

**SUMMARY:**  
**YEAR 2. DEVELOPMENT OF A BIOSENSOR FOR DETECTING ODORS AT LANDFILLS**

**Daniel E. Meeroff (PI)<sup>1</sup>**

**Sharmily Rahman<sup>2</sup>**

In 2021, the Bill Hinkley Center for Solid and Hazardous Waste Management funded a follow-up study at the Florida Atlantic University Laboratories for Engineered Environmental Solutions (FAU Lab.EES) to continue working on developing a novel biosensor technology using human odorant binding protein (hOBPIIa) that has the potential to objectively and rapidly measure odor concentrations in real-time. The currently accepted understanding of the human sense of smell is based on a mechanism of chemical binding to proteins that facilitate transport to specific receptors located in the membranes of human olfactory cilia. These receptors then generate impulses to olfactory nerves and trigger a response in the brain, which is then interpreted as a particular smell. By taking advantage of the nearly universal chemical binding sites of the recently isolated human odorant binding protein 2A (hOBPIIa), a biosensor can be designed by modifying the protein with a biomolecular fluorescent marker. Upon exposure to odorant compounds, the biosensor provides an objective concentration-dependent response that can be quantified spectrofluorometrically.

Over the last several years, FAU Lab.EES has been working with the Hinkley Center and the Environmental Research and Education Foundation (EREF) to conduct research on testing the effectiveness of the biosensor with a number of common odorants found in landfills (hydrogen sulfide, ammonia, methane, methyl mercaptan, and mixtures) demonstrating encouraging results that signal the potential of the biosensor to be a game changing solution for objectively measuring odorants in the atmosphere in near real-time. As part of the experiments in Year 1, a method to mass produce the protein was developed, and it was found that around 180 µg of protein was able to quantify approximately 35-45 µg of hydrogen sulfide, 12-18 µg of ammonia, 83-95 µg of methyl mercaptan, and 15 µg of methane depending on the flow rates of the gases used in the experiments.

The objective of this current research is to build on previous results by carrying out further spectrofluorometric analyses of the prototype biosensor with a wider range of pure odorants and their mixtures. This will include redesigning the reactor chamber as a flow-through system for increased accuracy and real-time measurement using a highly sensitive and portable spectrofluorometer. An investigation will be carried out to determine the reversibility of the protein-odorant bond to promote reuse of the biosensor cartridge, thus making the quantification process more efficient and even more cost-effective, while promoting adaptability for field usage at low cost.

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## QUARTERLY PROGRESS REPORT

(May 1, 2022 - May 30, 2022)

**Project Title:** Year 2. Development of a Biosensor for Detecting Odors at Landfills

**Principal Investigator:** Daniel E. Meeroff, Ph.D.

**Affiliation:** FAU

**Phone number:** (561) 297-2658

**Project website:** <http://labees.civil.fau.edu/leachate.html>

**Student:** Sharmily Rahman, Ph.D. Candidate

### **Work Accomplished During This Reporting Period:**

#### **TASK 1. Perform protein sensitivity experiments on an expanded list of pure odorant compounds.**

Dr. David Binninger, Professor of Biology at FAU has prepared a fresh batch of hOBPIIa protein to be used in the new round of experiments. Dr. Binninger's team is currently in the process of preparing additional hOBPIIa, and to that extent, an additional purification column (Cytiva His SpinTrap TALON) and protein size markers (Thermo Scientific Pierce Unstained Protein MW Marker) were purchased. To properly store the new batches of hOBPIIa, a -20°C freezer was purchased. The new modified experimental setup (explained in more detail in Task 3) has just been deployed in the lab, and S. Rahman will start the experimentation plan outlined in Task 1 during June-July. Previous experiments with pure compounds, e.g., hydrogen sulfide and ammonia, the two most commonly found landfill odorants, will be repeated using the modified flow-through setup set up (described in Task 3) and using much lower volumes of the biosensor solution to simulate practical usage in real-world scenarios. The expanded list of pure odorants has been selected and ordered for this testing:

- Formaldehyde, 25 ppm gas balanced with nitrogen
- Toluene, 24 ppm gas balanced with air
- Tert-butyl mercaptan, 3 ppm gas balanced with nitrogen

**TASK 2. Perform protein sensitivity experiments on landfill gas mixtures.** Once Task 1 is complete, S. Rahman will conduct experiments using gas mixtures. In Year 1, a mixture of hydrogen sulfide and methane and another mixture of ammonia with methane had been tested with the biosensor. In this study, initially a mixture of hydrogen sulfide and ammonia will be tested. Following this, S. Rahman will conduct another experiment using a mixture of compounds selected from the list tested in Task 1.

