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.

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PROGRESS REPORT
(December 2016)

Project Title: INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES
Principal Investigators: Daniel E. Meeroff, Ph.D.
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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 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. Another meeting with Craig Ash and Jeff Roccapriore of Waste Management Inc. of Florida was organized on September 19, 2016, and historical data sets have been collected. Installation of a wireless weather station (Figure 1) provided by FAU iSENSE has been arranged with Mr. Jeff Roccapriore and was installed by Mr. Chancey Kelley from FAU on October 21, 2016 (Figure 2-3). Meteorological data started coming on November 2, 2016. It provides information about wind direction, wind speed, temperature, humidity, pressure, precipitation intensity, etc.

Figure 1. Mr. Chancey Kelley installing the weather station at Monarch Hill landfill site

Figure 2. Mr. Jeff Roccapriore and Mr. Chancey Kelley during installation of weather station

Figure 3. Installed weather station

The online ENVIROS system (Environmental Inquiry and Resource System) of Broward County will be reviewed as well, in order to collect more information on odor complaints to the county environmental protection unit. Mrs. Damaris Lugo has been contacted to assist with this endeavor, and she has agreed to assist the research team in

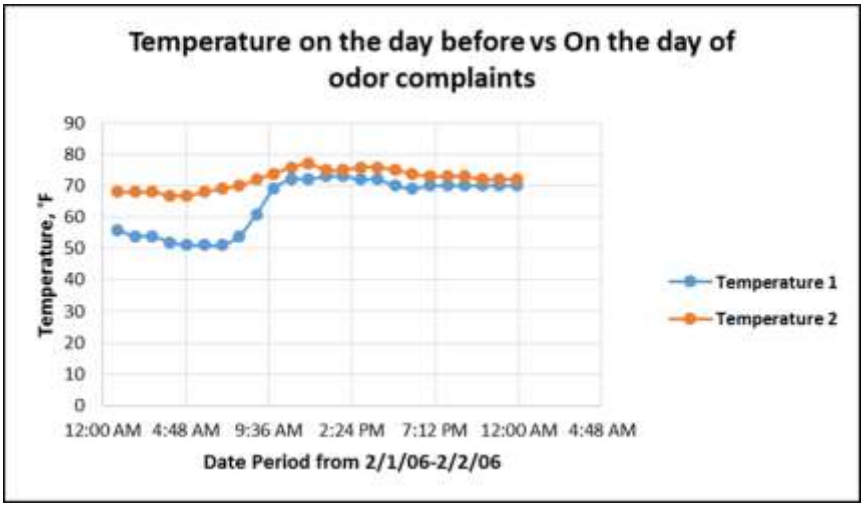
collecting this data for further analysis. She provided data sets on odor complaints for Monarch Hill Landfill site. Medley Landfill site has been also contacted but no success in collecting any usable data. Mateja Vidovic participated in odor data collection surveys on November 14, 2016 with landfill personnel Mr. Manglio with permission. Odor surveys started at 7am and four different points around the landfill were monitored: point 200 – Wiles Rd Banyon Pointe Entrance, point 360- Lyons Rd SW Corner in Promenade Parking Lot, point 500- Sample Rd Festival Flea market and point 720- 4640 Powerline Rd The Smoke Inn Cigars Parking Lot. The instrument used for measuring H_2S concentrations was Jerome 631, and meteorological data was retrieved from weather application. Mr. Manglio assisted in explaining how to use instrument GEM 5000 and how the concentration for methane from wells are collected using the same instrument. Also, Mr. Roccapriore provided data on solid waste operations occurred for the dates when 3 or more odor complaints were received in the same day. Another visit to Monarch Hill was set on November 21st, 2016 with Mr. Manglio. Four different sites on the fence line of the landfill were used to measure concentrations of H_2S with GEM 5000 (Figure 4) to see if the instrument is appropriate for measuring that specific odor compound. Concentrations were not accurate and for the next visit, team will borrow the Jerome 631 instrument from Monarch Hill personnel and measure the concentrations.

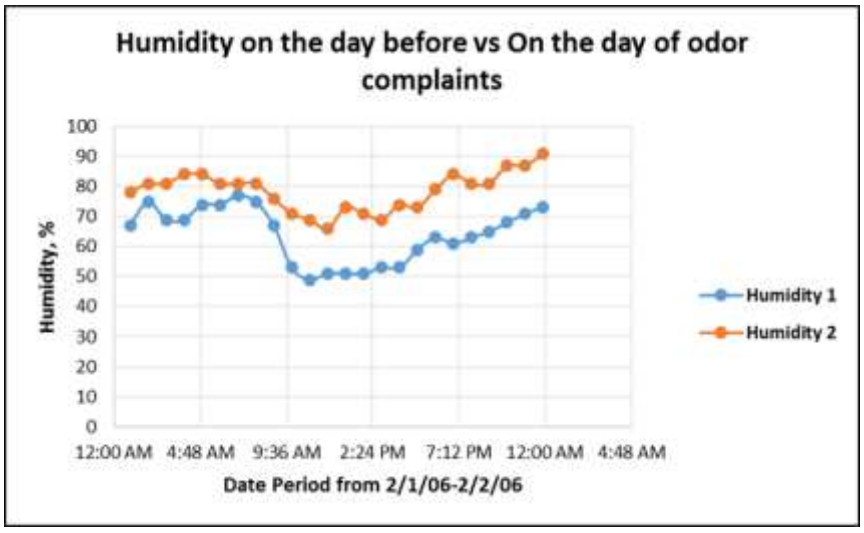
Figure 4. GEM 5000

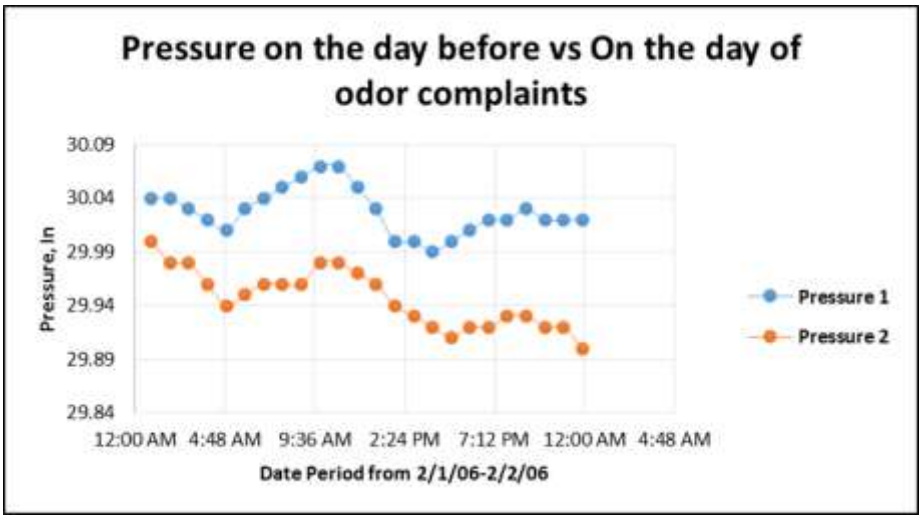
Figure 5. Mr. Manglio and Mateja Vidovic participating in odor survey

