

SUMMARY:
INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES
Daniel E. Meeroff (PI)¹
Julia Roblyer and Mateja Vidovic

In 2015, the Bill Hinkley Center for Solid and Hazardous Waste Management funded FAU Lab.EES to find ways to improve and standardize odor identification, evaluate additional methods to establish reasonable, objective standards for odor severity, and explore other options for mitigation and detection including a novel technology that will attempt to use human odorant binding protein to quantify odors. Areas of application include policy development, land use strategic planning, odor regulation, complaint assessment, odor impact assessment, odor master planning, odor control efficiency assessment, and process design.

Nuisance odor levels produced by solid waste management operations such as landfill facilities, wastewater treatment plants and confined animal feeding operations are subject to regulatory standards because of their impacts on the quality of life of the public living within range. 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.

The objective of the proposed research is to develop a standardized, non-subjective measurement of nuisance odors using human odorant binding protein 2a (OBP2A) or similar analog. Since OBP2A binds a wide range of odorants, it may be used singularly as an odorant detection method for municipal solid waste facilities whose odors are caused by a vast array of chemicals in varying proportions.

The OBP2A will be synthesized and isolated using standard laboratory methods. Following isolation, OBP2A will be labeled with fluorescent markers to indicate when odorant molecules have been bound to the protein. After fluorescent marking, OBP2A will be exposed to known odorants within a vacuum chamber. Fluorescence will be measured using a fluorometer and analyzed for fluorescence – concentration responses during odorant binding. If the relationship follows Beer's Law, then concentrations of odorants can be accurately determined using fluorometric measurements.

As a starting point, the fluorescently tagged OBP2A will be exposed to model compounds that generate specific responses in human olfactory cells such as formic acid and dimethyl disulfide, detected at concentrations as low as 0.1 ppm, to determine a positive response and concentration dependence.

¹ Prof., Dept. of Civil, Environmental & Geomatics Engineering, Florida Atlantic University, 777 Glades Road, 36/206, Boca Raton, FL 33431-0091, Phone: (561) 297-3099, E-Mail: dmeeroff@fau.edu

PROGRESS REPORT

(February 2016)

Project Title: INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES

Principal Investigators: Daniel E. Meeroff, Ph.D.

Affiliation: FAU

Phone number: (561) 297-2658

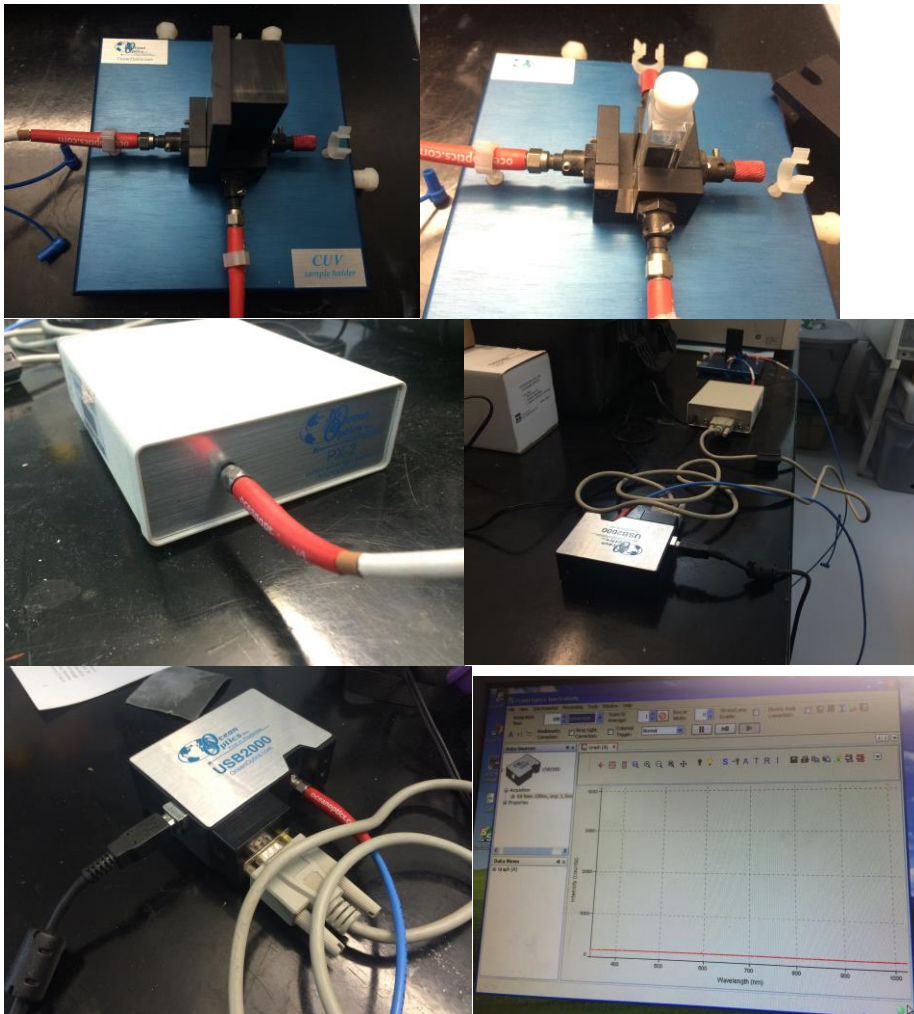
Project website: <http://labees.civil.fau.edu/leachate.html>

