

**Development of A Biosensor for Measuring Odorants in the Ambient Air
Near Solid Waste Management Facilities
Final Report**

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Report # (leave blank)

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ABSTRACT:

The objective of the study is to investigate 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. The Bill Hinkley Center for Solid and Hazardous Waste Management funded this follow up study in 2017 to find ways to improve odor detection including development of a novel technology that uses human odor binding proteins to detect and potentially quantify odors.

Key words:

Odors, landfills, biosensor, odor detection

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- Rahman, S., Meeroff, D.E. (2020). Development of a Biosensor for Objectively Quantifying Odorants. Odor and Air Pollutants Conference Proceeding 2020 (pp 427-463).

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

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- Rahman, S. (2020). Development of a Biosensor for Objectively Quantifying Odorants. Odor and Air Pollutants Digital Conference 2020, Cincinnati, OH, March 2020.
- Rahman, S. Biosensor Development to Quantify Nuisance Odors. Poster presented at: 11th Annual Graduate and Professional Student Association (GPSA) Research Day, Florida Atlantic University, FL, April 2020.

3. List who has referenced or cited your publications from this project.

None so far

4. How have the research results from **THIS** Hinkley Center project been leveraged to secure additional research funding? What grant applications have you submitted or are planning on submitting?

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5. What new collaborations were 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.

6. How have the results from **THIS** Hinkley Center funded project been used (not will be used) by the FDEP or other stakeholders?

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES	viii
LIST OF TABLES.....	xi
LIST OF ACRONYMS AND ABBREVIATIONS	xi
EXECUTIVE SUMMARY	xii
1.0 INTRODUCTION	1
1.1 Background of Physiology of Human Olfactory Perception	3
1.2 Structure and Properties of Odorant Binding Proteins (OBPs).....	4
1.2.1 Interaction of OBPs with Odorants	5
1.2.2 Human Odorant Binding Protein-2A (hOBPIIa).....	6
1.2.3 Biosensor Development Using OBP	8
1.3 Fluorophores.....	9
2.0 METHODS	13
2.1 Purified hOBPIIa.....	13
2.2 Fluorescence Binding Assay	14
2.3 Reactor Chamber.....	16
2.4 Fluorescence Measurements	17
2.5 Outlet Gas Concentration Detection	19
2.6 pH.....	19
3.0 RESULTS	21
3.1 Fluorescence Binding Assay with Extrinsic Fluorophore (1-AMA)	21
3.2 Biosensor Sensitivity Experiments on Model Compounds.....	23
3.2.1 Experimentation with Nitrogen (N_2)	23
3.2.2 Experimentation with Hydrogen Sulfide (H_2S)	23
3.2.3 Experimentation with Ammonia (NH_3)	28
3.2.4 Experimentation with Methyl Mercaptan (CH_3SH).....	30
3.2.5 Experimentation with Methane (CH_4)	31
3.2.6 Experimentation with Gas Mixture 1 ($NH_3 + CH_4$).....	32
3.2.7 Experimentation with Gas Mixture 2 ($H_2S + CH_4 + CO$)	33

3.3	Interpretation of Results for Different Odorant Gases	35
3.4	Binding Affinity of Pure Odorant Gases.....	37
3.5	Results from Experiments with PID Sensor.....	38
3.6	Biosensor Reversibility Experiment	38
4.0	CONCLUSIONS.....	40
4.1	Major Findings	40
4.2	Recommendations	40
	BIBLIOGRAPHY.....	43
	APPENDIX A: ADDITIONAL LITERATURE REVIEW	58
	APPENDIX B: EXPERIMENTAL DATA	76
	APPENDIX C: PUBLICATIONS	81

LIST OF FIGURES

Figure 1: Mechanism of quantifying odorant gases with hOBPIIa by means of spectrofluorometric analysis	2
Figure 2: Cells within nasal epithelium allowing humans to perceive odors (Jasper 2013).....	3
Figure 3: Illustration of the mechanism of the sense of smell involving the action of odorant binding proteins (Stangor 2012)	4
Figure 4: Percentages showing identical amino-acid sequence between various members of lipocalin superfamily including OBPs and PBPs (Pheromone Binding Protein) (Tegoni et al. 2000)	5
Figure 5: Tertiary structure of bOBP (bovine OBP) and pOBP (porcine OBP) modeled using DeepView software (Pelosi et al. 2014)	6
Figure 6: Tertiary structure of hOBPIIa (left) and hOBPIIb (right). β sheets, α -helices and disulfide bridge are indicated in blue, green, and yellow respectively (Lacazette et al. 2000)	7
Figure 7: Tertiary structure of hOBPIIa bonded with the aldehyde, undecanal (carbon atoms are indicated in grey, oxygen is in red, and nitrogen is in blue), in the middle of the ligand binding pocket (Heydel et al. 2013).....	8
Figure 8: 1-AMA competitive binding assay of pOBP-m2 (pig OBP mutant) with different polycyclic aromatic hydrocarbons. 1 μ M 1-AMA and 1 μ M protein were used in an increasing concentration of methanolic solutions of aromatic compounds starting from 1 mM (Wei et al. 2008).....	9
Figure 9: Mechanism of fluorescent turn-on probe where the ligand attaches to the specific hydrophobic ligand binding site of the protein and the surrounding hydrophobic environment allows the environment-sensitive fluorophore to emit strong fluorescence (Zhuang et al. 2013).....	10
Figure 10: Titration curve of 2 μ M hOBPIIa against the fluorescent probes NPN (left) and DAUDA (right) at increasing concentrations (Briand et al. 2002).....	11
Figure 11: Fluorescence curve of 1-AMA at 485 nm with increasing solvent concentration. 1-AMA is displaced the least by methanol, leading to relatively high fluorescence emission even though the solvent concentration is increased (Triangle: methanol, Circle: ethanol, Square: dimethyl sulfoxide) (Briand et al. 2000).....	12
Figure 12: Bradford curve showing the relationship of optical density to protein concentration between induced and uninduced samples	13
Figure 13: (a) 12.5% SDS-PAGE Gel of crude protein (b) 12.5% SDS-PAGE Gel of purified protein	14
Figure 14: Schematic diagram of the experimental setup.....	15
Figure 15: Reactor chamber using a centrifuge tube (a) and 3-way stopcock used on the lid of the exposure chamber (b). The different ports and other parts are labeled	17
Figure 16: Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (a) and Fluorescence software screenshot (b)	18

Figure 17: 'Z' dimension (distance from the base to the center of the sample chamber window) of the quartz cuvette (a) and a 100 μ L capacity quartz cuvette containing 100 μ L of sample each time (b)	18
Figure 18: Experimental setup for verifying whether there is any gas present at the outlet of the reaction chamber without filter (a) and with filter circled in red (b)	19
Figure 19: Spectrofluorometric emission spectra for four different solutions (a) and magnified curves of the other three solutions except solution #4 (b)	21
Figure 20: Binding curve of 1-AMA at different concentrations with hOBPIIa.....	22
Figure 21: Graph showing peak emission intensity against time for nitrogen gas flowing at 0.5 slpm.....	23
Figure 22: Spectrofluorometric emission spectra at 380 nm excitation for 0.5 slpm hydrogen sulfide found by Roblyer (2017) (a), and graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)	24
Figure 23: Spectrofluorometric emission spectra for excitation at 380 nm for the verification experiment at 0.5 slpm hydrogen sulfide for 25 mL sample (a), and graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b).....	25
Figure 24: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.5 slpm hydrogen sulfide and 10 ml sample (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b).....	26
Figure 25: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.7 slpm hydrogen sulfide (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b).....	26
Figure 26: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.9 slpm hydrogen sulfide (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b).....	27
Figure 27: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for hydrogen sulfide (a), and a graph showing change in peak fluorescence intensity with mass flow of hydrogen sulfide at all three flow rates (b)	28
Figure 28: Graph showing peak emission intensity against time for ammonia gas flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c).....	29
Figure 29: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for ammonia gas (a), and a graph showing change in peak fluorescence intensity with mass flow of ammonia gas at all three flow rates (b)	29
Figure 30: Graph showing peak emission intensity against time for methyl mercaptan gas flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c).....	30
Figure 31: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for methyl mercaptan gas (a), and a graph showing change in peak fluorescence intensity with mass flow of methyle mercaptan gas at all three flow rates (b).....	31
Figure 32: Graph showing peak emission intensity against time for methane gas flowing at 0.5 slpm (a), and a graph showing change in peak fluorescence intensity with mass flow of methane gas at 0.5 slpm (b)	32

