SUMMARY:
INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES

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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.

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PROGRESS REPORT
(May 2016)

Project Title: INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES
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Project website: http://labees.civil.fau.edu/leachate.html
Students: Julia Roblyer, Mateja Vidovic

Methodology/Scientific Approach

- **TASK 1. Conduct literature review**. Mateja Vidovic is continuing to conduct and update an exhaustive literature review focused on identifying sources of odor in landfills, non-subjective odor monitoring techniques, and methods of odor control including best odor management practices. To date, databases have been created and are being constantly updated with: 1) lists of specific odor causing compounds in solid waste operations; 2) lists of parameters that can impact the efficiency of data collection; 3) lists of parameters which have the greatest influence on creating and spreading of nuisance odors; 4) lists of odor monitoring technologies that are used in solid waste operations; and 5) lists of case studies and best management practices for odor mitigation technologies.
- **TASK 2. Collect data on Florida-specific odor management strategies.** The strategy of this study is to target partner landfills located in an urban setting, therefore, several solid waste management facilities in those locations have been contacted in order to collect data about odor complaints. So far, data has been provided by the Solid Waste Authority of Palm Beach County as well as the real-time access to their weather station has been approved. Meteorological parameters such as, temperature, wind speed, wind direction, precipitation accumulation and pressure can be monitored via a wireless connection. Also, the meteorological data from previous years can be retrieved as well. A meeting to secure data from Monarch Hill is scheduled for June 2016.
- **TASK 3. Pattern identification and trend analysis.** A meeting with Craig Ash, Jeff Roccapriore and Jim Christiansen of Waste Management Inc. of Florida is planned for the first week of June 2016 to discuss odor response data as well as to propose an installation of a wireless weather station (or access to an existing one) from which every day meteorological parameters that have impact on the strength of malodorous odors can be monitored. Based on the odor complaints data received from the Solid Waste Authority of Palm Beach County and databases from Waste Management Inc. of Florida, as well as using appropriate qualifiers for meteorological measurements and landfill operations, the datasets can be analyzed to determine the existence of patterns or trends that could lead to the development of effective management strategies.
- **TASK 4. Perform protein sensitivity experiments**. Figure 1 represents the general sequence of events of the protein sensitivity experiments. The flow chart will be updated with more specific information as it becomes available. The bacterial expression plasmid was obtained, absorbed in filter paper from Artur Ribeiro, Professor of

Biological Engineering at the University of Minho in Braga, Portugal. The plasmid was eluted from the filter paper using tris-EDTA buffer in a heat sealable bag to maintain minimum volume of buffer. The buffer was allowed to saturate for 1 day in refrigeration at 4°C. The next step to isolate the plasmid is ethanol precipitation. Prior to ethanol precipitation, the eluted plasmid-tris-EDTA buffer solution was stored in deep freeze at -80°C.

Figure 1. Protein sensitivity experiment sequence

The drug selection marker on the bacterial expression plasmid is Kanamycin. We must obtain Kanamycin to proceed with cloning the plasmid into *E. coli* for expression (BL21, BLR) of the recombinant protein. There are several protocols for transformation, one of which is outlined in the "TSS Transformation Method." The specific type of vector used is pET-28a. The affinity tag on the vector is a Hexa-His Tag.

The method used to transform competent *E. coli* cells is as follows: The TSS "Transformation and Storage Solution" method is a combination of two steps. The first step is to obtain competent cells. The second step is to transform the cells resulting in transformation efficiency that goes up to the $107\text{-}10^8$ CFU (colony forming units) per microgram of DNA. This method was applied for the preparation of competent cells of the *E. coli* BL21(DE3) and BLR(DE3) expression strains (Silva et al. 2013).