#### **TASK 3. Upgrade the reactor chamber as a flow-through system for improved real-time result accuracy.**

In the previous work associated with Year 1, an enclosed reactor chamber had been used that only allowed extracted subsamples to be analyzed at specific time intervals for the fluorescence analysis using a Horiba Jobin Yvon spectrofluorometer. That experimental setup did not allow for real-time analysis of the odorant-biosensor binding assays. The goal of this task is to increase the accuracy of the results without removing any of the biosensor molecules from the reaction chamber, while obtaining real-time fluorescence measurements to observe the odorant-biosensor interaction. The modified setup (schematic diagram shown in Figure 1) will allow a constant flow of odorant gases to be directed to the reactor chamber containing the biosensor to provide real-time fluorescence data while allowing the full amount of biosensor to remain in the chamber during testing, potentially increasing the accuracy of the experiment.

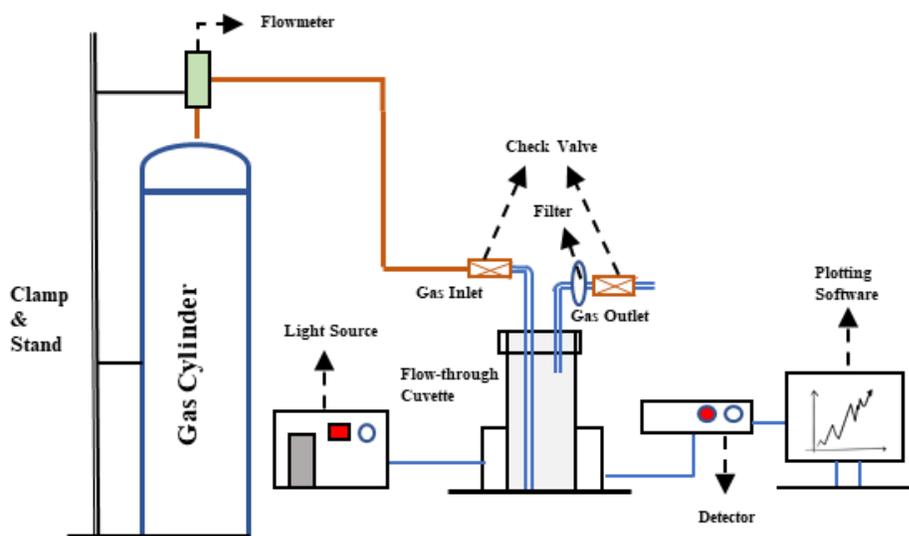


Figure 1: Schematic diagram for experimentation with a flow-through system

The miniaturization of the new experimental setup as a flow-through system for real-time fluorescence analysis will take the technology one step closer towards the ultimate goal of deploying the biosensor in a handheld device in real-world scenarios. FAU Lab.EES has obtained additional funding of \$23,000 in February 2022 to procure a sensitive, small scale spectrofluorometer (QE Pro-FL from Ocean Insight) as shown in Figure 2a to allow miniaturization of the experimental setup and real-time, flow-through spectrofluorometric measurements to improve accuracy of the concentration-dependence relationship. The flow cell cuvette (refer to Figure 4a) used in this spectrofluorometer, placed in a specialized cuvette holder (Figure 2b), will itself serve as the reactor chamber, further miniaturizing the setup compared to previous experiments.



Figure 2: (a) QE Pro-FL High Performance Spectrofluorometer from Ocean Insight (b) SQUARE ONE (SQ1-ALL) Cuvette Holder to be used with QE Pro-FL for flow-through system (Ocean Insight)

