SUMMARY:
DEVELOPMENT OF A BIOSENSOR FOR MEASURING ODORANTS IN THE AMBIENT AIR NEAR SOLID WASTE MANAGEMENT FACILITIES

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In 2017, the Bill Hinkley Center for Solid and Hazardous Waste Management funded FAU Lab.EES to develop a novel biosensor technology that has the potential to objectively and rapidly measure odor concentrations in real-time, transforming how nuisance odors are monitored and regulated. This is a follow up study to a project entitled, "*Investigation of effective odor control strategies*" that was completed in 2017 that in part studied ways to improve odor detection including development of a novel technology that uses human odorant binding proteins as a biosensor to quantify odors.

Nuisance odor levels produced by solid waste management operations such as landfill facilities are subject to regulatory standards because of their impacts on the quality of life of the residents living within close proximity to the facility. Failure to meet such standards may result in costly fines, litigation, inability to acquire permits, mitigation, and re-siting operations. Since measurement of environmental nuisance odors is currently limited to subjective techniques, monitoring odor levels to meet such standards is often problematic. This is becoming more acute as increasing residential populations begin to encroach on properties adjacent to landfills. Odors can cause relations between the facility and the surrounding population to deteriorate. In order to ensure that nuisance odor issues are minimized, it is necessary to provide an objective measurement. However, until now, we did not have any objective methods of monitoring or recording nuisance odors. Moreover, there are usually a number of odorants that interact with each other, further complicating quantification.

The objective of the current research is to develop a biosensor for providing an objective, standard measurement of odors. Our approach will modify hOBP2A, a human odorant binding protein, isolated using published biomolecular techniques by either fluorescently tagging it with a chromophore functional group or a monoclonal antibody. Then the biosensors will be exposed to selected model odorants (single and mixtures) to determine positive/negative spectrophotometric response and concentration dependence/Beer's Law quantitation for a specific set of odorants typically encountered at solid waste facilities.

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PROGRESS REPORT
(December 2018)

Project Title: DETECTION OF NUISANCE ODORS USING ODOR BINDING PROTEIN SENSOR
Principal Investigator: Daniel E. Meeroff, Ph.D.
Co-Principal Investigator: David M. Binninger, Ph.D.
Affiliation: FAU
Phone number: (561) 297-2658
Project website: http://labees.civil.fau.edu/leachate.html
Student: Sharmily Rahman, MSCV Candidate

Methodology/Scientific Approach

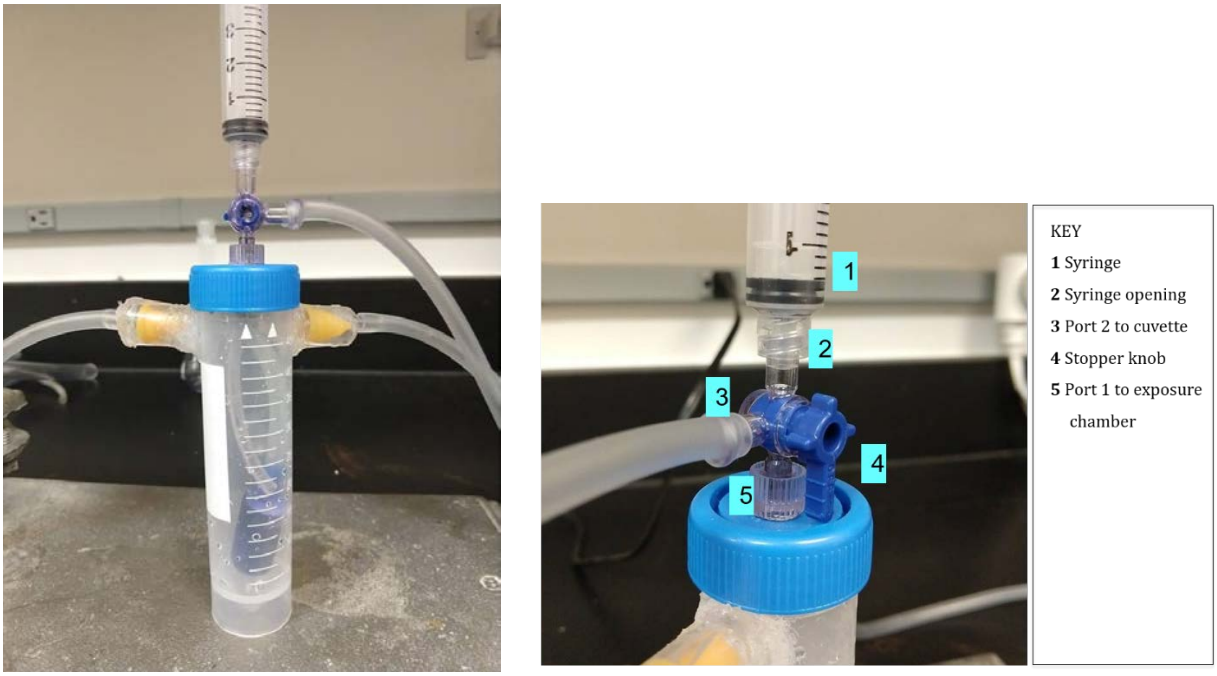
TASK 1. Conduct literature review. Sharmily Rahman continues to update the literature review focused on objective measurement techniques for detecting landfill odors with the main goals of: 1) identifying specific odor causing compounds in solid waste operations to create a database of odorant candidates for testing with several viable candidates found for testing; and 2) identifying literature that would improve the efficiency of the analysis technique proposed. A list of parameters has been identified which can improve said efficiency.