Students: Julia Roblyer, Mateja Vidovic

Methodology/Scientific Approach

- **TASK 1. Conduct literature review.** Mateja Vidovic and Julia Roblyer continue to conduct and update an exhaustive literature review focused on identifying sources of odor, non-subjective odor monitoring techniques, and methods of odor control including best odor management practices. So far, we have 1) identified specific odor causing compounds in solid waste operations and created a database of odorants; 2) identified factors that can impact the efficiency of data collection; and 3) examined case studies and best management practices for odor mitigation technologies for Palm Beach County.
- **TASK 2. Collect data on Florida-specific odor management strategies.** Dr. Meeroff and graduate students, Julia Roblyer and Mateja Vidovic, attended the FWEA air quality workshop in Boynton Beach, FL (February 11, 2016) to gather information about typical nuisance odorants, methods of sample collection and measurement, challenges of odor control, and methods of odor mitigation. Information sessions were provided by Dick Pope, Robert Bowker, Philip Wolstenholme, Chris Hunniford, and Bruce Singleton. Multiple facilities in Palm Beach County were visited to observe existing odor control applications in the field.
- **TASK 3. Pattern identification and trend analysis.** Craig Ash and Jim Christiansen of Waste Management Inc. of Florida have scheduled a meeting for the week of March 1 to discuss odor response data from partner landfills located in an urban setting. Using appropriate qualifiers for meteorological measurements and landfill operations, the data will be analyzed to determine the existence of patterns or trends that could lead to the development of important parameters with respect to effective management strategies.
- **TASK 4. Perform protein sensitivity experiments.**
 1. The Ocean Optics Spectrometer was configured for future fluoroscopy of fluorescently marked OBP2A as it binds odorants. Updated software called SpectraSuite was acquired and advanced operator training was conducted with the vendor. <http://www.spectroscopytv.com/the-basics-of-spectrasuite/>
<http://oceanoptics.com/wp-content/uploads/px-2.pdf>

Photos of OceanOptics Spectrometer and accompanying SpectraSuite software interface:



2. The research team met with Dr. Binniger, Biology Professor at FAU, regarding cloning, synthesis and expression of OBP2A. He recommended contacting other researchers who have previously synthesized OBPA2A to request a clone containing the amino acid sequences to use as a target for PCR amplification either in bacterial form with plasmid or DNA, which then can be transformed into *E. coli*, or CDNA sequence from open reading frame for PCR template, and the expression vector. The key component is the piece of DNA that encodes the amino acid sequence. This should be the nucleotide sequence that encodes just the amino acid sequence of OBPAII:

Nucleotide Sequence (513 nt):

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ATGAAGACCCTGTTCCCTGGGTGTCACGCTCGGCCTGGCCGCTGCCCT
GTCCTTCACCCTGGAGGAGGAGGATATCACAGGGACCTGGTACGTG
AAGGCCATGGTGGTCGATAAGGACTTTCCGGAGGACAGGAGGCC
AGGAAGGTGTCCCCAGTGAAGGTGACAGCCCTGGGCGGTGGGAAC
TTGAAGCCACGTTACCTTCATGAGGGAGGATCGGTGCATCCAGA
AGAAAATCCTGATGCGGAAGACGGAGGAGCCTGGCAAATTCAGCG
CCTATGGGGGCAGGAAGCTCATATACCTGCAGGAGCTGCCCGGGAC
GGACGACTACGTCTTTTACTGCAAAGACCA

