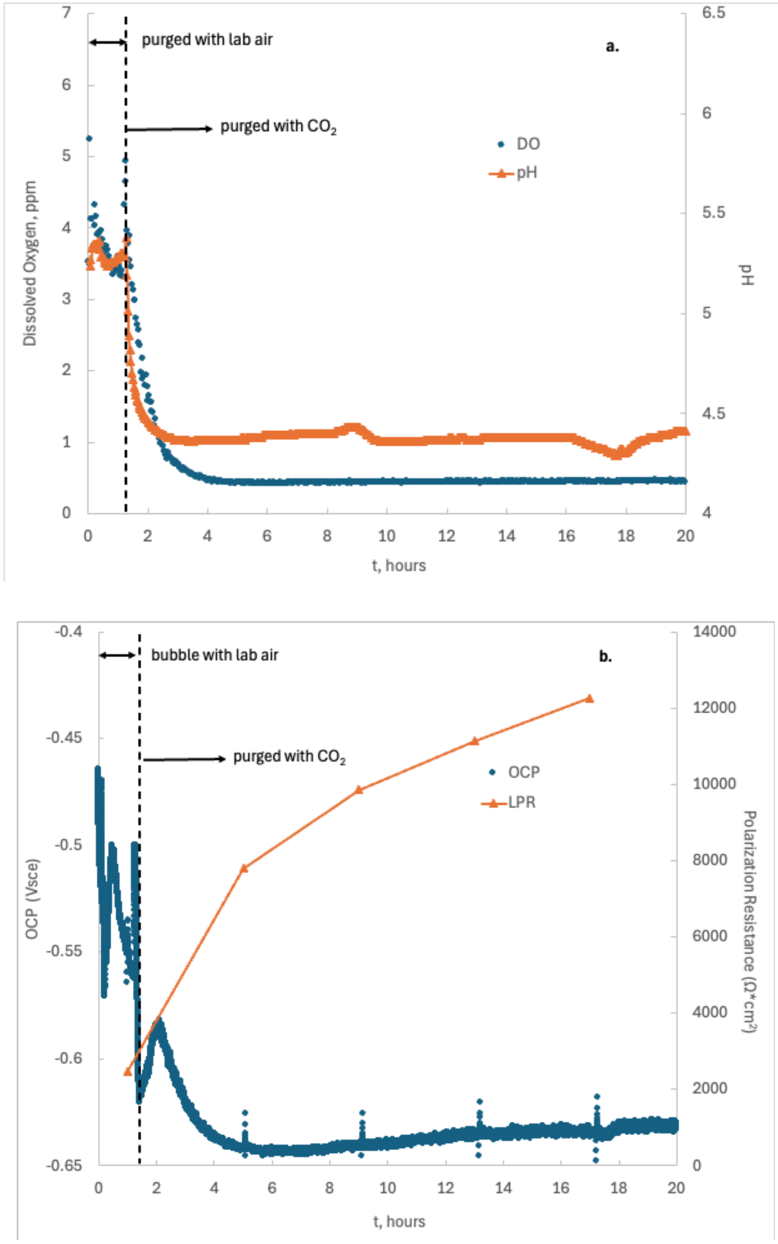

Figure 7. 1% (mass) NaCl solution + 88 ppm RhL: a) solution dissolved O_2 and pH with time and b) corresponding C1018 open circuit potential and polarization resistance with time.