TASK 2. Prepare biosensor molecules. Dr. Binninger, is supervising the production of the first large batch of purified hOBP2A with the assistance of Yasmeen Amanza Ampuero and Cynthia Raaijmakers (graduate students in his laboratory). The research team has cultured and induced *E. coli* containing the human odor binding protein gene. The protein was then isolated from the batch and a Bradford assay was conducted to determine the presence of the target protein in the sample. After obtaining a positive result from the Bradford assay, a SDS-PAGE electrophoresis was conducted to confirm the presence of the protein. Currently, the team is in the final stages of purifying the protein using the SpinTrap TALON column of Dr. Scheurle in the FAU Department of Chemistry.

TASK 3. Develop an experimental exposure chamber. Sharmily Rahman is in the process of updating the prototype reactor. The following important changes have been made:

- The previous setup consisted of a reactor chamber that had a relatively large base. This meant that the exposed surface area of the protein complex was large and caused the odorant to escape fast. In the new setup (Figure 1), the reactor chamber has taken the form of a centrifuge tube. This has a twofold benefit. The first is that the exposed surface area of the protein complex has been reduced to limit the amount of odorant escaping from the surface. The second benefit comes from the increased length of the centrifuge tube, which should improve the likelihood of successful binding of the target odorant with the protein complex as it travels a greater distance to reach the surface. These changes in the design of the reactor chamber are expected to increase the efficiency of the process.
- In the previous setup, a pipette was used to collect samples from the exposure chamber for fluorometric tests. This created a number of issues. Collecting samples using a pipette by opening the cap of the sample bottle may have introduced external contaminants. In the new setup, a 3-way stopcock is attached to the cap of the centrifuge tube. One of the ports (shown as #2 in

Figure 1b) is only opened for sample collection using a syringe, which can be screwed on using a Luer-lok mechanism to draw a sample of the solution from the exposure chamber. The other port (shown as #3 in Figure 1b) is used to direct the sample already in the syringe into the cuvette for subsequent fluoremetry readings. This setup ensures that the inside of the reaction chamber is never exposed to the external environment, thus eliminating the introduction of any external contaminants. Also, the syringe never needs to be removed during the whole experiment which makes it very convenient. However even if we need to remove the syringe for any reason, the opening has a cap which ensures that it remains sealed.



(a) (b)

Figure 1: (a) Revised reactor chamber using a centrifuge tube **(b)** 3-way stopcock used at the mouth of the exposure chamber. The different ports and other parts are labeled.

S. Rahman has already completed her training for laboratory safety as well as training on the Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (Figure 2) and FluorEssence software interface used to read fluorescence emission needed for testing in Dr. Deguo Du's lab in the FAU Department of Chemistry.