Figure 33: Graph showing peak emission intensity against time for gas mixture 1 (NH₃ 25 ppm +/- 5% and CH₄ 25 ppm +/- 5%) flowing at 0.5 slpm (a), and a graph showing change in peak fluorescence intensity with mass flow of gas mixture 1 along with its individual component gases at 0.5 slpm (b)..... 33

Figure 34: Graph showing peak emission intensity against time for gas mixture 2 (H₂S 25 ppm +/- 5%, CO 50 ppm +/- 5%, and CH₄ 2.5% +/- 2%) flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)..... 34

Figure 35: Graph showing peak emission intensity against time for gas mixture 2 for all three flow rates..... 34

Figure 36: Graph showing peak emission intensity against time for gas mixture 2 (H₂S 25 ppm +/- 5%, CO 50 ppm +/- 5%, and CH₄ 2.5% +/- 2%) along with individual component gases (from previous experiments) flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)..... 35

Figure 37: Quantitation range of different odorant gases (a), and slope of emission intensity curves of different odorant gases tested at different flow rates (b)..... 36

Figure 38: Graph showing peak emission intensity against time for passing hydrogen sulfide gas through the biosensor solution at 0.5 slpm (first 240 seconds) followed by nitrogen gas at the same flow rate (final 240 seconds) 39

Figure 39: Schematic diagram for experimentation with a flow-through cuvette 42

LIST OF TABLES

Table 1: Mass of pure odorant gases combining with hOBPIIa and of gas mixtures passed into the solution.....	36
Table 2: Detection ranges of mass and concentration of the odorants	37

LIST OF ACRONYMS AND ABBREVIATIONS

MSW	Municipal Solid Waste
LFG	Landfill Gas
HAP	Hazardous Air Pollutant
VOC	Volatile Organic Compound
USEPA	United States Environmental Protection Agency
FIDOL	Frequency (F), intensity (I), duration (D), offensiveness (O), and location (L)
OU	Odor Unit
OUE	European Odor Unit
ppm	Parts per million
ppb	Parts per billion
ppt	Parts per trillion
OR	Odds Ratio
CI	Confidence Interval
NIOSH	National Institute for Occupational Safety and Health
ASTM	American Society of Testing and Materials
GC/MS	Gas Chromatography/Mass Spectrometry
MOSFET	Metal–Oxide–Semiconductor Field-Effect Transistor
ANN	Artificial Neural Networks
PCA	Principal Component Analysis
DFA	Discriminant Function Analysis
OBP	Odorant-Binding Protein
ORs	Odor Receptors
PBPs	Pheromone Binding Proteins
bOBP	Bovine Odorant-Binding Proteins
pOBP	Porcine Odorant-Binding Proteins
hOBP	Human Odorant-Binding Proteins
1-AMA	1-Aminoanthracene
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
OD	Optical Density
PID	Photo-Ionization Detector

EXECUTIVE SUMMARY

Nuisance odor levels produced by solid waste management operations are subject to regulatory standards due to their impacts on the quality of life of the residents living nearby the facility. Failure to meet regulatory standards may result in 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. In order to ensure that nuisance odor issues are minimized, it is necessary to provide an objective measurement. The objective of the current research is to develop a biosensor for providing an objective, standard measurement of odors. The approach is to modify the human odorant binding protein (hOBPIIa), isolated using published biomolecular techniques, by fluorescently tagging it with a chromophore functional group. When this protein is tagged with a fluorophore marker and excited in a spectrofluorometer, it emits light of a certain wavelength that can be detected and quantified. Once odorant molecules are exposed to this complex, they start replacing the fluorophore, and as a result, the emitted light intensity decreases in proportion to the number of odorant molecules. Since the protein response depends on odorant concentration, following an inverse Beer's Law relationship, the odorants can be quantified accurately and rapidly using fluorometric measurements. The results establish quantitation ranges for different pure and mixture of odorant gases as well as the amount of gas that can be quantified across various flow rates.

1.0 INTRODUCTION

Unpleasant odors are considered as environmental pollution, and one of the most prominent sources of odors are municipal solid waste (MSW) landfills, which have a variety of waste components in varying quantities undergoing complex biological and physicochemical reactions (Palmiotto et al. 2014). Fang et al. (2012) investigated the odor emissions from different areas in a solid waste disposal site, and identified dozens of specific odorants, some of which are known to have very high odor intensity even at extremely low, difficult-to-detect levels (Details in Appendix A). The emissions from landfills are unavoidable because of the nature of the waste materials and its anaerobic decomposition. Moreover, such emissions can last at least three decades after the landfill ceases its operation, complicating the odor control strategy (Ritzkowski et al. 2006). Ideally, solid waste management facilities are to be located far from further human contact, but population growth, sprawling development, and transportation optimization have led to public encroachment in areas surrounding urban solid waste management facilities, with this essential infrastructure now having to operate in closer proximity to potential receptors than initially intended. This situation can lead to sharp increases in odor complaints (Vidovic 2017). Additionally, the uncertainty associated with odor measurement has added to the increased pressure on regulators to introduce more stringent rules regarding odor control. Considering such problems, controlling odor emissions have become a major challenge to solve for the solid waste management industry.

While odor complaints from industrial facilities have increased over time, the technology for detecting and characterizing complex odors has not developed to the level of a reliable surveillance method, which has hindered regulatory frameworks. Current approaches to odor measurement and regulation vary greatly among local jurisdictions, states, and countries. Regulatory tools have ranged from relatively simple qualitative measurements or checklists of perceived odor and/or specific chemicals to the more complex use of electronic nose technology and atmospheric dispersion models to predict odor impacts on neighboring receptors. State of the art analytical measurements that characterize a specific odorant in terms of its chemical composition and concentration are objective, repeatable, and accurate; however, they provide little information about odor perception by human receptors (Lebrero et al. 2011), and they can only detect known odorant compounds on an individual basis, making analysis time-consuming and expensive (Details in Appendix A). Hypothetically, the odor contribution of waste facilities should be measured by identifying, sampling, and quantifying the concentrations of all odorants from all odor sources. But this is extremely difficult to accomplish due to the nature of the wide variety of compounds known to cause odors at industrial facilities (Paxeus 2000; Bruno et al. 2007; Kjeldsen 2010).

Certainly great strides have been made with regard to understanding odor science, but there is still a need for continued research to overcome the uncertainties associated with odor monitoring and

prediction needed for development of an odor measurement technique that can objectively measure odor intensity and do so at a low cost. What is most needed is a way to standardize odor identification that will allow regulatory agencies to establish reasonable, objective standards for odor severity (i.e. what constitutes “objectionable”). One way to address this issue is to understand how the human sense of smell actually works. By understanding the mechanism behind human olfaction a biosensor can be designed that can objectively quantify odors using copies of the human odorant binding protein (hOBPIIa). These proteins bind with a wide range of odorants in the micromolar range. Recent advances in biotechnology (Silva 2014) have allowed hOBPIIa to be easily replicated in the lab to form the basis of an odorant-detecting biosensor. So by adding a biomolecular fluorescent marker, which in this research is the fluorophore 1-AMA (Figure 1), binding is quantified using a fluorescence spectrofluorometer. Since the human nervous system processes odor intensity based on the number of bound receptor sites, and the biosensor protein fluorescent response is concentration-dependent, this new technology will allow for authentic quantification of odors without subjective interpretation.