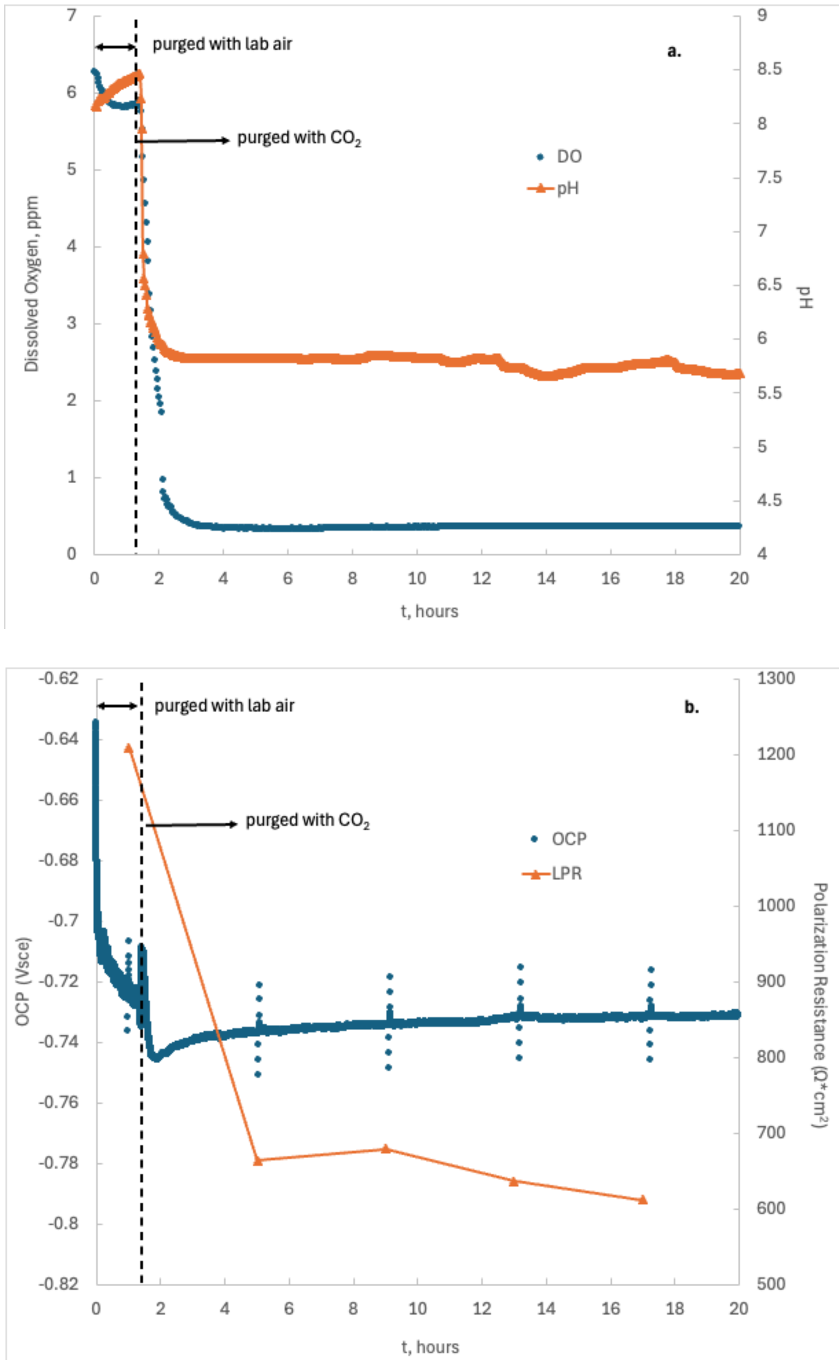

Figure 8. 1% (mass) NaCl solution + 0.01 M $NaHCO_3$: a) solution dissolved O_2 and pH with time and b) corresponding C1018 open circuit potential and polarization resistance with time.

Milestone 3, SRB-MIC.

The new batch of sulfate-reducing bacterial strain *Desulfovibrio vulgaris* (ATCC 7757) was revived and cultured in anaerobic Modified Baar's Medium (ATCC Medium: 1249): 2.0 g/l $MgSO_4$, 0.5.0 g/l Sodium Citrate, 1.0 g/l $CaSO_4 \cdot 2H_2O$, 1.0 g/l NH_4Cl, 0.5 g/l, K_2HPO_4, 3.5 g/l sodium lactate and 1.0 g/l yeast extract. The revival attempt was unsuccessful. Following submission of a report to ATCC, the institution agreed to replace the vial, acknowledging the challenges associated with reconstituting freeze-dried strains. It is anticipated that U. Akron will receive this new strain in early July and reconstituting it will begin shortly thereafter.

Project Financial Activities Incurred during the Reporting Period:
Since the beginning of the project, we have spent $217,217.25 (with encumbrances): $124,264 salary, $17,445 fringe, $8,393 supplies $75,871 indirect cost. The spending break down is shown below.

Ledger Account	Budget	LTD Actuals	Current Period Actuals	Total Spend	Remaining Balance	% Remaining
Salary	$234,584.00	$110,319.79	$25,000.82	$110,319.79	$124,264.21	52.97%
Fringe Benefits	$20,599.00	$17,445.56	$2,820.44	$17,445.56	$3,153.44	15.31%
Supplies & Services	$18,600.00	$10,206.53	$346.81	$10,206.53	$8,393.47	45.13%
Student Aid	$30,000.00	$7,500.00	$0.00	$7,500.00	$22,500.00	75.00%
Travel	$10,000.00	$0.00	$0.00	$0.00	$10,000.00	100.00%
Total Direct Costs	$313,783.00	$145,471.88	$28,168.07	$145,471.88	$168,311.12	53.64%
Indirect Cost	$147,567.00	$71,745.37	$14,647.40	$71,745.37	$75,821.63	51.38%
Total Direct & Indirect	$461,350.00	$217,217.25	$42,815.47	$217,217.25	$244,132.75	52.92%

Project Activities with Cost Share Partners:
None.

Project Activities with External Partners:
None.

Potential Project Risks:
None.

Future Project Work:
During Q4 of FY ’25, the PIs anticipate working on the following topics:

1) RhL fermentation will focus on 1. identify the limiting substrate in the current medium to inform future optimization and 2. methodology for separating mono- and di-rhamnolipid fractions in downstream processing.
2) QCM investigation to quantify RhL adsorption and benchmark EIS data for steel.
3) SRB-MIC testing: reconstituting newly acquired freeze-dried strain, EIS/LPR measurements with and without RhL.

Potential Impacts to Pipeline Safety:
FY ’25, Q3 – The results, thus far, are extremely encouraging. We have verified our initial experiments that show RhL may be an effective inhibitor crude pipelines.