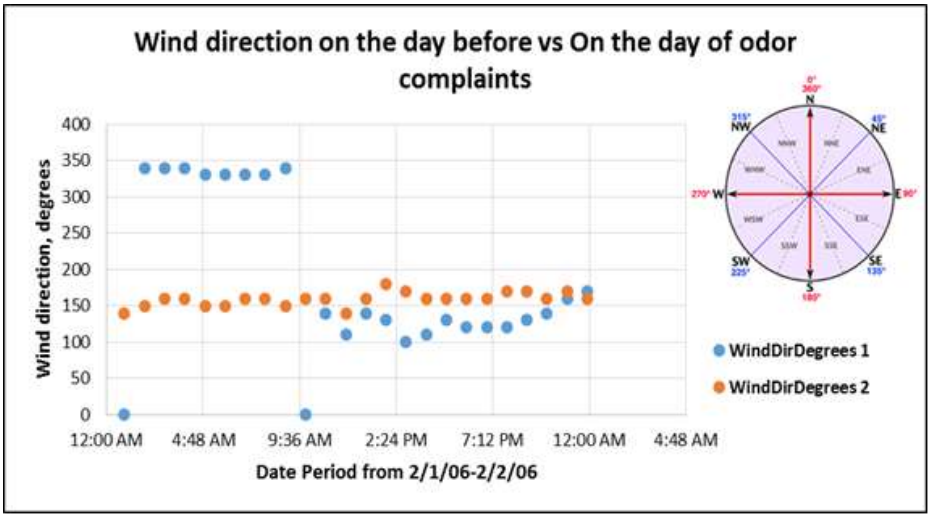
TASK 3. Pattern identification and trend analysis. After receiving the data from Monarch Hill personnel and Mrs. Damaris Lugo from Broward County on odor complaints, dates with three or more complaints in the same day were retrieved. The same was done for data provided from SWA of Palm Beach County. For the period from 2005-2016, Monarch Hill landfill had 17 days in total with 3 or more odor complaints in the same day. Data provided from SWA of Palm Beach County for the period of 2005-2015

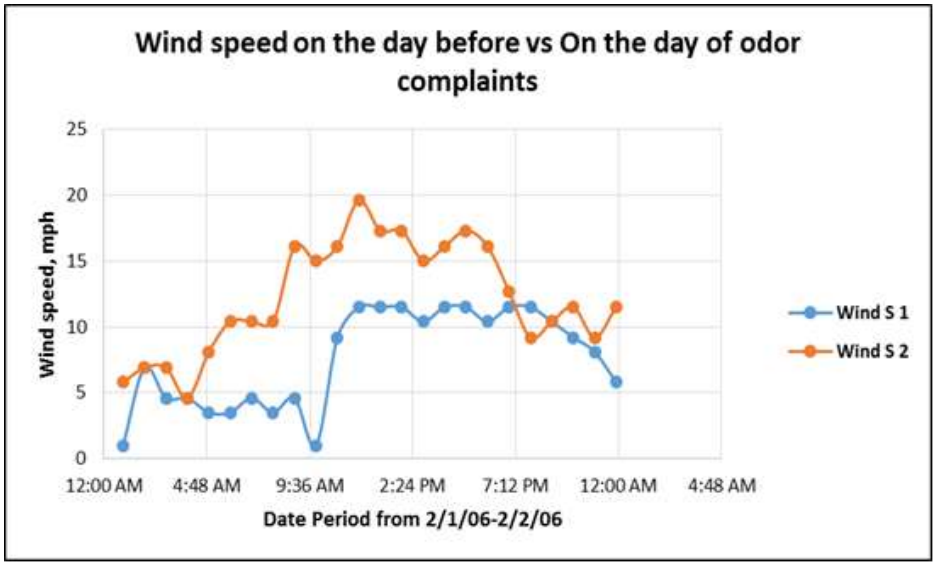
had a total of 25 days with 3 or more odor complaints in the same day. Meteorological data for both sites was collected from weather stations nearest to the location of sites for the day the odor complaint occurred as well as for the day before. Meteorological conditions taken into consideration are temperature, humidity, sea level pressure, wind direction, and wind speed. Data for each of the meteorological parameters was plotted for the day the complaints occurred and were compared to the corresponding values of the day before. Also, values are plotted for the exact time of received odor complaints and compared to the same time period on the previous day. Correlations between parameters showed that wind direction and wind speed are the parameters with lowest correlations when compared to the values from the day before, which is expected, while temperature, humidity, and pressure had constantly higher correlation values. Results for one of the dates for each site are presented in the figures that follow.