QE Pro has a number of unique features which makes it the most sensitive spectrometer offered by Ocean Insight. The integration time (the time taken to collect a single reading) of the QE Pro can be as low as 8

ms and can be up to 60 minutes, which greatly enhances the detection limit in low light level applications. Also, the thermoelectric cooling (TEC) featured in QE Pro can precisely control the temperature of the detector, which minimizes the effect of thermal noise and maximize the signal to noise ratio (SNR), increasing the accuracy of results to many folds. The fluorescence data obtained from the Ocean View software are updated continually to provide an uninterrupted real-time fluorescence measurement. The spectrofluorometer also comes with the added advantage of portability, even allowing it to be carried on-site for field measurements. Most currently available spectrofluorometers on the market cannot be used in a flow-through configuration and are not portable. Also, with its high quantum efficiency detector, it considerably increases the accuracy of the experiments. In QE Pro, the fluorescence measurements obtained from a sample automatically subtracts the fluorescence value obtained from dark measurements to reduce any background noise. This specialized piece of equipment has been purchased at a cost of \$23,000 and was delivered in April 2022. It has already been set up in the laboratory as shown in Figure 3. The light source and the spectrometer are connected to the cuvette holder at a 90-degree angle relative to each other for obtaining maximum sensitivity for the fluorescence measurements. The spectrometer is connected with a laptop to record the readings.

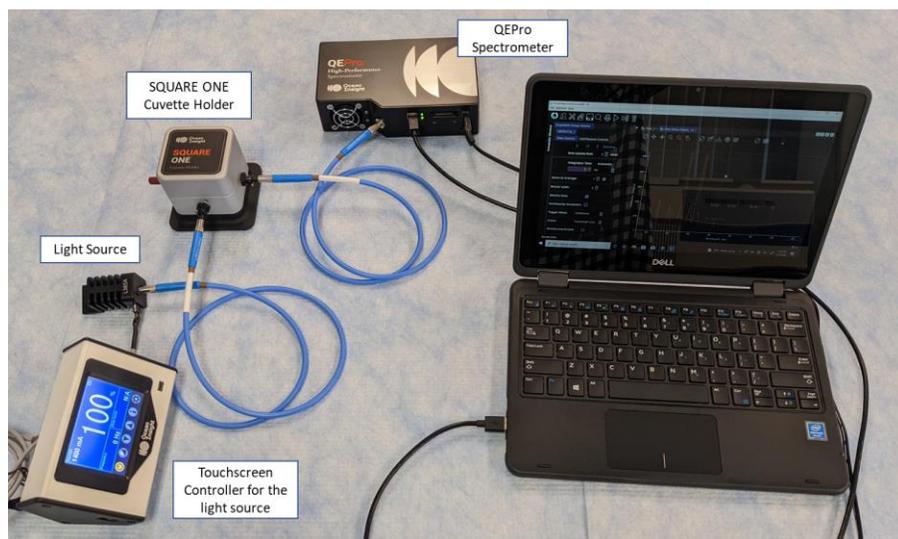


Figure 3: The QE Pro-FL spectrometer and the light source connected with the SQUARE ONE cuvette holder

The 3 mL flow cell cuvette to be used as the reactor chamber in the new modified setup has been procured in April 2022 from Starna Cell (Figure 4a). The flow cell has two protruding tubes at its top that can be used as an inlet and outlet during the experimentation. A vacuum air duster as shown in Figure 4b was purchased to dry out the cuvette after washing.

To establish the flow-through system with the flow cell, the lid of the cuvette holder can be kept open while taking the fluorescent measurements without incorporating any inaccuracy in the analysis due to the following reasons:

1. The cuvette holder has collimating lenses on both sides, so it only accepts light from a very specific angle.

2. Even if potentially interfering ambient light does make its way into the system, it would be subtracted out in the software when the dark measurement is taken.

