SUMMARY:

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

Daniel E. Meeroff (PI)[1]

Sharmily Rahman[2]

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
(March 1, 2022 - April 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 procured a fresh batch of hOBPIIa protein to be used in the new round of experiments. His team is currently in the process of preparing more hOBPIIa to be used in biosensor experiments. To that extent, an additional purification column (Cytiva His SpinTrap TALON) and protein size markers (Thermo Scientific Pierce Unstained Protein MW Marker) have been procured during this reporting period. Once the new experimental setup (explained in Task 3) is established, S. Rahman will use this protein as the stock solution for planned experiments. Initially, the previous experiment with pure compounds (e.g. hydrogen sulfide) will be tested using much lower volumes of the biosensor solution to simulate practical usage in real world scenarios for different gas flow rates using the flow-through setup. Then experiments will be conducted on the expanded list of pure odorants.

TASK 2. Perform protein sensitivity experiments on landfill gas mixtures. Once Task 1 is complete, S. Rahman will conduct experiments using gas mixtures commonly found in landfills from the expanded list 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. FAU Lab.EES has obtained an additional funding of $23,000 in February 2022 to procure a high-performance and sensitive spectrofluorometer (QEPro-FL from Ocean Insight) as shown in Figure 1a to allow miniaturization of the experimental setup and real-time, flow-through spectrofluorometric measurements to improve accuracy of the concentration-dependence relationship. The cuvette used in this spectrofluorometer, placed in a specialized cuvette holder (Figure 1b), will itself serve as the reactor chamber, further miniaturizing the setup compared to previous experiments.

(a) (b)

Figure 1: (a) QEPro-FL High Performance Spectrofluorometer from Ocean Insight (b) SQUARE ONE (SQ1-ALL) Cuvette Holder to be used with QEPro-FL for flow-through system (Ocean Insight)

This modified setup (schematic diagram shown in Figure 2) will allow a constant flow of odorant gases to be directed to the chamber containing the biosensor to provide real-time fluorescence data while allowing the full amount of biosensor to remain in the chamber, potentially increasing the accuracy of the experiment. This advancement will take the technology one step closer towards the ultimate goal of deploying the biosensor in a handheld device in real-world scenarios. The spectrofluorometer 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. With its high quantum efficiency detector, it will also considerably increase the accuracy of the experiments.