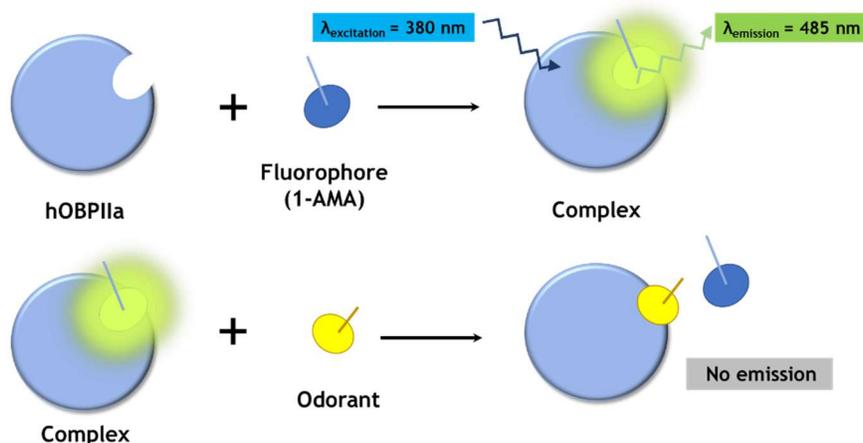


Figure 1: Mechanism of quantifying odorant gases with hOBPIIa by means of spectrofluorometric analysis

The objective of this study is to develop a non-subjective measurement technique for nuisance odors using human odorant binding protein 2A (hOBPIIa). The technology will involve designing the biosensor, testing for spectrofluorometric interference and concentration-dependence of the biosensor for a specific set of odorants typically encountered at solid waste facilities including hydrogen sulfide, ammonia, methyl mercaptan, etc. as well as mixture of odorants. The detection limit along with the quantitation ranges (i.e. the time up to which the biosensor solution remains effective) for these odorants have also been determined.

1.1 Background of Physiology of Human Olfactory Perception

Human perception of odor is considered to be a complex phenomenon. Figure 2 shows a longitudinal cross section of the human nasal cavity to illustrate the mechanisms involved in the human sense of smell.

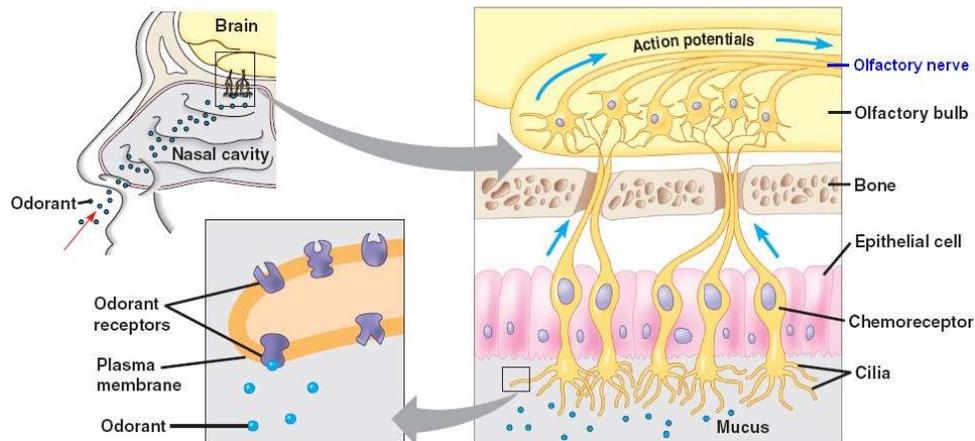


Figure 2: Cells within nasal epithelium allowing humans to perceive odors (Jasper 2013)

First, the air containing odorants are inhaled by the nose and warmed in the nasal cavity while passing through a series of small bones known as turbinates (ATSDR 2016; Ruth 1986). Inside the nasal cavity, olfactory membranes less than 1 square inch in size. Odorant molecules must travel through the aqueous nasal mucous and sensillar lymph that surround the olfactory membrane at the surface of the nasal epithelium (Tegoni et al. 2000) to reach a group of specialized nerve cells on small hairs called cilia. These nerve cells are known as olfactory neurons or receptor cells (chemoreceptors). The human nasal cavity contains 10-20 million of olfactory receptor neurons (Saladin 2004) that interact with odorant molecules and convey the sensation of smell through the olfactory bulb in the form of electric signals to the brain (ASTDR 2016). However, before reaching the receptor neurons, odorants that are typically hydrophobic must be conveyed through the hydrophilic mucosa in the nasal cavity to reach the olfactory neurons (Tegoni et al. 2000). Nasal mucus, secreted by the supporting cells in the epithelium along with Bowmans's glands of mammals and insects, consists of 95% water, 2% mucopolysaccharides, lysozyme, high-molecular-weight glycoproteins, antibodies, salts, odorant binding proteins (OBPs) and other enzymes (Heydel et al. 2013). The OBPs, secreted in the nasal mucus by the olfactory epithelium, bind with odorant molecules in the hydrophobic ligand binding pocket and accompany odorants across the membrane to facilitate transport towards the olfactory receptors (ORs) (Schiefner et al. 2015). As soon as the odorants reach the receptors, they are detected and converted into a signal to the brain that build an olfactory image (Heydel et al. 2013) as shown in Figure 3. OBPs also carry away odorant molecules to allow other odorants to interact at the receptor sites and protect receptors from experiencing excessive amounts of odorants.

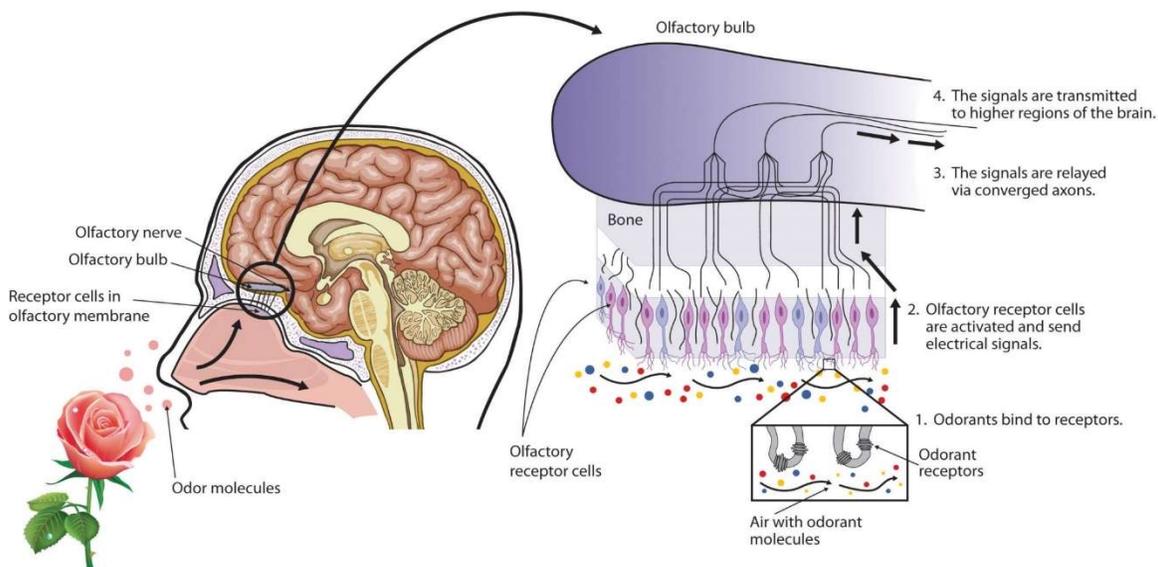


Figure 3. Illustration of the mechanism of the sense of smell involving the action of odorant binding proteins (Stangor 2012)

The quality of the olfactory signal depends on the mechanisms that convey the odorants or changes the ligand binding properties of the odorants during the detection process. Such mechanisms are called perireceptor events, which involve an active participation of proteins that are secreted in the nasal mucus to arouse an odorant stimulus (Heydel et al. 2013). Humans can detect up to 10,000 odors and are able to distinguish between odorants whose difference in concentration varies by as little as 7% (ATSDR 2016; Sela & Sobel 2010). Even the slightest change in the molecular structure can be perceived by humans. For example, odorants having equal number of carbon atoms but different functional groups or having the same functional groups but with a difference in chain length of only one carbon can be distinguished (Sela & Sobel 2010).