A single colony, isolated and grown in a LB-agar plate, is used to inoculate 10 mL of LB medium and is grown at 37ºC with shaking (250 rpm), until the media reaches an optical density (λ = 600 nm) of 0.3 – 0.4. At this point, the metabolism and cell growth is stopped by incubation on ice for 5 minutes. The cell suspension is centrifuged at 3000 rpm (1100G) for 10 minutes at 4ºC. The supernatant is discarded and the pellet is resuspended in 1 mL of cold x1TSS solution. About 1-10 ng of plasmid in final volume of 1-10 μL are added to the mix. The cellular suspension plus the plasmidic DNA are kept on ice for one hour and after that a heat shock is given to the cells by exposition of the mix at 42ºC for 2 minutes. The heat shock is stopped by immersion on ice for 2 minutes. After this, 1mL of warm LB is added and the suspension is incubated one hour at 37ºC with shaking (250 rpm) in order to activate and express the gene that confers resistance to the selective marker (antibiotic). Last, 50-200 μL of the transformation mix is plated on LB-agar plus the antibiotic plates that are incubated for 16-20 hours at 37ºC. The TSS (Transformation and Storing Solution) is prepared with LB with 10% (w/v) PEG Mw 3350, 5% (v/v) DMSO and 50 mM $MgCl_2$ (pH 6.5), sterilize by filtration. Figures 2 and 3 indicate restriction endonuclease sites used to clone the gene of interest into this vector.

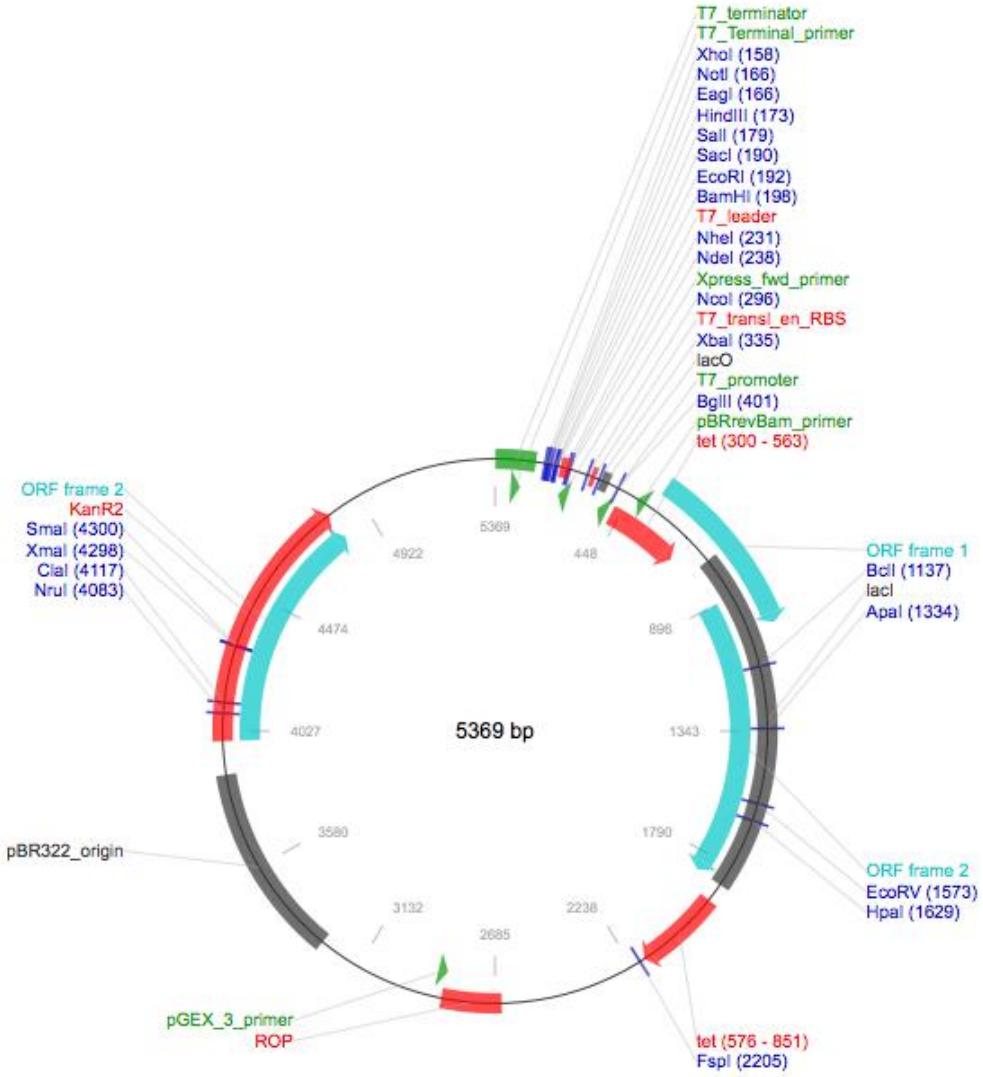

Figure 2. Generated plasmid map for pET28a vector.