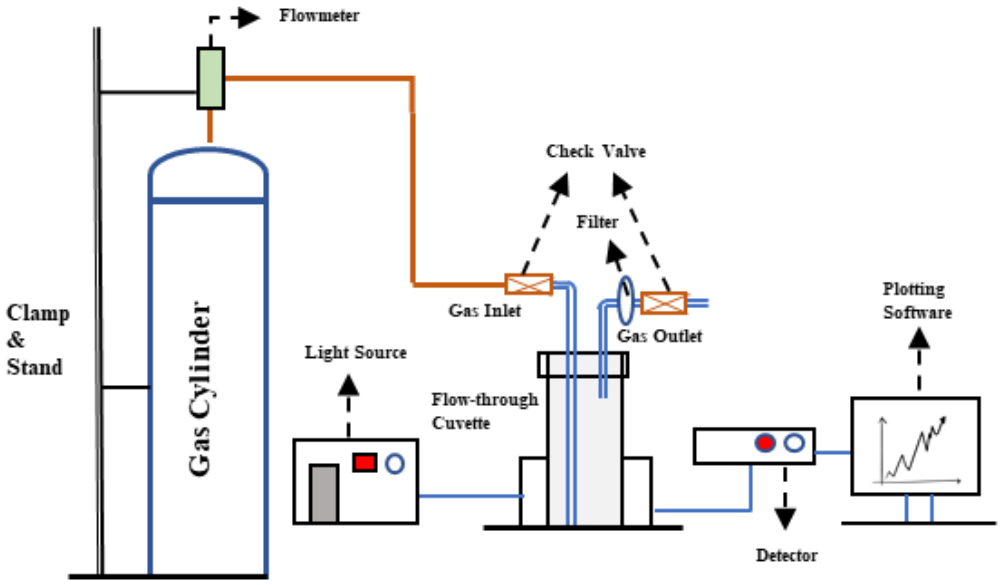
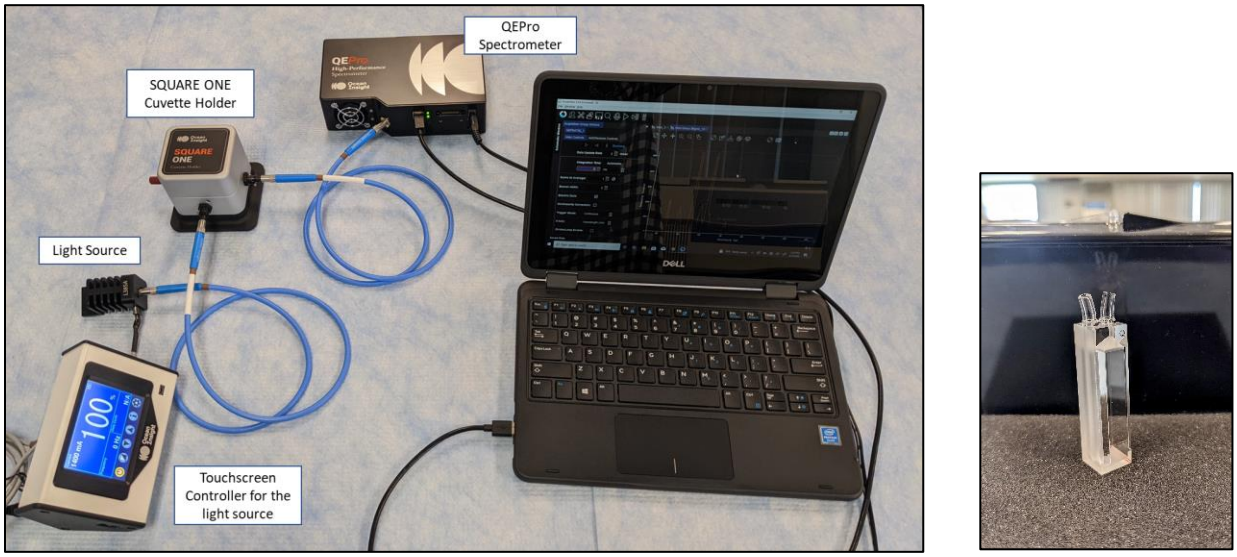


Figure 2: Schematic diagram for experimentation with a flow-through system

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 3a, where the light source and the spectrometer are coupled to the cuvette holder at a 90-degree angle relative to one another for obtaining maximum sensitivity for the fluorescence measurements. The QEPro is connected to a laptop through a data cable where the readings are recorded. The thermoelectric cooling (TEC) featured in QEPro 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. The integration time (the time taken to collect a single reading) of the QEPro can be as low as 8 ms and can be up to 60 minutes, making it the most sensitive spectrometer offered by Ocean Insight. To establish the flow-through system with the flow cell, the lid of the SQUARE ONE (SQ1-ALL) 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.

The flow-cell cuvette to be used in the upcoming experiments as the reactor chamber has been procured in April 2022 from Starna Cell (Figure 3b). This flow cell has a nominal volume of 3 mL and fits in the SQUARE ONE cuvette holder. The connection of the cell with the outside gas supply system is currently being designed by the research team pending procurement of a sensitive flowmeter, which has been ordered.



(a) (b)

Figure 3: (a) QEPro-FL spectrometer and the light source are connected with the SQUARE ONE cuvette holder (b) Flow cell to be used as the reactor chamber

TASK 4. Perform experiments to explore protein-odorant reaction reversibility. S. Rahman is conducting literature review on the viability and methods of reversing the reaction between odorant binding protein (OBP) and odorant gas. In case of successful regeneration, the spent biosensor could be used multiple times as the fluorescence intensity would come back after the initial odor detection as represented by Figure 4a. Initially in previous work, it was hypothesized that passing an odorless, inert gas through the biosensor-odorant complex would regenerate the biosensor 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 (as shown in Figure 4b). However, initial experiments indicated that full regeneration may take longer than initially anticipated.