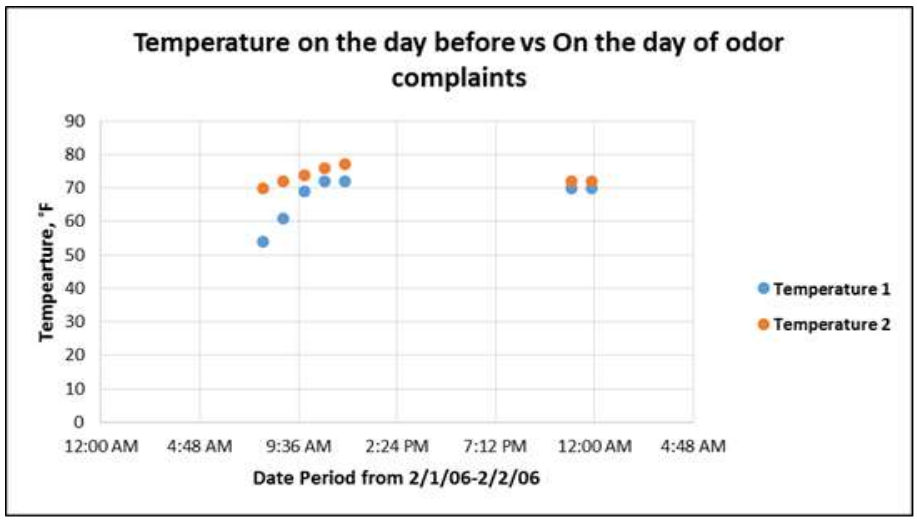


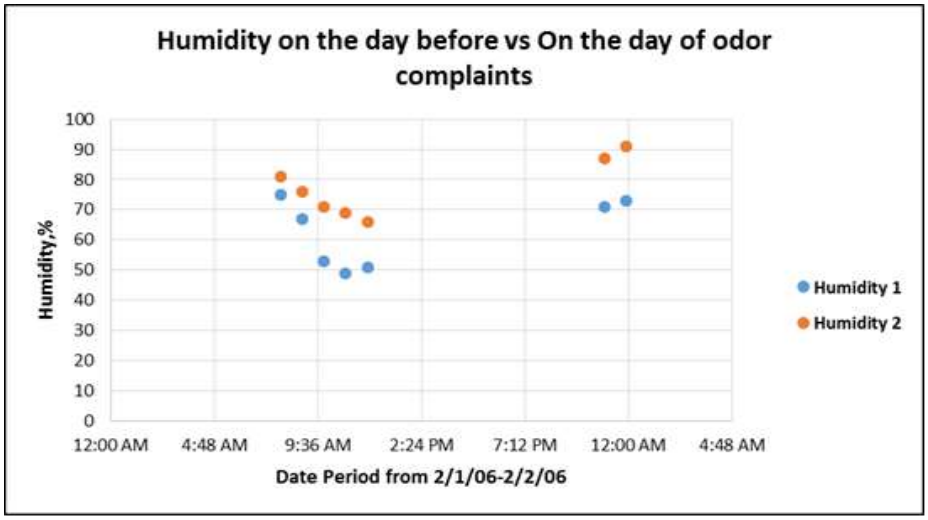


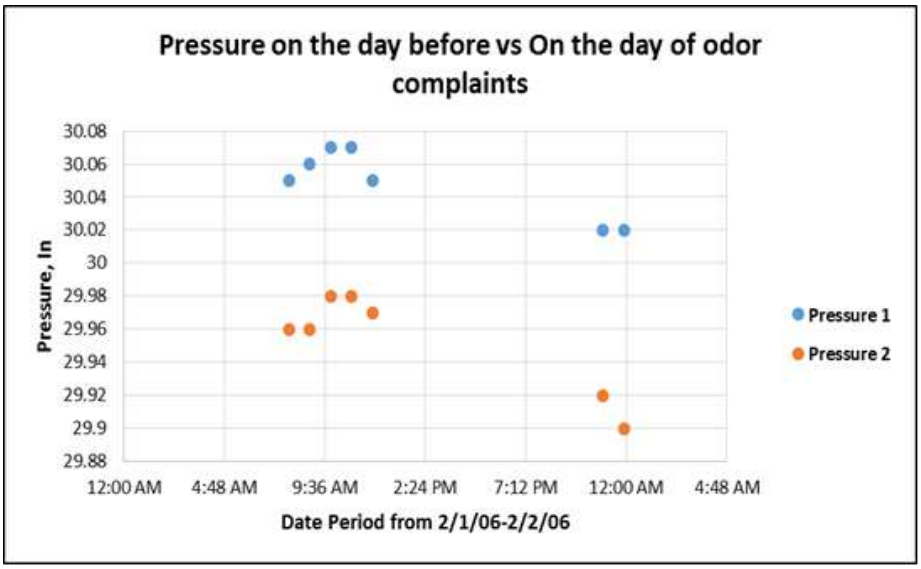

Pressure on the day before vs On the day of odor complaints
30.09
30.04
29.99
29.94
29.89
29.84
Pressure, In
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
Pressure 1
Pressure 2

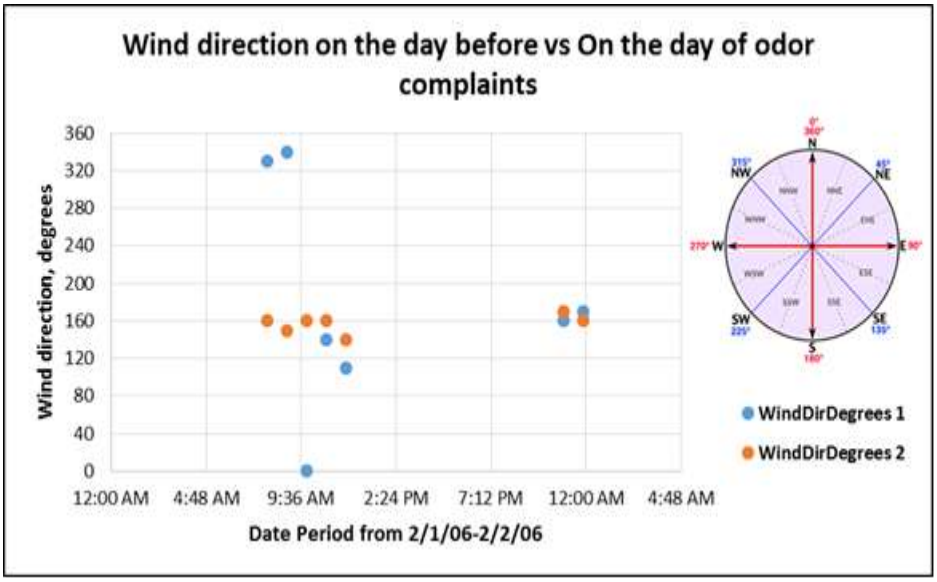

Wind direction on the day before vs On the day of odor complaints
400
350
300
250
200
150
100
50
0
Wind direction, degrees
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
N
NE
E
SE
S
SW
W
NW
WindDirDegrees 1
WindDirDegrees 2

