**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 3 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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[1] Professor & Associate Chair, Dept. of Civil, Environmental & Geomatics Engineering, Florida Atlantic University, 777 Glades Road, 36/206, Boca Raton, FL 33431-0091, Phone: (561) 297-2658, E-Mail: dmeeroff@fau.edu

[2] Research Assistant and Ph.D. candidate, Dept. of Civil, Environmental & Geomatics Engineering, Florida Atlantic University, E-Mail: rahmans2018@fau.edu

**QUARTERLY PROGRESS REPORT**
(January 1, 2022 - February 28, 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#Biosensor
**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. Once the new experimental setup (explained in Task 3 of our proposal) is established, S. Rahman will use this batch of 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. The experiments will then be expanded to include the expanded list of pure odorants planned out for this task.

**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.** Previous experiments used a Horiba Jobin Yvon spectrofluorometer, which 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, while obtaining real-time fluorescence measurements. FAU Lab.EES obtained separate funding to procure a high-performance, sensitive, flow-through spectrofluorometer, QEPRO-FL from Ocean Insight (Figure 1a) to allow miniaturization of the experimental setup and allow real-time fluoroscopic measurements to improve accuracy of measuring the concentration-dependence relationship. This specialized piece of equipment was ordered in February at a cost of $23,000. The cuvette used in this spectrofluorometer placed in a specialized cuvette holder (Figure 1b) can itself be used as the reactor chamber. This modified setup will allow a constant flow of odorant gases to be directed to the chamber containing the biosensor to provide real-time fluorescence data. This advancement will take the technology one step closer towards the ultimate goal of being able to deploy 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. The device is scheduled to be delivered in 6-8 weeks.


(a) (b)

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

**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. Initially it was assumed 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 2). However, initial experiments indicate that full regeneration may take longer than initially anticipated. Therefore, S. Rahman is currently preparing to conduct an experiment in which the regeneration period is extended from 4 minutes to 15 minutes using the previous reaction chamber setup. Then she will repeat the experiment for the miniaturized flow-through configuration to determine the regeneration time in the field.

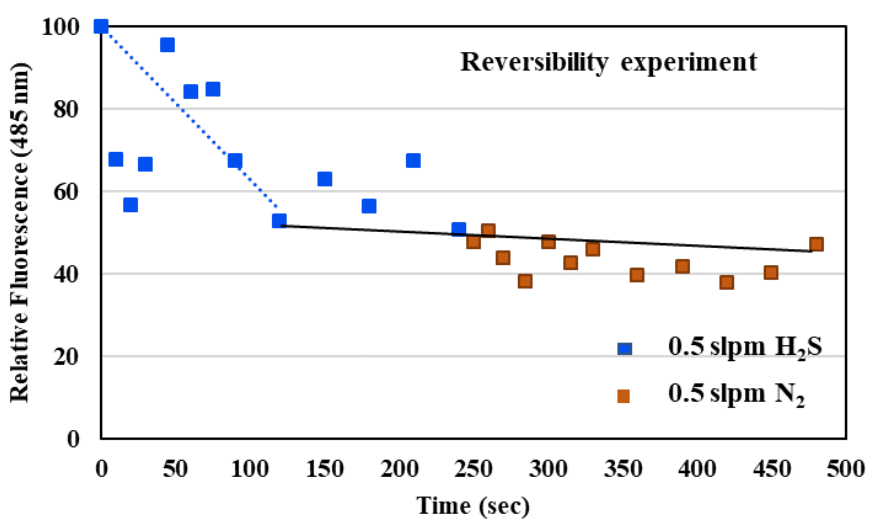


Figure 2: Graph of peak emission intensity against time obtained by passing 0.5 SLPM $H_2S$ gas through the biosensor solution for the first 240 seconds followed by 0.5 SLPM $N_2$ gas for the final 240 seconds

**TASK 5. Develop recommendations and prepare publication materials.** S. Rahman has already prepared a manuscript composed of the Year 1 findings 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.
**TASK 2. Perform protein sensitivity experiments on landfill gas mixtures.** Will be performed following Task 1.
**TASK 3. Upgrade the reactor chamber as a flow-through system for improved real-time result accuracy.** As soon as the spectrofluorometer is obtained, the S. Rahman will move forward with Tasks 1 and 2.
**TASK 4. Perform experiments to explore protein-odorant reaction reversibility.** Literature review will be continued to explore possible options and experimentations will be conducted accordingly.
**TASK 5. Develop recommendations and prepare publication materials.**

## TAG Meetings

No TAG meetings were conducted during this reporting period. The initial TAG meeting is being planned.

## 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**

Owrang 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