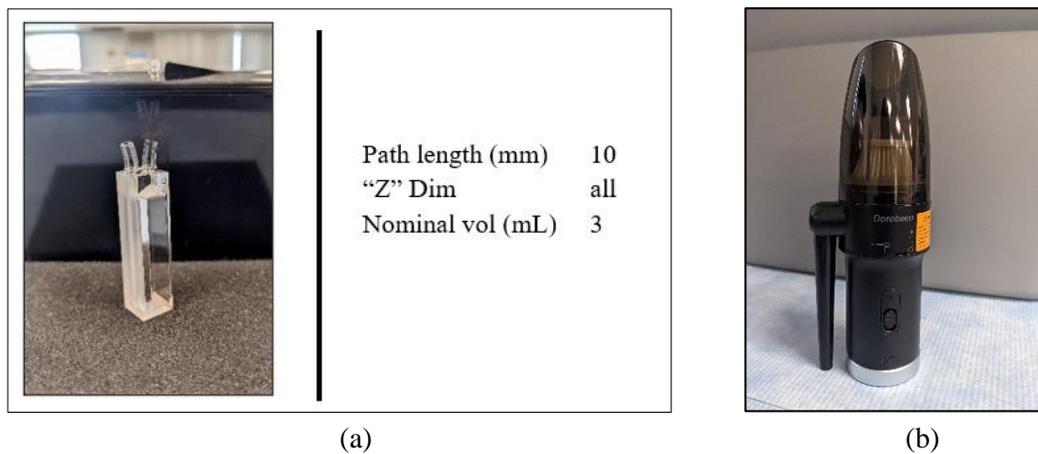


Figure 4: (a) Flow cell to be used as the reactor chamber in the modified experimental setup (b) Vacuum air duster used for cuvette drying

The modified experimental setup to establish the flow-through system with real-time fluorescence measurement as per the schematic diagram shown in Figure 1 has already been set up in the laboratory (shown in Figure 5). The basic concept is still the same, except that the modified setup utilizes the flow cell itself as the reactor chamber for real-time fluorescence analysis. Pressurized odorant gas is delivered to the flow cell containing the biosensor solution, which is placed in the cuvette holder. The flow cell is half-filled (1.5 mL) with the biosensor solution and the upper half is kept empty to allow the headspace gas to escape from the chamber so that the uncombined gas does not exert pressure on the cell. The cylinder uses a calibration gas regulator (0.1 Lpm constant flow rate) connected to a highly sensitive flowmeter (Flowmeter 2 in Figure 5) that controls gas flow to the flow cell at a very low rate (0-33 mL/min.). This flowmeter comes with four different floats, allowing for four different upper limits of flow rates as below:

- Glass Float: 6 mL/min
- Sapphire Float: 8 mL/min
- Stainless Steel Float: 17 mL/min
- Carboloy Float: 33 mL/min

With the different floats, the flow rate of the odorant gases can be controlled more accurately. Brass pipe fitting adapters are used at the NPT connections of the flowmeter and from there the other connections use flexible silicone tubing. The extra gas (difference in flow rate between cylinder regulator and flowmeter) coming from the cylinder is diverted through a Y-connector by means of another flowmeter (Flowmeter 1 in Figure 5) and exhausted in the fume hood. Check valves are attached on both sides of the Y-connector to prevent backflow and a third check valve is attached at the outlet of the flow cell that would allow the uncombined gas to escape from the chamber to prevent reverse contamination of the reaction zone with external air. After the setup is established, the flow cell containing the biosensor is excited by means of the light source at 385 nm when the spectrometer starts recording the fluorescence

measurements at a continuous pace as an increasing amount of odorant gases get combined with the complex. The whole experimental apparatus was set up under a fume hood to exhaust the gas safely.