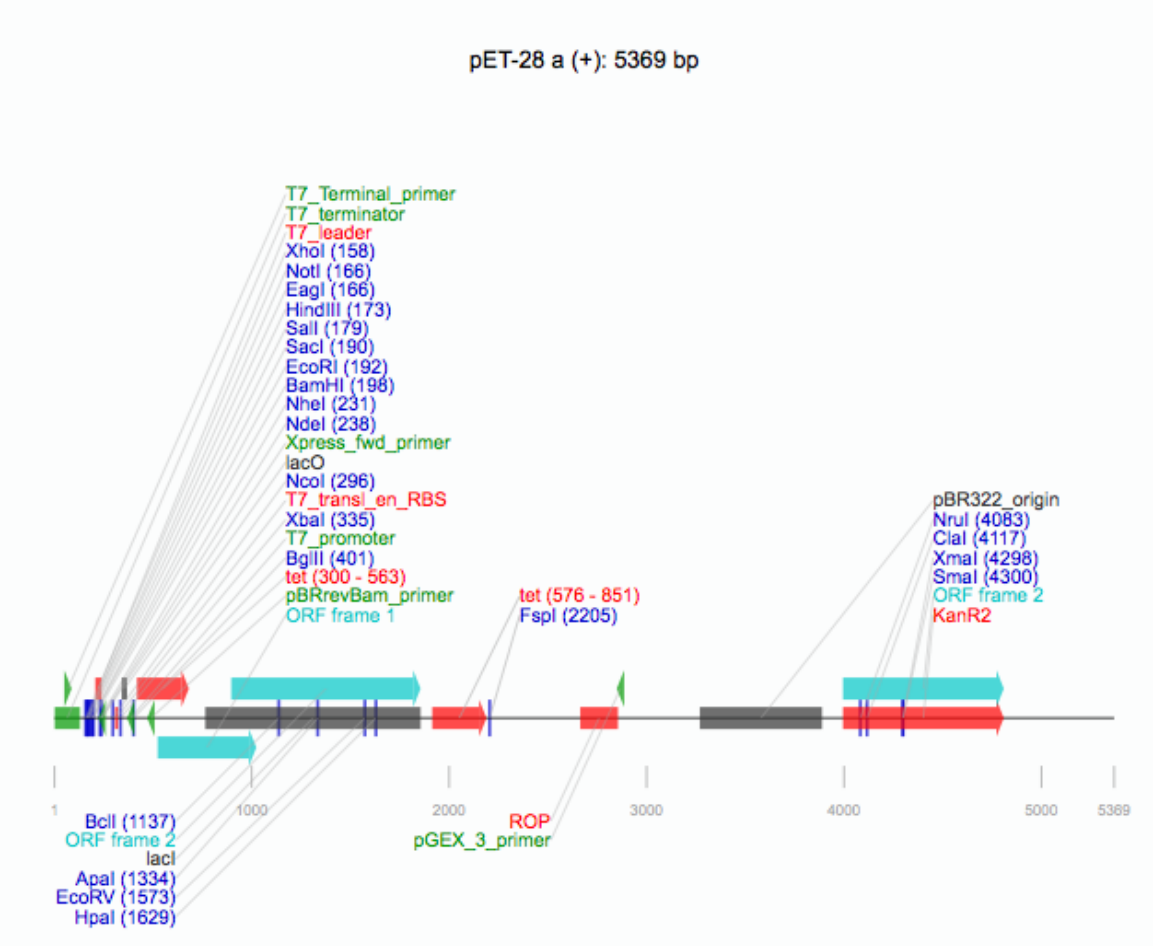

Figure 3. Linear map of analyze sequence: pET-28 a (+)