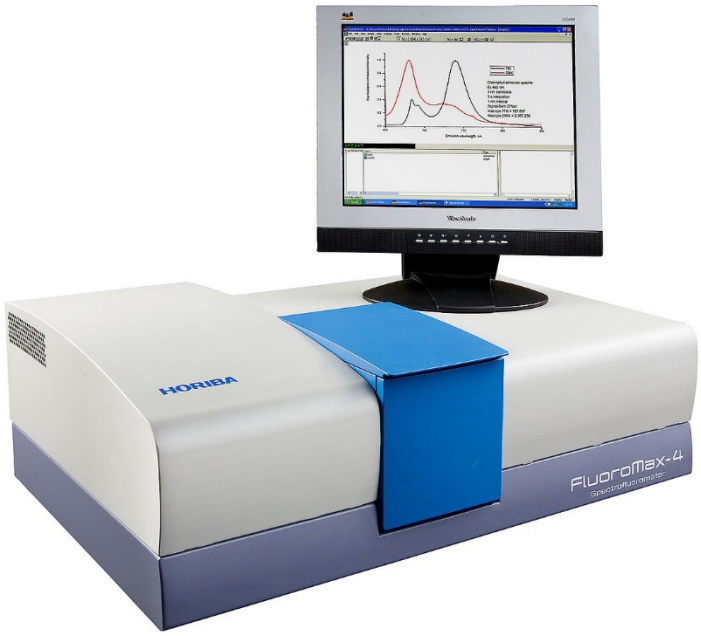


Figure 2: Horiba Jobin Yvon FluoroMax-4 spectrofluorometer and FluorEssence software interface *(image from Horiba website)*

TASK 4. Perform protein sensitivity experiments on model compounds. H_2S, NH_3 and N_2 gas cylinders have already been procured for experimentation with the biosensor complex. These gases will be used as model compounds. 1-aminoanthracene (1-AMA), which is an intrinsic fluorophore, is being used in conjunction with hOBP2A to quantify the amount of odorant binding with the protein. The 1-AMA was obtained from Sigma Aldrich in powder form. Since it is hydrophobic, 10% methanol was used in dissolving the powder.

S. Rahman is preparing to replicate the previous protein sensitivity experiments conducted by Julia Roblyer with H_2S. For now, the optimal ratio of hOBP2A to 1-AMA will be maintained at 1:1. The accuracy of the calibration and quantitation range will be checked. As before, the aqueous protein-AMA complex will be placed in the reaction chamber. A Y-connector will connect the gaseous cylinder and a flowmeter with a one-way valve at the top of the chamber. A pipe leads from the valve into the chamber and ends in an aquarium-grade, pumice stone bubbler, which increases the surface area of solution exposed to the odorant. The odorant will escape through the top of the chamber by means of a second one-way valve. Fluorescence spectra will be obtained using a Horiba Jobin Yvon FluoroMax-4 spectrofluorometer to reveal the relationship between fluorescence and odorous gas concentration.

The revised setup (Figure 3) was tested using an air pump to check whether it works and remains sealed as expected.

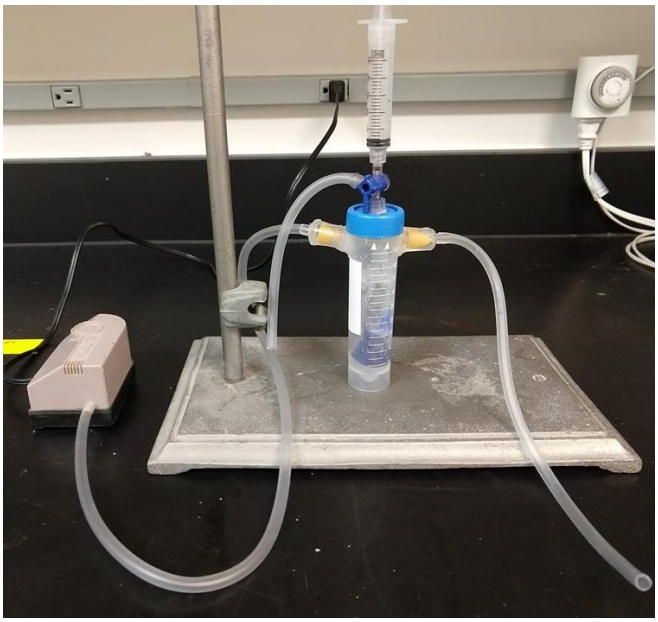


Figure 3: Revised reactor chamber being tested using an air pump. Air was successfully bubbled through a sample of water using a stone bubbler and exited through the valve at the top of the chamber.

TASK 5. Perform protein sensitivity experiments on mixtures. S. Rahman will compare the results and also check whether the protein shows a binding affinity only towards H_2S or whether the same applies to a mixture of "standard landfill gas," containing H_2S, CO_2, NH_3 and N_2 to confirm the Beer's Law relationship in the presence of other odorants. She will also check the binding capacity of the biosensor complex when exposed to acidic and basic odorants.