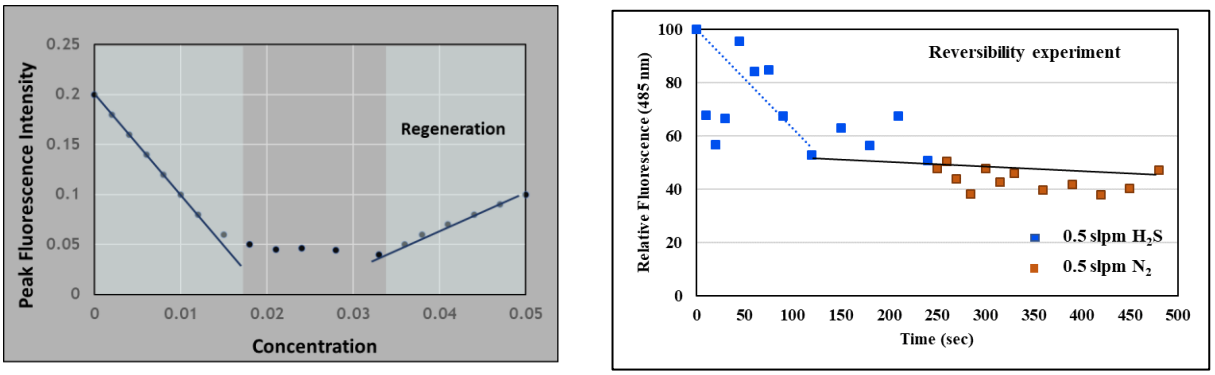


Figure 4: (a) Concept graph indicating successful regeneration shown by a bounce-back of the intensity (b) Graph of peak emission intensity against time obtained by passing 0.5 SLPM H_2S gas through the biosensor solution for the first 240 seconds (4mins) followed by 0.5 SLPM N_2 gas for the final 240 seconds (4mins)

In this study, 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 5a. In the next experiment, while passing the nitrogen gas, the temperature of the solution was adjusted to human body temperatures of 37°C using a hot plate to replicate a more real-world biological scenario analogous to temperatures typically found in the human nose. Figure 5b shows that adjusting the temperature had no impact in the regeneration of the sensor.

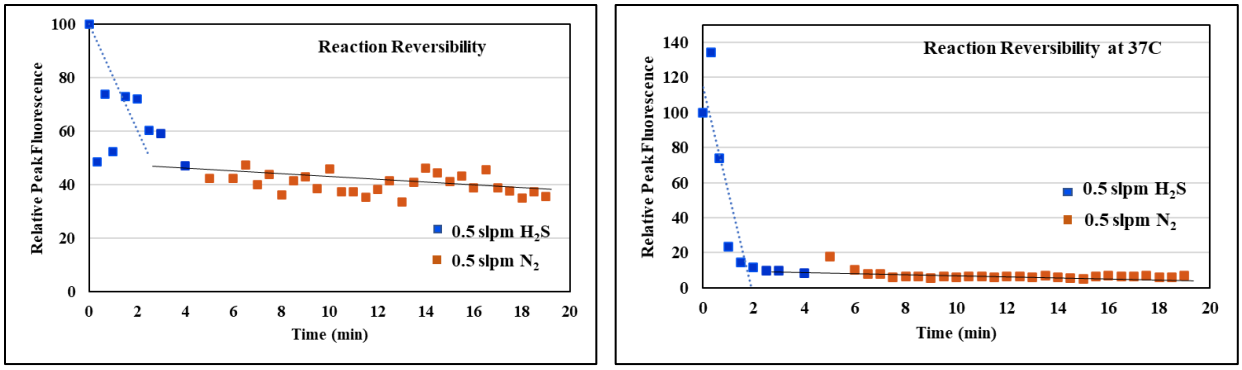


Figure 5: (a) Graph of peak emission intensity against time obtained by passing 0.5 SLPM H_2S gas through the biosensor solution for the first 4 mins followed by 0.5 SLPM N_2 gas for the final 15 mins (b) Same experiment condition but with temperature adjustment from ambient to 37°C

Additional experiments are planned to take advantage of Le Chatelier's principal by introducing additional fluorophore (1-AMA) in a bid to regenerate the biosensor. According to Le Chatelier's principal, if a dynamic equilibrium is disturbed by changing the conditions (pressure, temperature, or concentration), the position of equilibrium shifts to counteract the change to reestablish an equilibrium. In the case of a truly reversible reaction, adding more product, i.e., fluorophore 1-AMA, may shift the reaction to the left and regenerate the protein-fluorophore bond again as shown in Figure 6. Exploring this idea will shed light on whether it is possible to regenerate the biosensor.