1.2 Structure and Properties of Odorant Binding Proteins (OBPs)

Odorant binding proteins (OBPs) are small, soluble extracellular proteins that are found in the nasal mucus of a large variety of animals including insects, pigs, cows, mice, rats, elephants, and humans (Briand et al. 2002; Tegoni et al. 2000; Pelosi 2001). OBPs belong to the lipocalin superfamily that can bind with hydrophobic molecules and are secreted at a high concentration (10 mM) in the nasal epithelium (Heydel et al. 2013). They are known to reversibly bind with volatile chemicals i.e. airborne odorants with micromolar affinities and have dissociation constants in the micromolar range (Briand et al. 2002). They are thought to be good carriers of inhaled odorants towards the olfactory neurons and participate in the selection or deactivation of odorant molecules as well (Brind et al. 2002).

Although the structural pattern of vertebrate and insect OBP is quite dissimilar, their role in olfaction is similar (Wei et al. 2008). Commonly, less than 20% of the amino-acid sequence is identical among the members of lipocalin superfamily as shown in Figure 4 (Briand et al. 2002). Vertebrate OBP follow a generic structural pattern comprising of 8 stranded antiparallel β -barrels bounded on both sides with an α -helix (Briand et al. 2002). A central apolar cavity named “calix” exists within the barrel inside of which odorant binding takes place in the ligand binding site. The broad binding activity of OBPs having dissimilar amino acid sequences in the same animal species allows them to bind with odorants of different chemical structures. OBPs can be monomer, dimer or heterodimer, and their molecular weights vary between 18~20 kDa (Heydel et al. 2013; Briand et al. 2002). Most of these subtypes are acidic (pH 4~5) (Heydel et al. 2013).

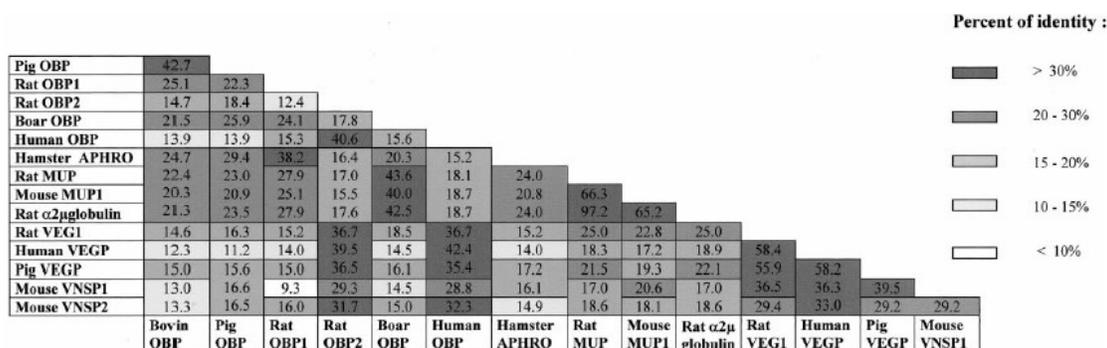


Figure 4: Percentages showing identical amino-acid sequence between various members of lipocalin superfamily including OBPs and PBPs (Pheromone Binding Protein) (Tegoni et al. 2000)

1.2.1 Interaction of OBPs with Odorants

The concept of OBP acting as an odorant carrier was hypothesized when Pelosi et al. (2014) first discovered that bovine OBP (bOBP) is able to bind with pyrazine (2-isobutyl-3-metoxypyrazine), an odorant of bell pepper with low detection threshold. Eventually a broad binding affinity towards medium-sized hydrophobic odorants has been specified for bOBP and porcine OBPs (pOBPs) (Tegoni et al. 2000). In case of the dimer bOBP, which was considered as the prototypic OBP for quite a while, two odorant molecules replace the naturally occurring endogenous ligands existing inside the inter-dimer open cavity (Spinelliet al. 1998; Paolini et al. 1999). Unlike bOBP, the monomeric pOBPs do not hold any natural ligand inside of their β -barrel cavities and one binding site per monomer has been reported by Paolini et al. (1999). The cysteine residue, that is commonly observed in most of the OBPs, is in the form of a disulfide bridge in the case of pOBPs whereas it is completely missing in bOBP (Paolini et al. 1999). A 3D structure of bOBP and pOBP is shown in Figure 5.

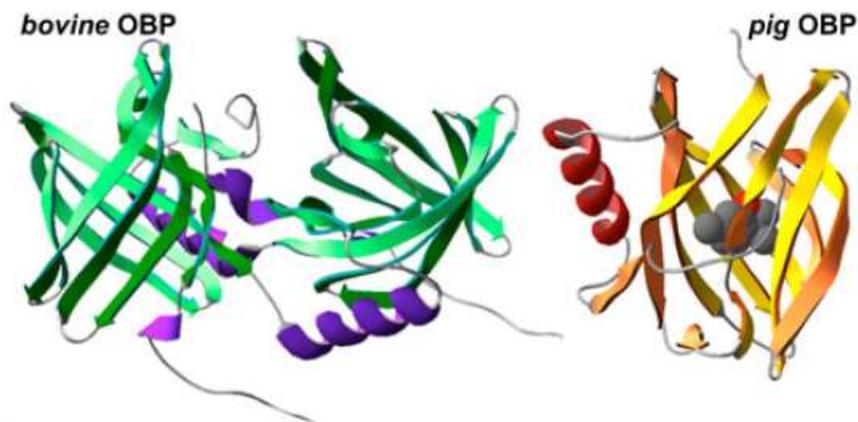


Figure 5: Tertiary structure of bOBP (bovine OBP) and pOBP (porcine OBP) modeled using DeepView software (Pelosi et al. 2014)

Researchers have established that heterocyclic derivatives have the highest affinity towards OBPs; whereas fatty acids with short-chain, spherically-shaped terpenoids (e.g camphor and its analogues) have less affinity (Tegoni et al. 2000). Thiazoles, pyrazine, terpenoids, menthol, thymol, aliphatic alcohols and aldehydes were found to exhibit good affinity for OBPs with dissociation constants within range of 0.1-1.0 μM (Tegoni et al. 2000). In the case of rat OBPs, the relative fluorescence intensity in displacement assays has proved that each type of the OBPs binds with a distinct class of odorants. For example, rOBP-1 tends to interact with heterocyclic compounds (e.g. pyrazine and its derivatives), rOBP-2 specifically prefers long-chain aliphatic aldehydes as well as carboxylic acids and rOBP-3 normally binds with odorants having a ring structure (Briand et al. 2002).

Silva et al. (2014) first used pOBP for odor control where a cationized cotton surface coated with pOBP was used to mask the smell of cigarettes by delaying the release of citronellol, a fragrance often uses in perfumes. This idea introduced the possibility that OBPs can be efficiently used to trap unpleasant odors from fabrics and can be more effective than cyclodextrins in this regard (Silva et al. 2014). Increasing the temperature was also observed to increase the affinity of OBP towards a particular fragrance, which demonstrated that textiles treated with OBP can be a good option for making perfumes last longer than usual as the temperature rises from ambient to human body temperature.