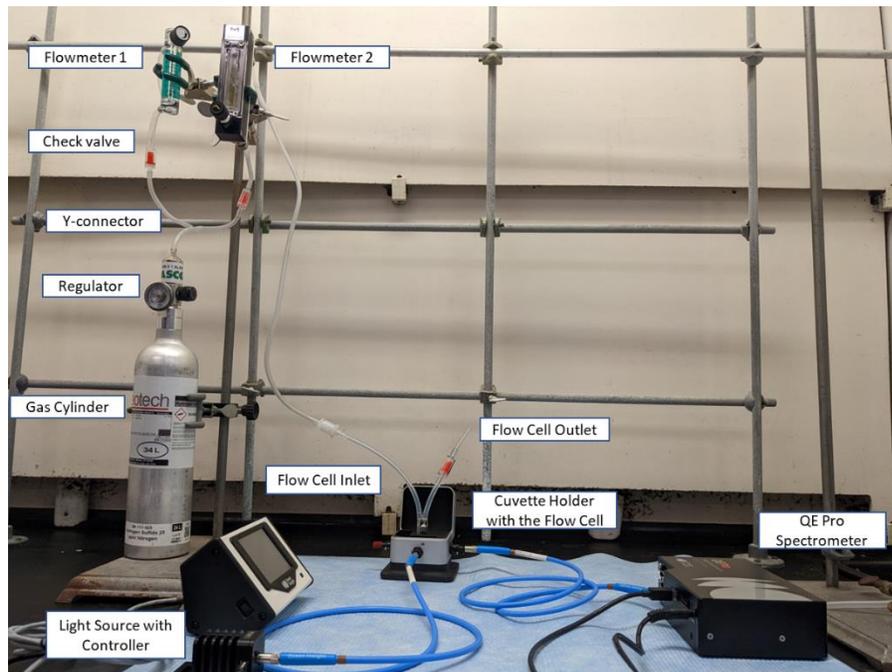
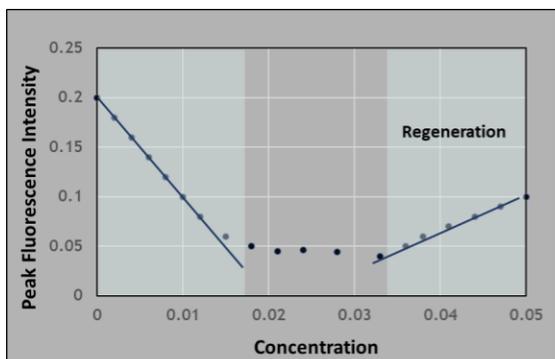
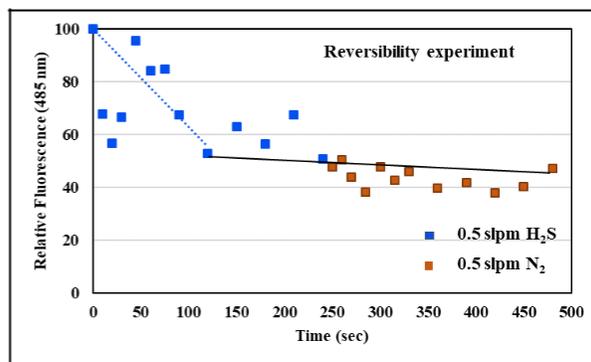


Figure 5: Experimental setup for real-time fluorescence analysis using flow-through system

**TASK 4. Perform experiments to explore protein-odorant reaction reversibility.** A number of reversibility tests to regenerate the odorant-protein binding were conducted to see if the protein can be reused multiple times to quantify the odorant gas, after its initial use. The expected result is shown by the concept graph in Figure 6a. Initially, in the Year 1 project, it was hypothesized that passing an odorless, inert gas through the previously bound odorant-biosensor complex would regenerate the sensor by reversing the reaction and purging the odorants. To that end, experiments were conducted in Year 1 by passing nitrogen through the bound odorant-biosensor complex, where hydrogen sulfide was used as the odorant (as shown in Figure 6b). However, initial experiments indicated that full regeneration might take longer than initially anticipated.



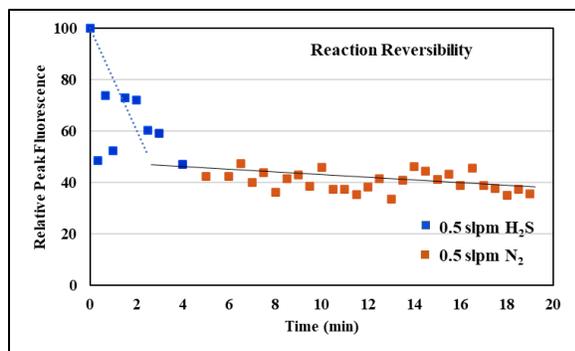
(a)



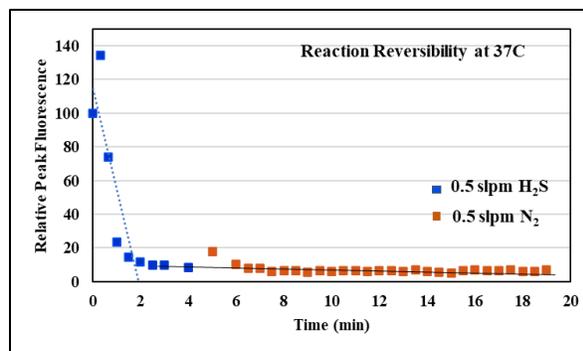
(b)

Figure 6: (a) Concept graph indicating successful regeneration shown by a bounce-back of the intensity (b) Graph of peak emission intensity against time obtained by passing 0.5 SLPM  $\text{H}_2\text{S}$  gas through the biosensor solution for the first 240 seconds (4mins) followed by 0.5 SLPM  $\text{N}_2$  gas for the final 240 seconds (4mins)

In this project, additional experiments for checking reversibility have already been conducted. In the first experiment, the nitrogen purging time was increased to 15 minutes, which unfortunately still did not regenerate the sensor as shown in Figure 7a. In the next experiment, while passing the nitrogen gas, the temperature of the solution was adjusted to human body temperatures of  $37^\circ\text{C}$  to replicate a more real-world biological scenario analogous to temperatures typically found in the human nose. Figure 7b shows that adjusting the temperature had no impact in the regeneration of the sensor.