Upcoming Research Tasks

- **TASK 1. Conduct literature review.** Continue to update the literature review.
- **TASK 2. Prepare biosensor molecules.** The buffer for isolating the protein is yet to be ordered. Once it is obtained by the research team at Dr. Binninger's lab, they would isolate the protein and hand it over to S. Rahman. S. Rahman will then convert them to the biosensor molecules using the fluorescent tagging markers.
- **TASK 3. Develop an experimental exposure chamber.** The new prototype would be tested to see whether it functions as expected and changes would be implemented if problems arise.
- **TASK 4. Perform protein sensitivity experiments on model compounds.** Once the protein is obtained, H_2S would be used as a model compound and the previous experiment run by Julia Roblyer would be verified. The optimal ratio of the protein and 1-AMA would be verified by testing with different mixtures of protein and fluorophore. The system would be exposed to higher concentrations of H_2S and more trials would be conducted.
- **TASK 5. Perform protein sensitivity experiments on mixtures.** After experimenting on model compounds, S. Rahman would analyze the results obtained from mixtures of odorants.
- **TASK 6. Develop recommendations and preliminary cost analysis.**
- **TASK 7. Prepare publication materials.**

PROJECT METRICS:

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

Last name, first name	Rank	Department	Professor	Institution
Rahman, Sharmily	MSCV Candidate	CEGE	Meeroff	FAU
Ampuero, Yasmeen	MS Biology Candidate	BIO	Meeroff, Binninger	FAU
Raaijmakers, Cynthia	MS Biology Candidate	BIO	Meeroff, Binninger	FAU

2. List undergraduate researchers working on **THIS** Hinkley Center project.

Last name, first name	Department	Professor	Institution

3. List research publications resulting from **THIS** Hinkley Center project.

None yet

4. List research presentations resulting from **THIS** Hinkley Center project

None yet

5. List research papers that have cited any publications (or the final report) resulting from this Hinkley Center project (use format for publications as indicated in the Hinkley Center Investigators Guide).

None so far

6. List additional research funding that has been secured due to leveraging the research results from this Hinkley Center project (give project title, funding agency, amount of funding, award date, and award period)

Year two funding from the Hinkley Center for Solid and Hazardous Waste Management was secured. "Development of a biosensor for measuring odorants in the ambient air near solid waste management facilities (this project)," Hinkley Center, $50,487. 12/01/2017 (delayed project start to 08/01/2018) – 05/31/2019.

Additional funding was secured from the Environmental Research and Education Foundation, "*Detection of nuisance odors using odor binding protein sensor*," Environmental Research and Education Foundation (EREF), $150,000. 12/01/2017 – 11/30/2019.

7. List submitted proposals which leverage the research results from this Hinkley Center project (give the proposal title, funding agency, requested funding, date submitted)

None yet

8. List new collaborations initiated based on this Hinkley Center project

- Deguo Du, Assistant Professor, Chemistry, FAU is allowing us to use his sophisticated fluorometry equipment for this project.
- Dr. Daniela Scheurle, Coordinator for Academic Support Services, Chemistry, FAU is allowing us to use her SpinTrap TALON column to purify the protein.

9. How have the results from this Hinkley Center funded project been used (not will be used) by the FDEP or other stakeholders in the solid waste field? Please note that the term "other stakeholders" is meant to broadly include any party or practitioner in the solid waste field. This includes county solid waste directors and their staff, municipal solid waste directors and their staff, solid waste facility design engineers, local/county/city solid waste management regulatory staff, federal solid waste regulatory staff, landfill owners and operators, waste haulers, waste to energy plant owners and operators, recyclers, composting plant owners and operators, yard waste operators, construction and demolition debris companies and organizations, county recycling coordinators, citizens and members of the academic community, etc. (1 paragraph maximum)

To date, the results have not been used by stakeholders yet.

TAG members:

Mark Eyeington, Mark Maclean, Mark Bruner, Owrang Kashef, D.V. Reddy, Craig Ash, Ravi Kadambala, Ron Schultz, Jeff Roccapriore, André McBarnette, Dan Schauer, Damaris Lugo, Amanda Krupa, Richard Meyers, Amede Dimonnay, Art Torvela, Ted Batkin

TAG meetings:

October 19, 2018 (Joint TAG meeting held at SWA in conjunction with UM)