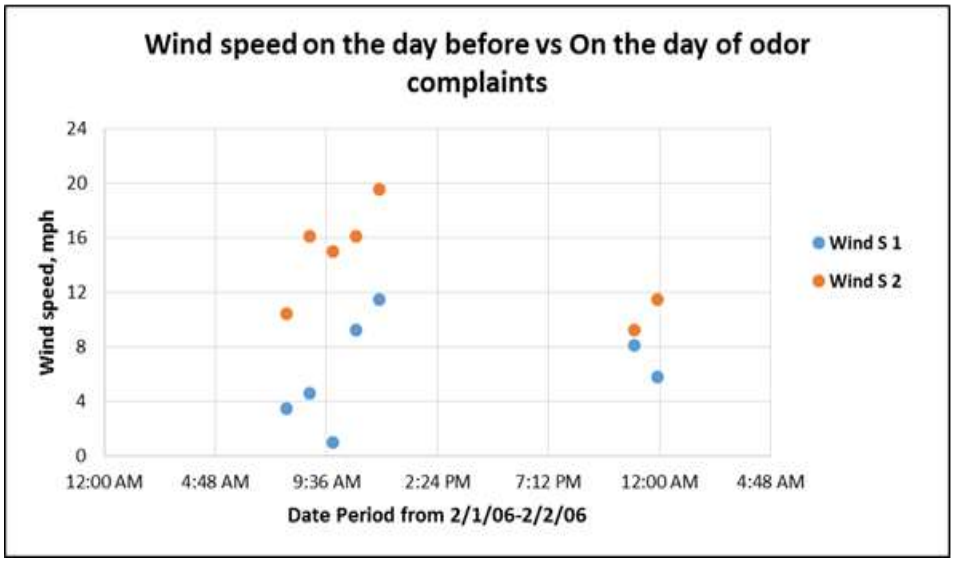

Wind speed on the day before vs On the day of odor complaints
25
20
15
10
5
0
Wind speed, mph
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
Wind S 1
Wind S 2


Temperature on the day before vs On the day of odor complaints
Tempearture, °F
90
80
70
60
50
40
30
20
10
0
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
Temperature 1
Temperature 2


Humidity on the day before vs On the day of odor complaints
Humidity,%
100
90
80
70
60
50
40
30
20
10
0
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
Humidity 1
Humidity 2

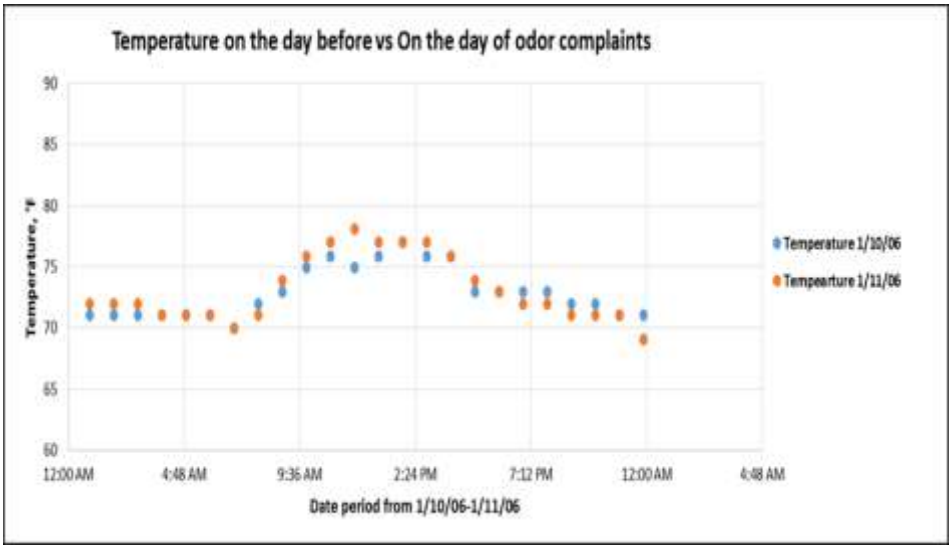

Pressure on the day before vs On the day of odor complaints
Pressure, In
30.08
30.06
30.04
30.02
30
29.98
29.96
29.94
29.92
29.9
29.88
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date Period from 2/1/06-2/2/06
Pressure 1
Pressure 2