(a)



(b)

Figure 7: (a) Graph of peak emission intensity against time obtained by passing 0.5 SLPM  $\text{H}_2\text{S}$  gas through the biosensor solution for the first 4 mins followed by 0.5 SLPM  $\text{N}_2$  gas for the final 15 mins (b) Same experiment condition but with temperature adjustment from ambient to  $37^\circ\text{C}$

The fluorescence curves obtained from regeneration tests indicated that the reaction reaches an equilibrium point after the saturation limit (after around 2 min). Keeping this in mind, an additional experiment was conducted to take advantage of Le Châtelier's principle by introducing additional fluorophore (1-AMA) at the reaction equilibrium point in a bid to regenerate the biosensor. If a dynamic equilibrium of a reversible reaction is disturbed by changing the conditions (pressure, temperature, or concentration), the position of equilibrium shifts to counteract the change to reestablish an equilibrium.

This means, in the case of a truly reversible reaction, adding more product, i.e., fluorophore 1-AMA at the reaction equilibrium point, may shift the reaction equilibrium to the left and regenerate the protein-fluorophore bond again as shown in Figure 8.

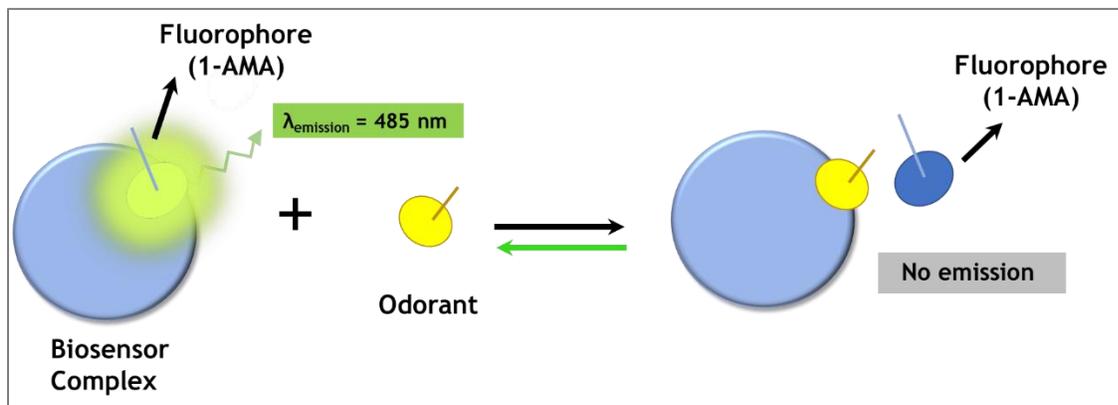


Figure 8: Adding more 1-AMA at the reaction equilibrium will favor more reactant formation (protein-fluorophore complex) to regenerate the sensor if the reaction is reversible

To test this hypothesis, an experiment was conducted by saturating the biosensor solution with hydrogen sulfide (first 4 min) and then passing nitrogen for the same period of time to purge any remaining hydrogen sulfide gas. Afterward, an additional  $1 \mu\text{M}$  1-AMA was added to the solution (protein and fluorophore initial conc. was  $1 \mu\text{M}$  as well), which was able to increase the fluorescence reading to some extent (graph not shown). The same experiment was then repeated by adding  $2 \mu\text{M}$  additional 1-AMA, which was able to return the intensity back to almost 90%, leading to successful regeneration of the biosensor. Afterward, another cycle of hydrogen sulfide was passed into the regenerated sensor, which produced a similar pattern of decrease in intensity of the peak fluorescence as the initial cycle, resulting in similar slopes as shown in Figure 9.