1.2.2 Human Odorant Binding Protein-2A (hOBPIIa)

Two possible odor binding protein genes, hOBPIIa and hOBPIIb being 95% identical, have recently been discovered in humans (Briand et al. 2002; Tegoni et al. 2000). The hOBPIIa gene, which codes for the protein hOBPIIa, has been transcribed in nasal mucosa as well as lung, lachrymal, and salivary glands. This is contrary to the hOBPIIb gene, which has been found mainly in the genital sphere organs (e.g. prostate and mammary glands) (Tegoni et al. 2000; Lacazette et

al. 2000). The amino acid sequence of hOBPIIa is 45.5% homologous to rat OBP-2; whereas hOBPIIb is 43% identical to that of human tear lipocalin-1 (Heydel et al. 2013). A central eight-stranded antiparallel β -barrel (strands A–H) with a C-terminal α -helix, which is a traditional lipocalin fold for all other OBPs, has also been observed in the case of the monomeric hOBPIIa (Schiefner et al. 2015). Additionally, another short β -barrel (strand I), running slightly antiparallel to strand A, at the downstream of the α -helix (Figure 6) and a disulfide bridge located between cys59 and cys151 have been detected in hOBPIIa. A positive charge exists in the entire cavity inside the β -barrel, more specifically at the entrance of the loop region, which is another common feature for all amino acid side chains pointing towards the cavity (Schiefner et al. 2015). Most of the OBPs are acidic, but the measured isoelectric point (PI) of a recombinant hOBPIIa manufactured by Briand et al. (2002) to characterize its odorant binding activity was found to be 7.8 (neutral).

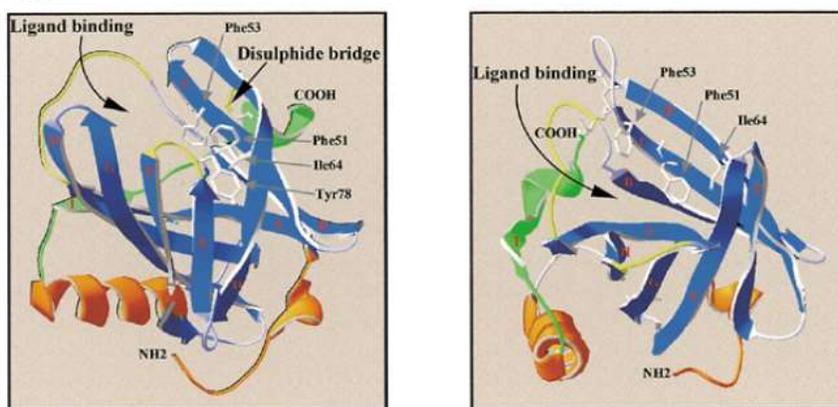


Figure 6: Tertiary structure of hOBPIIa (left) and hOBPIIb (right). β sheets, α -helices and disulfide bridge are indicated in blue, green, and yellow respectively (Lacazette et al. 2000)

The remarkably large ligand binding cavity inside the β -barrel of hOBPIIa allows the protein to bind a wide variety of hydrophobic odorants having different structures and sizes with affinities in the micromolar range though comparatively low affinity has been seen for some very strong odorants such as 2-isobutyl-3-methoxy pyrazine and eugenol ($K_{diss} > 10 \mu\text{M}$) (Schiefner et al. 2015; Briand et al. 2002; Heydel et al. 2013). In the case of hOBPIIa, a more restricted binding specificity than porcine OBP and rOBP-1 or rOBP-3 has been reported due to the fact that the protein shows very strong affinity for aldehydes (e.g. undecanal: $K_{diss} \sim 0.3 \mu\text{M}$, lilyal, the odor of the lily of the valley: $K_{diss} \sim 0.5 \mu\text{M}$, and vanillin: $K_{diss} \sim 1 \mu\text{M}$) and large chain fatty acids ($K_{diss} \sim 0.3 \mu\text{M}$) (Heydel et al. 2013; Briand et al. 2002). Moreover, the affinity is even stronger for aldehyde compounds with respect to acids, since the lysine residue located at the edge of the pocket binds strongly with aldehydes by forming a preferential hydrogen bond (Figure 7) (Heydel et al. 2013).

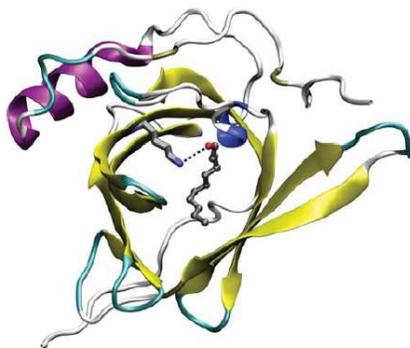


Figure 7: Tertiary structure of hOBPIIa bonded with the aldehyde, undecanal (carbon atoms are indicated in grey, oxygen is in red, and nitrogen is in blue), in the middle of the ligand binding pocket (Heydel et al. 2013)

The affinity of hOBPIIa for aldehyde compounds (either aliphatic or aromatic) increases as the molecular size of the odorants increases when compared with their chemical series (Tcatchoff et al. 2006). As the cavity opens to transfer the odorants towards olfactory receptors (ORs), water enters the cavity and recovers the chemical integrity of lysine and aldehydes (Charlier et al. 2009).

hOBPIIa has a number of characteristics that qualify the protein to be a good biosensor. As mentioned above, the β -barrel of hOBPIIa has a large ligand binding cavity which allows the protein to bind with a diverse array of hydrophobic odorants (Briand et al. 2002). Since hOBPIIa is non-specific, the protein can bind with a large number of volatile hydrophobic odorants of different concentrations, which is accomplished through formation of non-covalent bonds (Pelosi 2001). hOBPIIa is also stable at room-temperature, pH and proteolytic digestion (Whitson and Whitson 2014). The protein can also be manufactured and purified using recombinant bacterial DNA technology at a low cost (Silva et al. 2014). All these characteristics make the human odorant binding protein a viable option to be used in a biosensor application (Ko et al. 2010).

1.2.3 Biosensor Development Using OBP

OBPs have high thermal stability, which is ideal for environmental monitoring. They are also not affected by the presence of an increased concentration of organic solvents (Wei et al. 2008). OBPs can easily accommodate site-directed mutagenesis, which can make them bind specifically with certain compounds (Wei et al. 2008). For any solution to work commercially, cost is an important consideration, and this is alleviated in the case of using OBPs as biosensors since they can be readily synthesized from recombinant DNA in bacteria as per Silva et al. (2014). Re-engineered pOBP has been used to monitor polycyclic aromatic hydrocarbons in the environment due to its good affinity in binding with such compounds and its ability to modify specificity by changing amino acid residues. (Figure 8) (Wei et al. 2008).

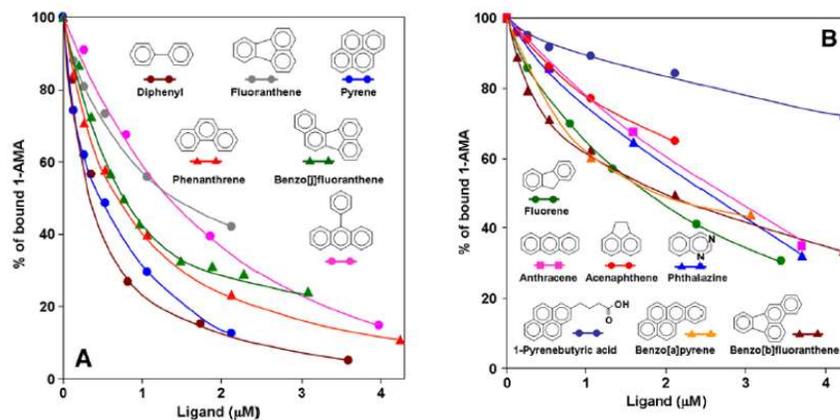


Figure 8: 1-AMA competitive binding assay of pOBP-m2 (pig OBP mutant) with different polycyclic aromatic hydrocarbons. 1 μ M 1-AMA and 1 μ M protein were used in an increasing concentration of methanolic solutions of aromatic compounds starting from 1 mM (Wei et al. 2008)

An effective biosensor needs to be sensitive enough to detect different compounds (Kim et al. 2008; Song et al. 2008; Mirmohseni et al. 2008), and there have been efforts to improve the sensitivity of systems relying on biosensors (Choi et al. 2004; Kang et al. 2006; Lee et al. 2009; Kim et al. 2009; Yoon et al. 2009). In the case of olfactory receptor-based biosensors, OBPs can be used to boost the sensitivity (Ko & Park 2008). This enhancement for OBP through interacting with the receptor has been described for *Drosophila* OBP (Xu 2005).