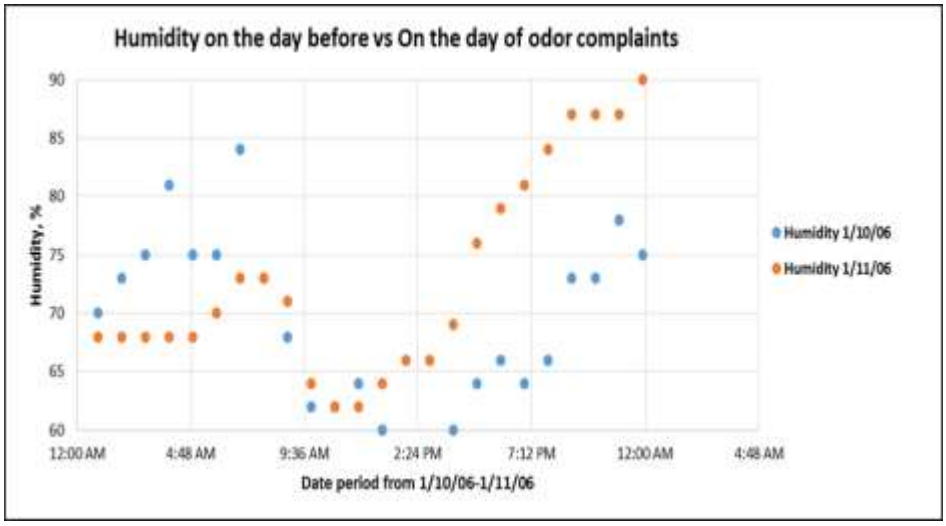


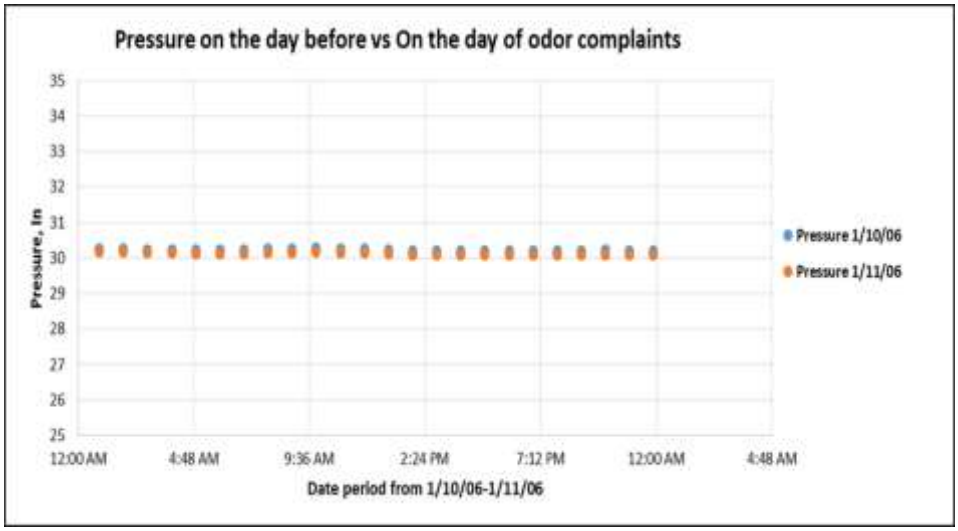


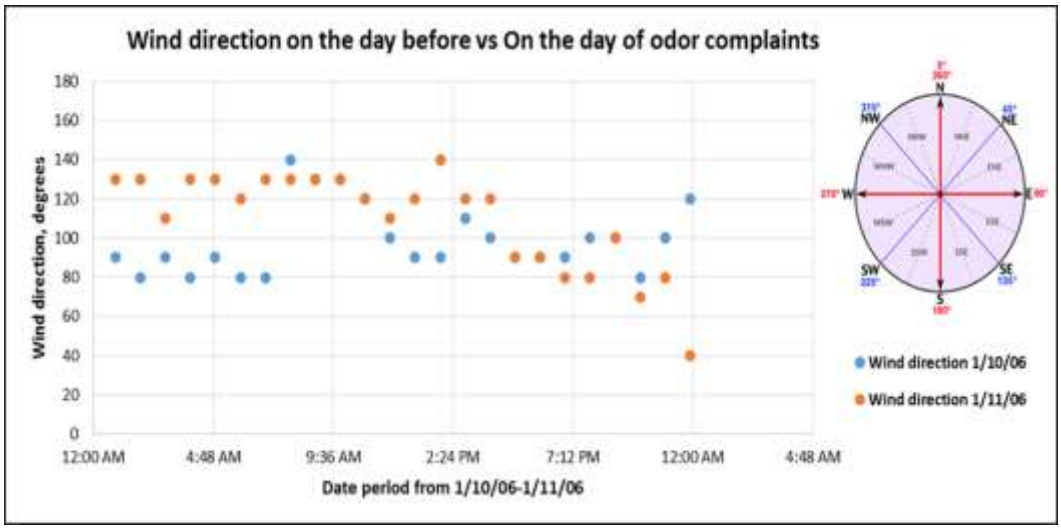
	T	H	P	W s	W d
Correlation 1	0.749037471	0.89097	0.9494	0.3604	-0.0622
Correlation 2	0.922759472	0.81613	0.71673	0.56778	-0.1714

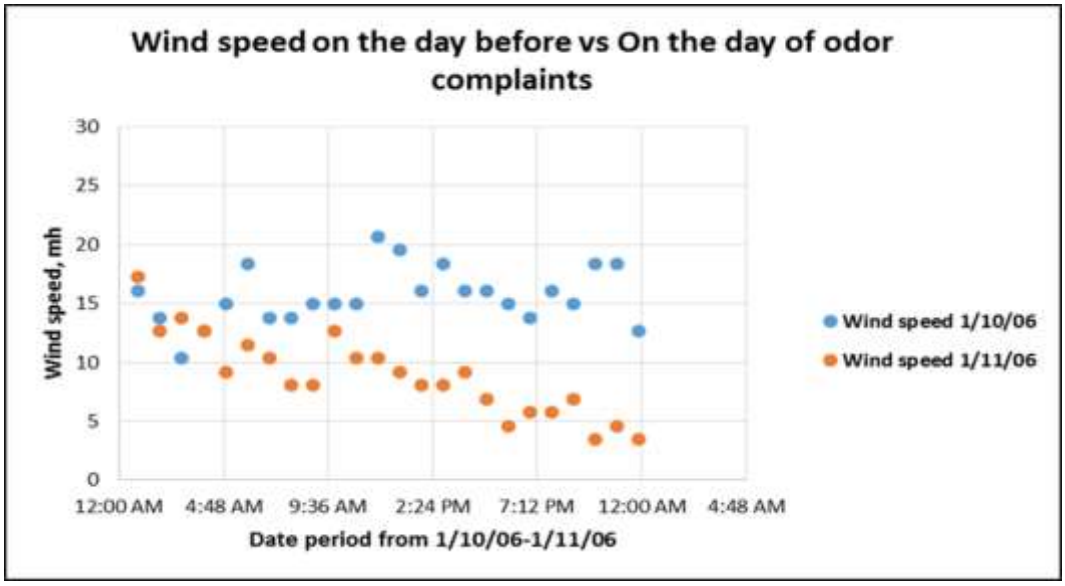
A similar analysis was conducted for the second set of data, as follows.