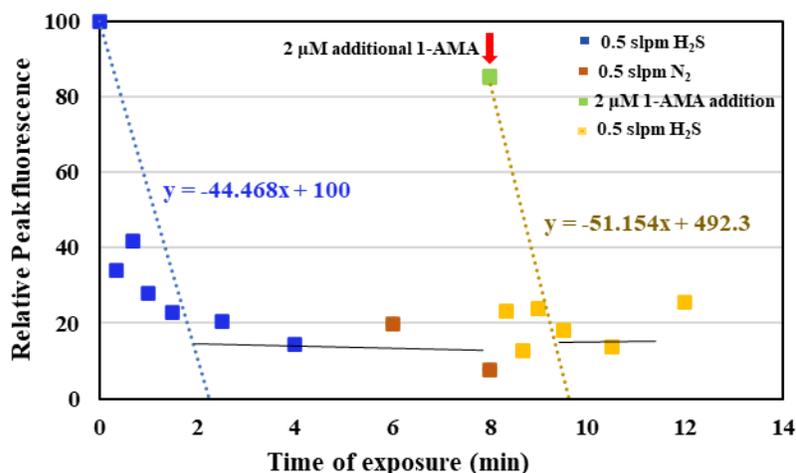


Figure 9: Graph of peak emission intensity against exposure time obtained by passing 0.5 SLPM H<sub>2</sub>S gas through the biosensor solution for the first 4 minutes followed by 0.5 SLPM N<sub>2</sub> gas for the next 4 minutes and then additional passage of H<sub>2</sub>S gas in cycle 2 after successful regeneration of the biosensor by adding  $2 \mu\text{M}$  additional 1-AMA

**TASK 5. Develop recommendations and prepare publication materials.**

A presentation was prepared for an invited panel session at WasteExpo 2022 in Las Vegas, NV.

**Upcoming Research Tasks**

**TASK 1. Perform protein sensitivity experiments on an expanded list of pure odorant compounds.** S.Rahman is about to start the experimentations laid in task 1 using the modified experimental setup.

**TASK 2. Perform protein sensitivity experiments on landfill gas mixtures.** Will be performed following Task 1 pure odorant tests with the expanded list.

**TASK 3. Upgrade the reactor chamber as a flow-through system for improved real-time result accuracy.** Task completed.

**TASK 4. Perform experiments to explore protein-odorant reaction reversibility.** Task completed.

**TASK 5. Develop recommendations and prepare publication materials.** Draft final report is in preparation.

## **TAG Meetings**

No TAG meetings were conducted during this reporting period. The initial TAG meeting was held on March 29, 2022.

- Video Link and Minutes: [TAG 1 \(video\)](#)

## **PROJECT METRICS:**

List graduate or postdoctoral researchers **funded** by **THIS** Hinkley Center project.

<b>Last name, first name</b>	<b>Rank</b>	<b>Department</b>	<b>Professor</b>	<b>Institution</b>
Rahman, Sharmily	Ph.D. Candidate	CEGE	Meeroff	FAU

1. List research publications resulting from THIS Hinkley Center project. Has your project been mentioned in any research and/or solid waste publication/newsletters/magazines/blogs, etc.?

A peer-reviewed manuscript was submitted and is under review: “Development of a biosensor for objectively quantifying nuisance odors” to an Elsevier Journal.

A two-part article was published online on Waste360 (November 11, 2021):

- <https://www.waste360.com/landfill-operations/odor-management-landfills-part-1-current-state-art>
- <https://www.waste360.com/landfill-operations/odor-management-landfills-part-2-novel-biosensor-measuring-odors-landfills>

A presentation was prepared for an invited panel session (M511) at WasteExpo 2022. “Innovative Solutions and Technologies for Keeping Odors at Bay” on May 9, 2022.

2. List research presentations resulting from (or about) THIS Hinkley Center project. Include speaker presentations, TAG presentations, student posters, etc.

- Dr. Meeroff was invited to speak at EREF Orlando on October 20, 2021, “Detection of Nuisance Odors using Odor Binding Protein Sensor: EREF Funded Project Update”
- Dr. Meeroff was invited to speak at WasteExpo 2022 on May 9-11 in Las Vegas, NV, “Innovative Solutions and Technologies for Keeping Odors at Bay”

3. List who has referenced or cited your publications from this project. Has another author attributed your work in any publications?