OBPs have also been used for detecting important ligands in complex environments. In work by Lu et al. (2014), honeybee OBPs were designed to detect ligands found in floral odors and pheromones. Ramoni et al. (2007) investigated the use of advanced nano-biosensors derived from bOBP and immobilized into carbon nanotubes to detect the presence of hazardous compounds in luggage storage facilities, airports and other public places. The same author also investigated the detection of explosive compounds using the protein scaffold of the lipocalin OBP (Ramoni et al. 2007). However, none of these applications address the challenge of measuring odorant concentrations. Moreover, none of the applications described here use human odorant binding protein for their purpose.

1.3 Fluorophores

Fluorophores are used to study the interaction of different ligands with proteins, among proteins themselves as well as in the study of protein properties and structures (Mikhailopulo et al. 2008; Abdurachim et al. 2006; Sreejith et al. 2009; Kazakov et al. 2009). Addition of an extrinsic fluorophore such as TNS (6,P-toluidinylnaphthalene-2-sulfonate) and fluorescein to a protein does

not modify the structure of the protein in any way, and this property makes such fluorescent compounds effective probes for the purpose of study (Kmieciak & Albani 2010).

In biology and medical science, small-molecule fluorescent turn-on probes are used to detect specific proteins. Such probes are especially capable of detecting and monitoring enzyme activities such as glycosidases, proteases, lactamases, and kinases (Kobayashi et al. 2009, Sakabe et al. 2012, Xing et al. 2005, Shults & Imperiali 2003). Generally, such fluorescence mechanisms work by reacting with the enzyme and converting from a non-fluorescent product to a fluorescent one. However, there are also some environmentally sensitive fluorophores with emission properties depending on the environment. Such fluorophores display weak fluorescence in polar environments but show strong fluorescence in hydrophobic environments (Figure 9) (Zhuang et al. 2013). Most of the ligand binding sites of proteins are hydrophobic in nature. So, binding of ligands to the hydrophobic ligand-binding domain will cause an environmentally-sensitive fluorophore to be closer to the hydrophobic region of the protein and emit high fluorescence. However, not all ligands bind with all proteins. Differences exist in the nature of hydrophobic molecules bound to the proteins. This difference arises because of the amino acid residue surrounding the hydrophobic pocket in proteins (Flower 1995). An example of this case will be progesterone binding to α 1-acid glycoprotein but not to β -lactoglobulin (Kmieciak & Albani 2010).

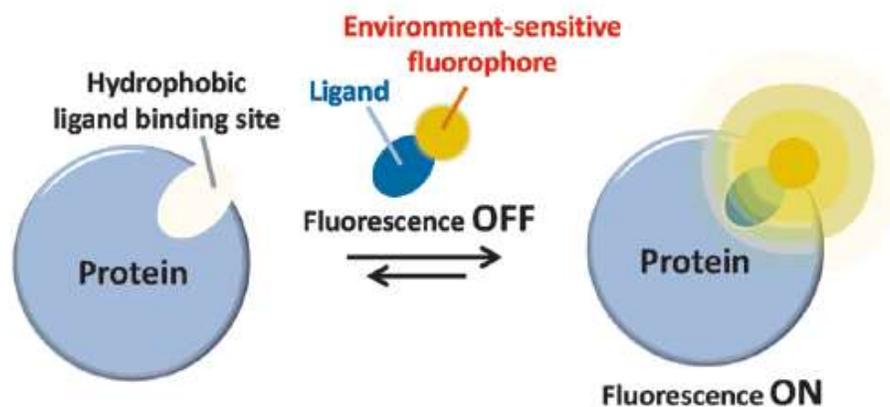


Figure 9: Mechanism of fluorescent turn-on probe where the ligand attaches to the specific hydrophobic ligand binding site of the protein and the surrounding hydrophobic environment allows the environment-sensitive fluorophore to emit strong fluorescence (Zhuang et al. 2013)

To date, the interaction of several fluorophores have been investigated with hOBPIIa (Briand et al. 2002). The binding capability of a recombinant hOBPIIa has been tested with a number of extrinsic fluorescent probes such as DAUDA (11-(5-(dimethylaminonaphthalenyl-1-sulfonyl) amino) undecanoic acid), NPN (*N*-phenyl-1-naphthylamine), DACA (dansyl-DL- α -(aminocaprylic acid) and ASA ((\pm)-12-(9-anthroyloxy)stearic acid). (Mei et al. 1997; Lechner et

al. 2001). The results showed that DAUDA shows a weak emission spectrum with a peak at 490 nm, while DACA, ASA and NPN had their maximas at 475 nm, 425 nm and 400 nm respectively (Briand et al. 2002). Figure 10 shows the titration curve of NPN and DAUDA binding with 2 μ M hOBPIIa. The spectral properties of probe fluorescence emission confirm that the binding site of the recombinant hOBPIIa is present in the hydrophobic pocket within the β -barrel.

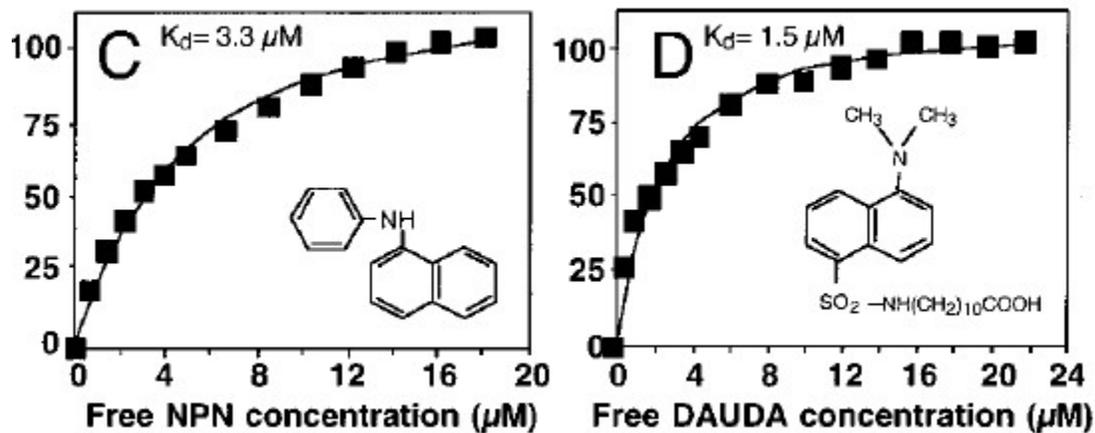


Figure 10: Titration curve of 2 μ M hOBPIIa against the fluorescent probes NPN (left) and DAUDA (right) at increasing concentrations (Briand et al. 2002)

1-aminoanthracene (1-AMA) is a widely used fluorophore to study the interaction among lipocalin family proteins. As a hydrophobic ligand, 1-AMA only shows fluorescence when bound to a hydrophobic site in a protein. While studying the fluorescence binding assays of several OBPs using 1-AMA, it has been showed in a number of studies that the peak intensity of the OBP-1-AMA complex increases with a blue-shifted emission compared to that of free 1-AMA solution just by itself (Gonçalves et al. 2018; Silva et al. 2014; Paolini et al. 1999; Briand et al. 2003).

Since 1-AMA is a hydrophobic fluorophore, various experiments have been performed with different alcohols as solvents to find out the displacement of 1-AMA by a particular solvent. Experiments with ethanol, methanol and dimethyl sulfoxide have revealed that methanol has a relatively low rate of displacement of the fluorophore, which leads to methanol being an attractive solvent for using 1-AMA as a marker in OBP experiments (Figure 11) (Briand et al. 2000). The mixture of 1-AMA with methanol has the potential to be used as a fluorescent probe to study the interaction of hOBPIIa and various odorants in more detail as used in the cases of rat and porcine OBPs.