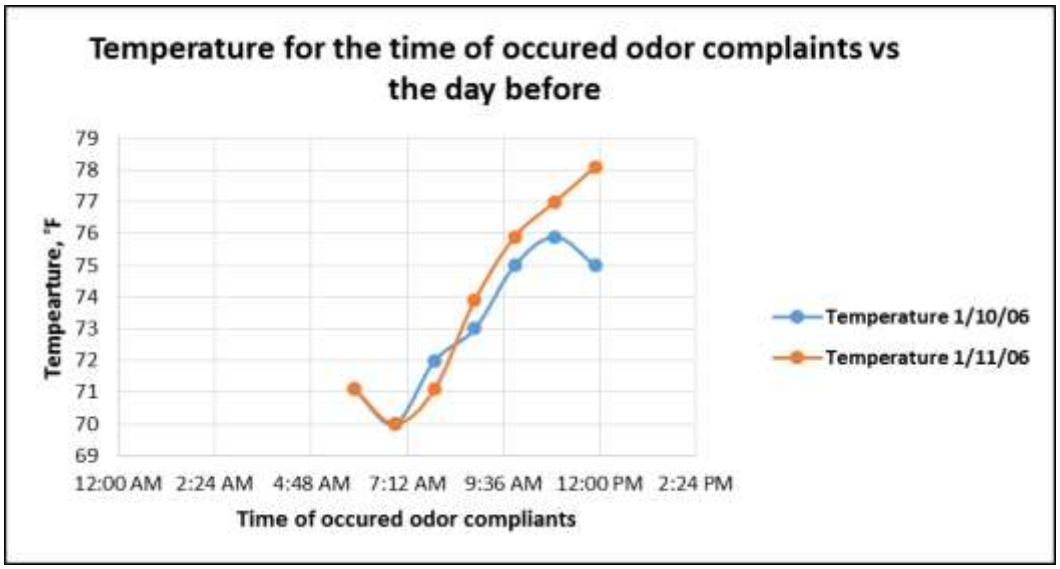


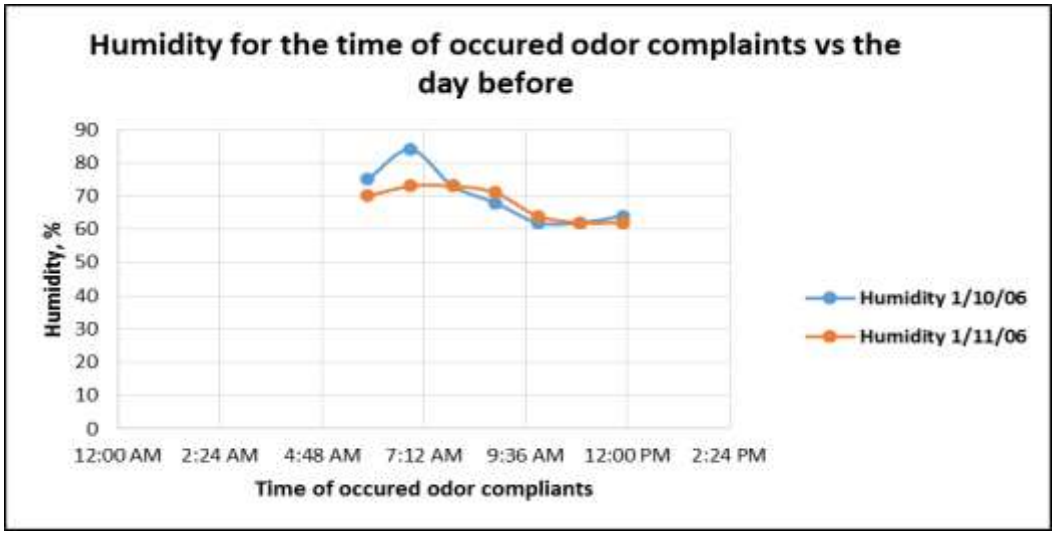


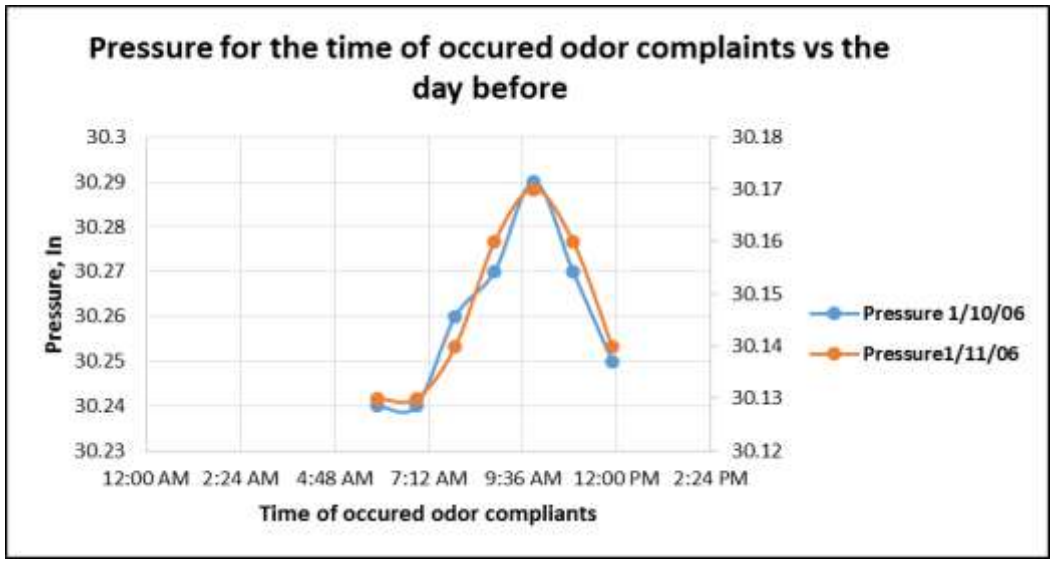


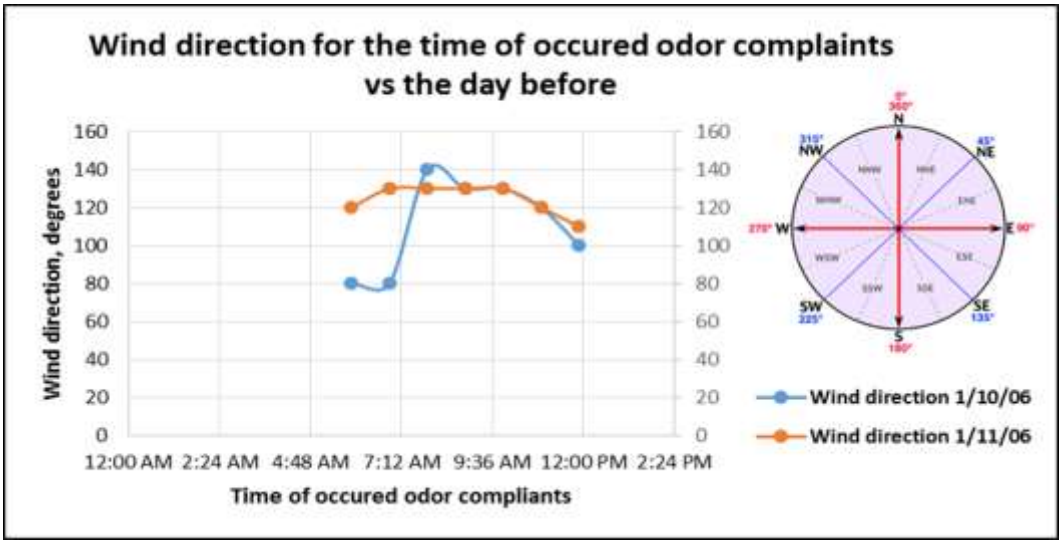

Wind direction on the day before vs On the day of odor complaints
Wind direction, degrees
180
160
140
120
100
80
60
40
20
0
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date period from 1/10/06-1/11/06
N
NE
E
SE
S
SW
W
NW
Wind direction 1/10/06
Wind direction 1/11/06