Plasmid where the gene is cloned: https://www.addgene.org/vector-database/2565/

Source/Vendor:	EMD Biosciences
Alt Name:	pET28a
Analyze:	Sequence
Plasmid Type:	Bacterial Expression
Expression Level:	High
Cloning Method:	Unknown
Size:	5369
5' Sequencing 1 Primer:	T7 Fwd
5' Sequencing 1 Primer Sequence:	5'd[TAATACGACTCACTATAGGG]3'
Tag 1:	His (Nterm and Cterm)
Bacterial Resistance:	Kanamycin
Notes:	Nterm thrombin cleavage site; a,b,c vary by MCS
Catalog Number:	69864-3
Stable:	Transient

Constitutive:	Constitutive
Viral/Non-Viral:	Nonviral

Following synthesis of odorant binding protein, a fluorescent marker will be attached to the protein. The marker will fluoresce when the protein is bound to an odorant and will be visualized with a fluoroscopic spectrometer. The protein will be exposed to pure odor gases such as are common to solid waste facilities, hydrogen sulfide and mercaptins and those common to algal blooms, dimethyl sulfide. The protein will be exposed to these gases using the FIA Series Fluorescence Flow Cell in Figure 4 such that the gas will be able to flow through a chamber containing the liquid protein and simultaneously be observed through fluorescent spectroscopy. It will likely be the flow cell used in combination with our fluorometer to detect odorant binding protein bound to odorants. FIA fluorescence flow cells combine flow injection analysis with optical components for fluorescence. A fiber sends excitation energy through a fused silica window into the sample compartment. Emitted energy is collected by a second fiber, oriented at 90 degrees, that connects to an Ocean Optics spectrometer configured for fluorescence. (http://oceanoptics.com/product/fia-series-fluorescence-flow-cells/).

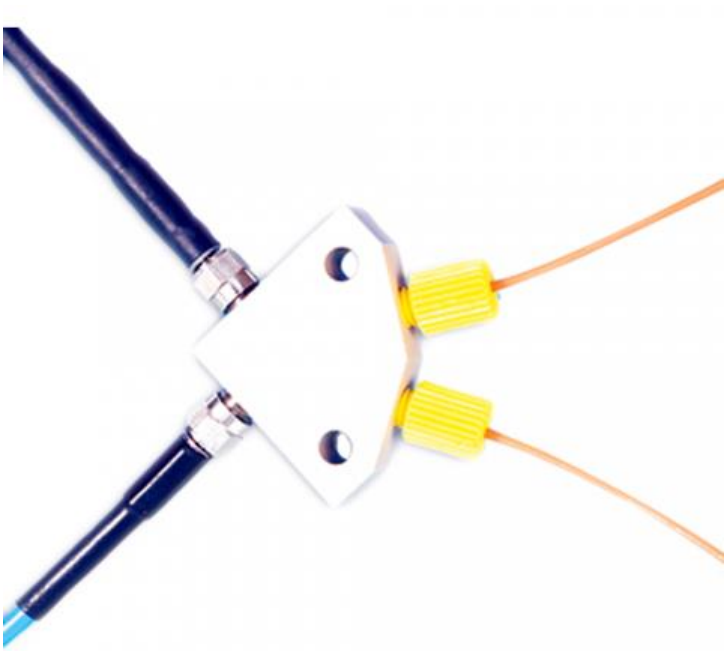

Figure 4. FIA series fluorescence flow cell is a chemically resistant cell for fluorescence applications made by Ocean Optics

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. Participate in odor data collection surveys with landfill personnel, with permission.
- **TASK 3. Pattern identification and trend analysis.** Meet with representatives from Waste Management, collect data, and analyze data.
- **TASK 4. Perform protein sensitivity experiments**. Procedures to clone, synthesize and express the protein are underway. 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 Asoociates), 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 Roccapriore, André McBarnette, Dan Schauer, Damaris Lugo, Amanda Krupa, Richard Meyers, Amede Dimonnay, Art Torvela, Ted Batkin, Roshan Jachuk, Fred Bloetscher