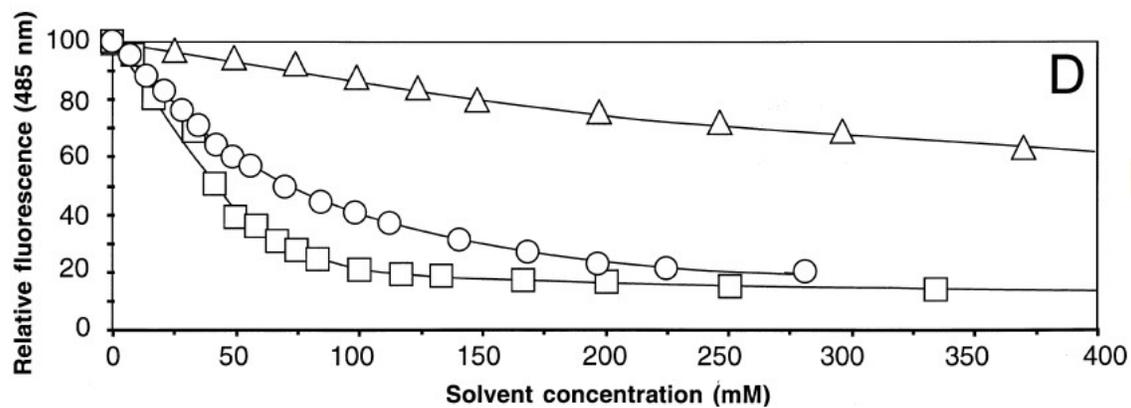


Figure 11: Fluorescence curve of 1-AMA at 485 nm with increasing solvent concentration. 1-AMA is displaced the least by methanol, leading to relatively high fluorescence emission even though the solvent concentration is increased (Triangle: methanol, Circle: ethanol, Square: dimethyl sulfoxide) (Briand et al. 2000)

2.0 METHODS

2.1 Purified hOBPIIa

The purified human odorant binding protein (hOBPIIa) was produced following the protocol described by Roblyer (2017). The bacterial expression plasmid containing the coding sequence for the recombinant protein variant hOBPIIa was cultured and induced in *E. coli*. The protein was then isolated from the batch, and a Bradford assay analysis was conducted to determine the concentration of the induced, purified protein samples (Figure 12).

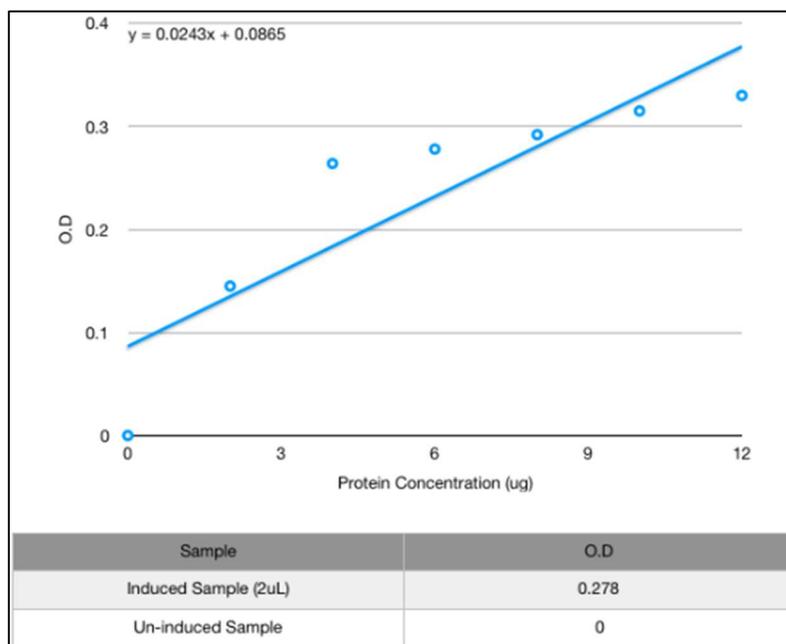


Figure 12: Bradford curve showing the relationship of optical density to protein concentration between induced and uninduced samples

After obtaining a positive result from the Bradford assay, an SDS-PAGE electrophoresis was conducted to verify the base-pair size of the purified protein (Figure 13). The measured optical density (OD) and linear equation were used to calculate the amount of isolated protein to be 3.94 $\mu\text{g}/\mu\text{L}$ in 50 mM Tris-HCl, pH 7.4 solution, with a molecular weight of around 17 kDa.

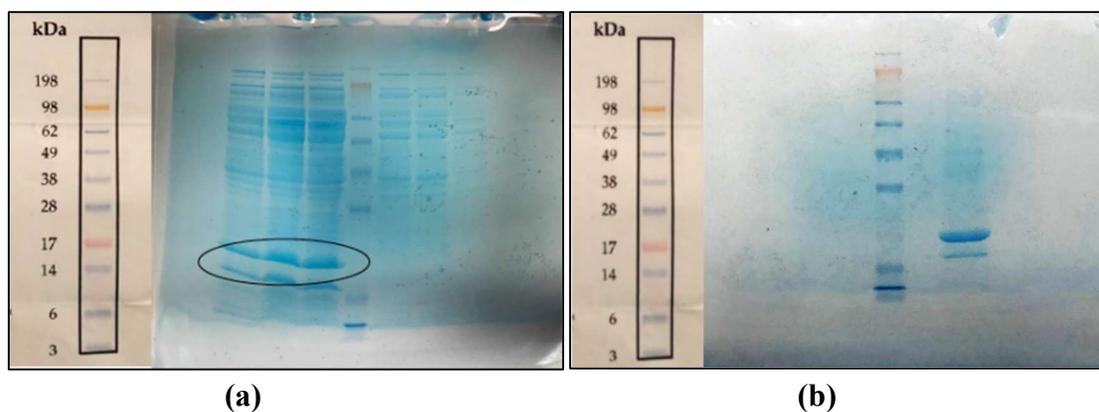


Figure 13: (a) 12.5% SDS-PAGE Gel of crude protein (b) 12.5% SDS-PAGE Gel of purified protein

2.2 Fluorescence Binding Assay

For analyzing the competitive binding assay of the biosensor complex to the odorants, 1-AMA was used as the fluorescent probe, and pure gases such as hydrogen sulfide, ammonia, methane and methyl mercaptan were selected as model compounds/odorants. Also, two gas mixtures were used whose components are present in typical landfill gas. The following gas cylinders were procured from Geotech Environmental Equipment, Inc. (Colorado, USA):

- A. 29 L containing H₂S, 25 ppm (+/- 5%) gas balanced with N₂
- B. 34 L gas cylinder containing NH₃, 25 ppm (+/- 5%) gas balanced with N₂
- C. 34 L gas cylinder containing CH₄, 25 ppm (+/- 5%) gas balanced with air
- D. 34 L gas cylinder containing N₂ (99.998%)
- E. 34 L gas cylinder containing a mixture of H₂S, 25 ppm (+/- 5%), CO, 50 ppm (+/- 5%) and CH₄, 2.5% (+/- 2%) gas balanced with air

And one more 34 L gas cylinder containing methyl mercaptan, 50 ppm gas balanced with N₂ was obtained from ShopCross (North Carolina, USA).

The mixtures provided a starting point for laboratory scale testing with known odorant gas mixtures and were selected based on their market availability.

The fluorophore, 1-AMA (technical grade, 90%) was obtained from Sigma Aldrich in powdered form. Since 1-AMA is hydrophobic, it was first dissolved in 100% methanol and then deionized water was added to the solution for a final methanol concentration of 10%. Methanol is used as a solvent for 1-AMA, since it displaces the fluorophore from the protein in lower amounts compared to other options such as ethanol and dimethyl sulfoxide (see Section 1.3). This in turn allows a better measurement of the effect of odorant displacement of 1-AMA. Due to light sensitivity, 1-AMA was stored in an amber colored glass bottle when not in use. Previously, in an experiment conducted by Roblyer (2017), it was evaluated that the optimal ratio of hOPBIIa to 1-AMA is

approximately 1:1. Following up from that experiment, to prepare the biosensor complex, 1 μ M hOBPIIa was mixed with 1 μ M 1-AMA (1:1 ratio of protein to fluorophore) solution in 50mM potassium phosphate-KOH, pH 7.5 buffer solution. The solution was then transferred to an enclosed reactor chamber (refer to Section 2.3).