None so far

4. How have the research results from THIS Hinkley Center project been leveraged to secure additional research funding? What additional sources of funding are you seeking or have you sought? Please list all grant applications and grants and/or funding opportunities associated with this project. Indicate if additional funding was granted.

The FAU Technology fee competitive grant was applied for in Spring 2021 and was awarded and received in February 2022. The grant funded purchase of a flow-through spectrofluorometer \$23,000.

5. What new collaborations were initiated based on THIS Hinkley Center project? Did any other faculty members/researchers/stakeholders inquire about this project? Are you working with any faculty from your institution or other institutions?

Other faculty include Dr. D. Binninger (FAU-Biology) and Dr. M. Jahandar Lashaki (FAU-CEGE)

6. How have the results from THIS Hinkley Center funded project been used (not will be used) by the FDEP or other stakeholders? (1 paragraph maximum). Freely describe how the findings and implications from your project have been used to advance and improve solid waste management practices

None so far

### Pictures:

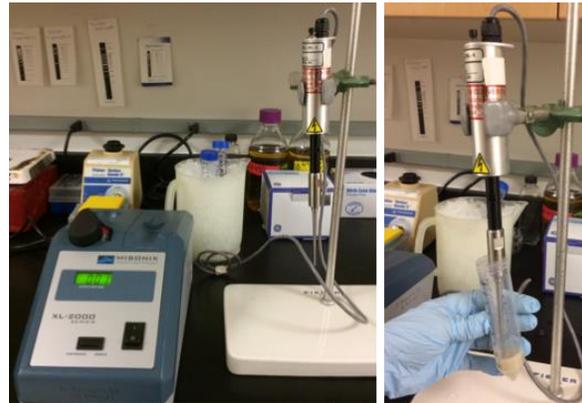
Please provide photographs and videos of your progress during this reporting period. Photographs can be copy and pasted below; please give a brief description of each photo. Videos should have links provided. (Both photos and videos are encouraged; please provide as many as you would like.)

Photo examples include:

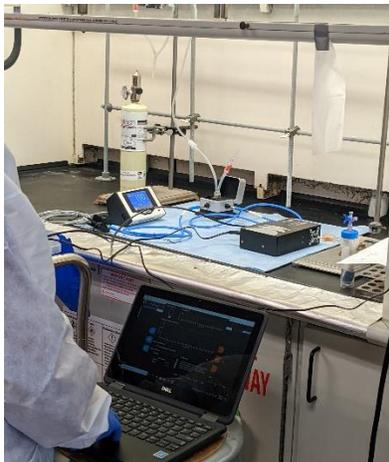
- A group picture of you and your student team
- Fieldwork (w/ student working)
- Lab work (w/ student working)
- Poster Presentations



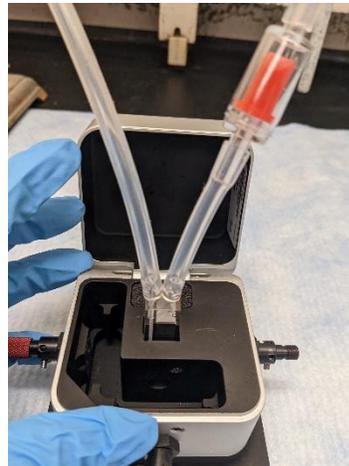
Preparation of hOBPIIa in Dr. Binninger's lab. The supernatant in the centrifuge tube was decanted while the cell pellets containing the protein gene was washed during the next stage.



Misonix XL-2000 Series Sonicator was used on the maximum setting to lyse cells and extract cell contents during the protein purification process.



S. Rahman is running fluorescence analysis using the new flow-through setup. The fluorescence data is constantly being displayed by the software.



S. Rahman is setting up the flow cell for running experiments.

**TAG Member List:**

Owring Kashef (CDM)

Craig Ash (WM)

Ravi Kadambala (CDM)

Jeff Roccapriore (WM)

André McBarnette (Stantec)

Dan Schauer (Geosyntec)

Damaris Lugo (Broward County)

Amanda Krupa (SWA)

Amede Dimonnay (Broward County)

Jarod Gregory (Trinity Consultants)

Hanting Wang (Greeley and Hanson)

Catherine Vanyo (Brown and Caldwell)

Sally Gordon (King County, WA)

Bishow Shaha (Geosyntec)

**Project Website:**

<http://labees.civil.fau.edu/leachate.html#Biosensor>