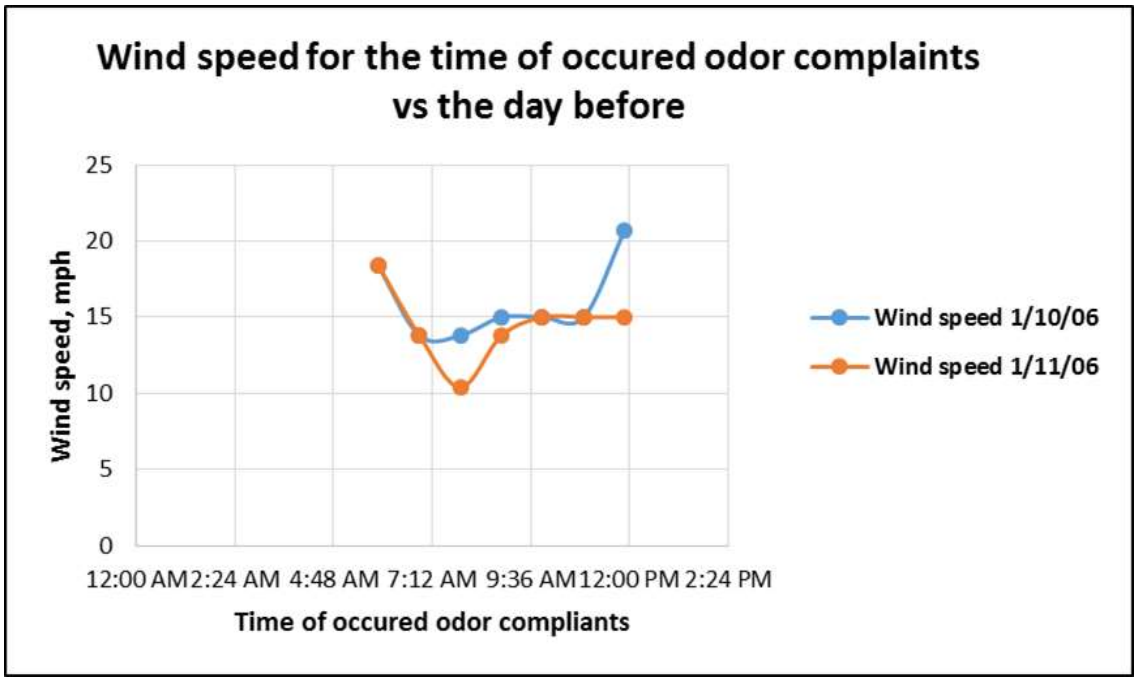

Wind speed on the day before vs On the day of odor complaints
Wind speed, mh
30
25
20
15
10
5
0
12:00 AM
4:48 AM
9:36 AM
2:24 PM
7:12 PM
12:00 AM
4:48 AM
Date period from 1/10/06-1/11/06
Wind speed 1/10/06
Wind speed 1/11/06


Temperature for the time of occured odor complaints vs the day before
Tempearture, °F
79
78
77
76
75
74
73
72
71
70
69
12:00 AM
2:24 AM
4:48 AM
7:12 AM
9:36 AM
12:00 PM
2:24 PM
Time of occured odor compliants
Temperature 1/10/06
Temperature 1/11/06


Humidity for the time of occured odor complaints vs the day before
Humidity, %
90
80
70
60
50
40
30
20
10
0
12:00 AM
2:24 AM
4:48 AM
7:12 AM
9:36 AM
12:00 PM
2:24 PM
Time of occured odor compliants
Humidity 1/10/06
Humidity 1/11/06


Pressure for the time of occured odor complaints vs the day before
Pressure, In
30.3
30.29
30.28
30.27
30.26
30.25
30.24
30.23
30.18
30.17
30.16
30.15
30.14
30.13
30.12
12:00 AM
2:24 AM
4:48 AM
7:12 AM
9:36 AM
12:00 PM
2:24 PM
Time of occured odor compliants
Pressure 1/10/06
Pressure1/11/06


Wind direction for the time of occured odor complaints vs the day before
Wind direction, degrees
160
140
120
100
80
60
40
20
0
12:00 AM
2:24 AM
4:48 AM
7:12 AM
9:36 AM
12:00 PM
2:24 PM
Time of occured odor compliants
N
NE
E
SE
S
SW
W
NW
Wind direction 1/10/06
Wind direction 1/11/06



	T1	T2
T1	1	
T2	0.930906	1

	Hum 1	Hum 2
H1	1	
H2	0.836057	1

	P1	P2
P1	1	
P2	0.939247	1

	Wd 1	Wd2
Wd 1	1	
Wd2	0.018405	1

	Ws1	Ws2
Ws1	1	
Ws2	-0.17854	1

- **TASK 4. Perform protein sensitivity experiments**

For the first attempt at cloning, a culture of *E. coli* XL1Blue II was grown in an overnight culture. Frozen DNA previously eluted from filter paper was concentrated by centrifugation and ethanol removed as supernatant. Nanopure water was added to the concentrated DNA. The *E. coli* culture was concentrated to prepare for electroporation. Transformants were inoculated on Kanamycin + Luria Broth Agar Plates and incubated at 37°C. No transformants grew on the first attempt. Future strategies to ensure successful transformation include: ensure adequate amount of starting plasmid, ensure preservation of plasmid, test electroporator to ensure adequate functioning, and ensure *E. coli* is healthy enough to survive the process.

A transformed BL21 *E. coli* strain already containing the plasmid to make OBP was incubated overnight on LB Agar + Kanamycin plates at 37°C and grew healthy colonies (Figure 1). The transformed BL21 clones were sent from the same researcher as the original plasmid. The difference is that instead of a plasmid that needed to be transformed into *E. coli*, transformants were already prepared and sent as agar stabs, which is a very stable format in which to transport clones. Following incubation, colonies were inoculated into fresh media and an overnight culture grown. The overnight culture was prepared with glycerol to be frozen at -80ºC for use indefinitely.

Figure 1. Healthy *E. coli* cultures following incubation.

Clones from the frozen stock will be centrifuged and run on acrylamide gels with and without IPTG to observe whether the protein remains soluble in the supernatant or ends up in the cell pellet. Once it is confirmed that the protein remains soluble and is of the expected base-pair length, protein expression and purification will be done using the cobalt-based IMAC medium from GE Healthcare (Figure 2). His SpinTrap TALON® is a single-use column for histidine-tagged proteins and comes prepacked with 100 µl TALON® Superflow™ cobalt-IMAC (immobilized metal affinity chromatography) medium. The benefit of cobalt based media rather than nickel-charged is that it offers better selectivity and purity. One run takes approximately 10 minutes with a microcentrifuge.