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GCGCCGTGGGGGCCTGCGCTACATGGGAAAGCTTGTGGGTAGGAAT
CCTAATACCAACCTGGAGGCCCTGGAAGAATTTAAGAAATTGGTGC
AGCACAAGGGACTCTCGGAGGAGGACATTTTCATGCCCTGCAGAC
GGGAAGCTGCGTTCTCGAACACTAG

Translation (170 aa):

MKTLFLGVTLGLAAALSFTLEEEEDITGTWYVKAMVVDKDFPEDRRPR
KVSPVKVTALGGGNLEATFTFMREDRCIQKKILMRKTEEPGKFSAYGG
RKLIYLQELPGTDDYVFYCKDQRRGGLRYMGKLVGRNPNTNLEALEE
FKKLVQHKGLSEEDIFMPLQTGSCVLEH

<https://www.ncbi.nlm.nih.gov/CCDS/CcidsBrowse.cgi?REQUEST=CCDS&DATA=CCDS6992>

The DNA sequence that would be inserted into an expression vector could come from a clone that could be obtained from a lab that has been working on this gene or it may be feasible to have it synthesized. Then we have to decide on the expression vector to use (i.e. *E. coli* or a eukaryotic host). FAU has experience with a vector called pGEX for expression in *E. coli*.

3. Roblyer contacted several researchers who have synthesized OBPA2A and published findings in the scientific literature. Dr. Loic Briand, Research Director of the Center for Taste and Feeding Behaviour in Dijon, France said he has the OBP2A cDNA sequence cloned in bacteria with *Pichia pastoris* expression vectors and agreed to send it by airmail. This clone is currently en route to FAU. Artur Ribeiro, Professor of Biological Engineering at the University of Minho in Braga, Portugal said he would send the wild type of their running OBP in a dried filter paper form. This clone is currently en route to FAU, as well. Dr. Chelsea Smartt, Associate Professor of UF's Florida Medical Entomology Laboratory says she has a partial odorant gene isolated but it is from the mosquito, *Culex nigripalpus*. We are focusing on human odorant binding protein but requested the mosquito protein clone to possibly use as a control. She says she has attempted to express other mosquito genes but has never tried with the odorant gene. She said she has expressed proteins in *E. coli* and has obtained proteins that formed crystals so she believes using *E. coli* is a great place to start.

Upcoming Research Tasks:

- **TASK 1. Conduct literature review.** Continue to update the literature review.
- **TASK 2. Collect data on Florida-specific odor management strategies.** Continue to update the database.
- **TASK 3. Pattern identification and trend analysis.** Meet with representatives from Waste Management, collect data, and analyze data.
- **TASK 4. Perform protein sensitivity experiments.** Upon arrival of OBP2A clone, synthesize and express the protein. Design and build or purchase a vacuum chamber or other appropriate chamber in which to expose fluorescently marked OBP2A to volatile odorants individually and then from a field sample of solid waste odorants. Calibrate and test spectrometer to ensure proper functioning.
- **TASK 5. Assess odor mitigation strategies.**

- **TASK 6. Develop recommendations and preliminary cost analysis.**
- **TASK 7. Prepare publication materials.**

Project Metrics

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

Last name, first name	Rank	Department	Professor	Institution
Julia Roblyer	MSCE candidate	CEGE	Meeroff	FAU
Mateja Vidovic	MSCE Candidate	CEGE	Meeroff	FAU

2. List undergraduate student/researchers working on this Hinkley Center project

Last name, first name	Department	Professor	Institution
Katharine Mesa	Business	Meeroff	FAU

3. List research publications resulting from this Hinkley Center project (use format for publications as indicated in the Hinkley Center Investigators Guide).
None yet
4. List research presentations resulting from this Hinkley Center project (use format for listing presentations as indicated in the Hinkley Center Investigators Guide).
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 yet
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)
None yet
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

Dr. Binninger (FAU College of Science), Craig Ash and Jim Christiansen (Waste Management), Dick Pope (Hazen and Sawyer), Robert Bowker (Bowker and Associates), Philip Wolstenholme (Brown and Caldwell), Chris Hunniford (V&A Consulting Engineers), and Bruce Singleton (CDM Smith), Dr. Loic Briand, Research Director of the Center for Taste and Feeding Behaviour in Dijon, France, Artur Ribeiro, Professor of Biological Engineering at the University of Minho in Braga, Portugal, and Dr. Chelsea Smartt, Associate Professor of UF's Florida Medical Entomology Laboratory.

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)

None yet to our knowledge

TAG Members:

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