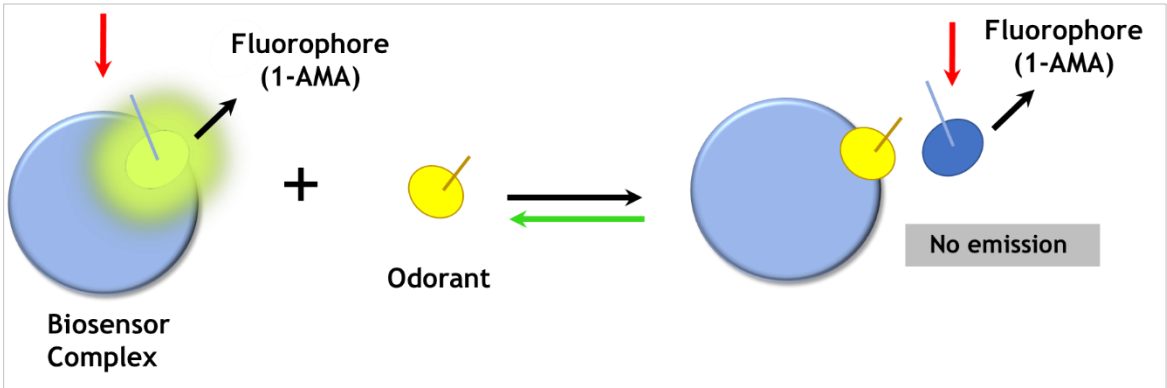


Figure 6: Adding more 1-AMA at the reaction equilibrium will favor more reactant formation (protein-fluorophore complex) if the reaction is reversible

TASK 5. Develop recommendations and prepare publication materials. S. Rahman has already prepared a manuscript and is in the process of submitting it to an Elsevier journal.

Upcoming Research Tasks

TASK 1. Perform protein sensitivity experiments on an expanded list of pure odorant compounds. Will be performed after completion of Task 3 to test the prototype flow-through reaction vessel.

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. The connection of the flow cell with the entire system will be established. To that extent, connecting tubes, unions and flowmeter have been purchased.

TASK 4. Perform experiments to explore protein-odorant reaction reversibility. Further experiments applying Le Chatelier's principal will be conducted accordingly.

TASK 5. Develop recommendations and prepare publication materials. We plan to submit the manuscript for peer review.

TAG Meetings

- Date of meeting: March 29, 2022

- Names of the participants: Daniel Meeroff, David Binninger, Craig Ash, Nathan Mayer, Masoud Jahandar Lashaki, Sharmily Rahman, Patrick Carroll, Damaris Lugo - BCSD, Daniel Courcy, Mateja Vidovic Klanac, Monica Mejia, Myles Clewner, Rakib Ahmed Chowdhury, Richard Meyers, Bishow Shaha, David Bromfield, Wanda Parker, David Dalton, Joseph Ullo, Jane Gregory, Samuel Levin.

- List of TAG members unable to attend this meeting: Owrang Kashef (CDM): Ravi Kadambala (CDM), Jeff Roccapriore (WM), André McBarnette (Stantec), Dan Schauer (Geosyntec), 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)

- Video Link and Minutes: TAG 1 (video)

PROJECT METRICS:

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

Last name, first name	Rank	Department	Professor	Institution
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 is in preparation "Developing a biosensor for objectively quantifying landfill odors" to submit 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

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

TAG Member List:

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Catherine Vanyo (Brown and Caldwell)
Sally Gordon (King County, WA)
Bishow Shaha (Geosyntec)

Project Website:

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