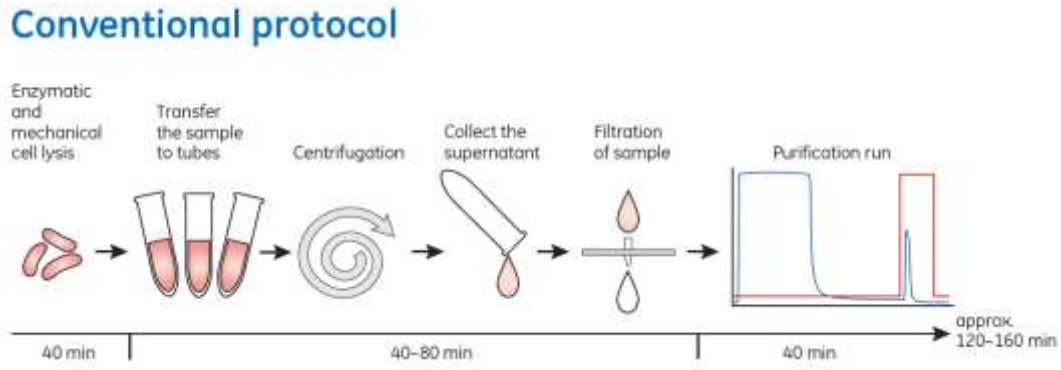


Figure 2. Conventional protocol for protein purification from GE Healthcare.

1-aminoanthracene (1-AMA) is an intrinsic fluorophore that will be used to observe binding of OBP to the pure odorous gasses, hydrogen sulfide, mercaptans and dimethyl sulfide. By monitoring fluorescence excitation spectra, Kmiecik and Albani (2010) studied the binding effect of 1-AMA on odorant binding protein structure. Results show that the conformation of OBP is modified by binding 1-AMA at low probe concentrations. Fluorescence excitation spectra incurs a red shift.

A gas reactor prototype was built and air was bubbled through water at the bottom of the reaction chamber (Figure 3). The aqueous protein-AMA complex will exist in the bottom of the chamber. A Y-connector will connect the gaseous cylinder and a flowmeter with the one way valve at the bottom of the chamber. Gas will escape the top of the chamber through a second one way valve. Fluorescence spectra will reveal the relationship between fluorescence and odorous gas concentration.

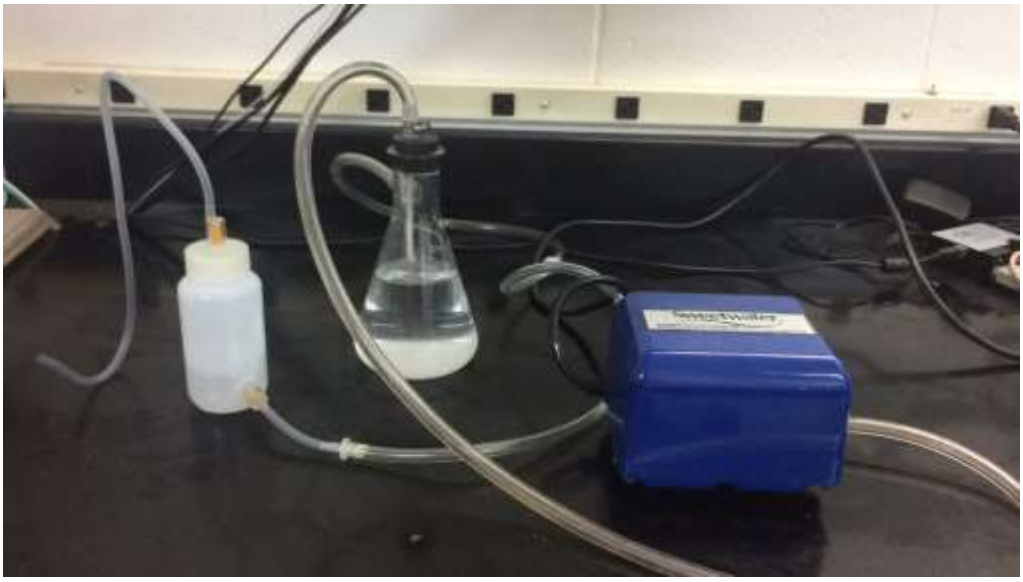


Figure 3. Gas reactor prototype being tested using an air compressor and beaker of water for humidifying the air connected to the valve at the bottom of the chamber. Air successfully bubbled through the water at the bottom of the chamber and out the valve at the top of the chamber.

- **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 odor complaint database.
  - **TASK 3. Pattern identification and trend analysis.** Further analyze data from Waste Management Inc. of Florida and other sources. Exclude meteorological parameters that do not affect number of odor complaints. Work on analysis of overnight pressure drop and precipitation accumulation as indicators of odor complaints. Measure concentrations of H_2S to see if there is any trend or correlation between the concentrations and number of odor complaints.
  - **TASK 4. Perform protein sensitivity experiments**. Procedures to clone, synthesize and express the protein have been successful. The DNA amplification procedure is underway and preliminary work to test monoclonal antibody detection are also underway. The vacuum chamber is built and is ready for testing with pure hydrogen sulfide gas and zero air.
  - **TASK 5. Assess odor mitigation strategies**. Waste Management and SWA personnel have provided information about mitigation strategies, and the next step is to conduct an alternative analysis.
  - **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
Martinez, Angel	CEGE	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).
   TAG meetings on March 2016 and December 2016. Julia Roblyer is presenting at the Section conference of the Air and Waste Management Association meeting on January 17, 2017.

   Roblyer, J. and Meeroff, D.E. (2017). Biosensor development for odor measurement. Florida Southeast Chapter of Air and Waste Management Association. January 17, 2017.

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)
   "Detection of nuisance odors using fluorescently labeled odor binding proteins", EREF, $169,569, August 2016

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

Dr. Jason Hallstrom (FAU I-SENSE Center), 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; however, a progress report presentation was made to Waste Management personnel to show the results of preliminary analysis of odor complaints relationship with meteorological data. We plan to continue to work with our partners to share our results and refine how odor complaints are dealt with by the industry.

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