To conserve protein, the total volume of the aqueous protein/1-AMA solution in each experiment was limited to 10 mL. The headspace gas was allowed to escape from the chamber during this process through a one-way valve, so that the gas would not exert any undue pressure on the chamber. The buffer solution keeps the pH of the system at a constant level of 7.5, so that the properties of the solution remains unchanged when combining with acidic or basic gases. The reactor chamber containing the buffered protein/1-AMA complex in solution was then exposed to pressurized gas for a controlled amount of time. Three different flow rates (0.5 standard liters per minute or slpm, 0.7 slpm, and 0.9 slpm) were used for each of the gases (i.e. three separate experiments for each gas) to examine the competitive binding assay of the biosensor complex. In each experiment, at periodic intervals of exposure time, 100 μ L subsamples were drawn separately from the reaction zone by means of disposable syringes attached to the top of the chamber. The samples were then transferred to a quartz cuvette for spectrofluorometric analysis. The schematic diagram of the experimental setup is illustrated in Figure 14.

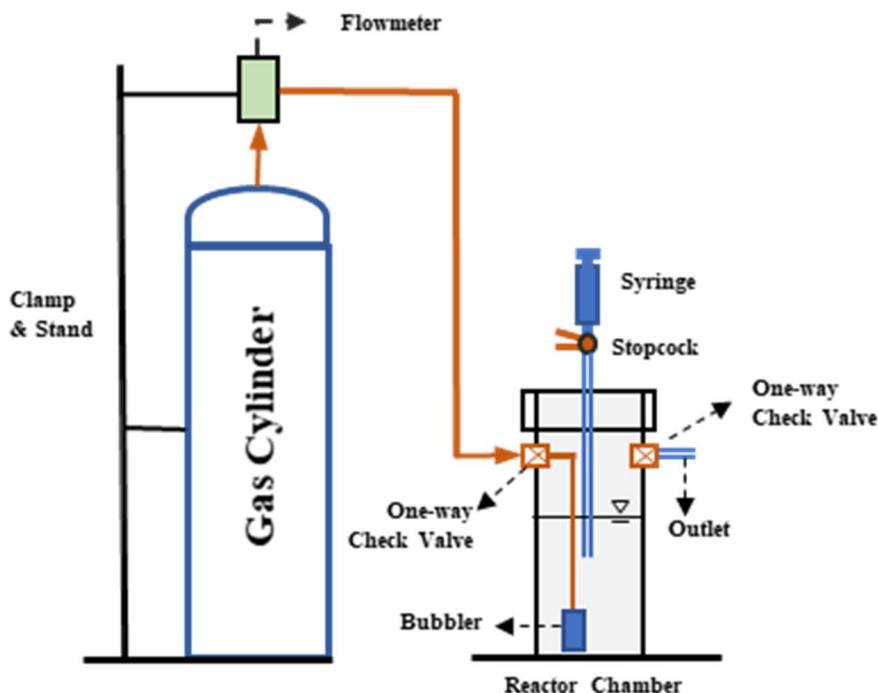


Figure 14: Schematic diagram of the experimental setup

2.3 Reactor Chamber

A flowmeter (0.1-1 L/min capacity) connects a gas cylinder with specialized regulator to the reactor chamber with an inflow check valve, to prevent backflow, using quarter-inch flexible pipe tubing. Another flexible pipe tubing section leads from the inflow check valve into the top of the reaction chamber and ends in an aquarium-grade pumice stone bubbler used to increase the surface area of exposure to the dispersed influent gas. The reactor chamber was built using a modified 50 mL centrifuge tube. By means of a second one-way check valve to prevent reverse contamination of the reaction zone with outside air, the gas escapes through the top of the reactor chamber. The biosensor complex exists in the 10 mL buffered solution located at the bottom of the reaction chamber. The use of a narrow centrifuge tube as the reactor chamber increases the efficiency of the process by exposing a reduced surface area of the protein/1-AMA complex to the headspace above. Due to this fact, the amount of odorant escaping the reaction chamber is reduced. Another benefit comes from the tube being elongated, which improves the likelihood of successful binding with the protein complex as the target odorant gas travels a greater distance to reach the surface. This design minimizes short circuiting of odorant gas in the reaction chamber.

To collect samples for fluorometry, a 3-way stopcock is inserted into the cap of the centrifuge tube (Figure 15a). One of the ports (shown as #4 in Figure 15b) of the stopcock was only opened to draw a sample of the solution from the chamber using a gas-tight syringe, which could be screwed on using a Luer-lok mechanism. Each time after collecting sample, an empty new syringe was substituted for the old one with the sample in it. The other port was kept closed for the duration of the experiment. This setup ensures that the inside of the reaction chamber is never exposed to the external environment, thus eliminating the introduction of possible contaminants. The experimental apparatus was set up under a fume hood to safely exhaust the odorant gases after passing through the reaction chamber.

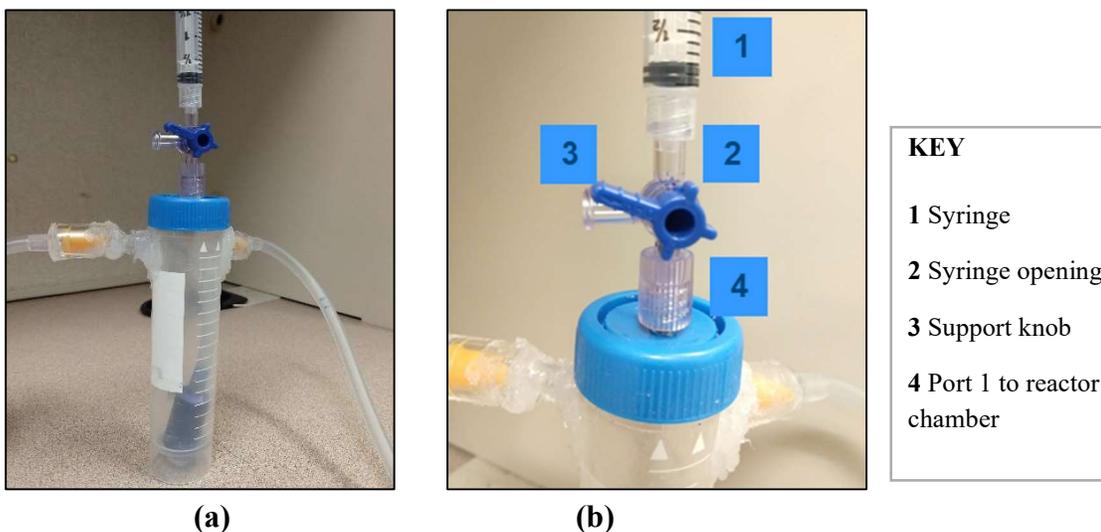


Figure 15: Reactor chamber using a centrifuge tube (a) and 3-way stopcock used on the lid of the exposure chamber (b). The different ports and other parts are labeled

For experiments with gas mixture 1, a Y-connector was used to supply the two gases into the reactor chamber from two separate cylinders in the same way that a single cylinder is used for the other pure gases or single cylinder mixtures.

2.4 Fluorescence Measurements

Fluorescence spectroscopy (i.e. spectrofluorometry) was conducted using a Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (Horiba-Jobin Yvon, Longjumeau, France) (Figure 16a). Two hours prior to starting the experiments, the spectrofluorometer was powered up so that ample time was provided to the instrument for warm up. The fluorescence emission spectra were recorded using FluorEssence software (Figure 16b) at room temperature using an excitation wavelength of 380 nm. The emission spectrum was recorded between 410 and 700 nm at 1 nm intervals.

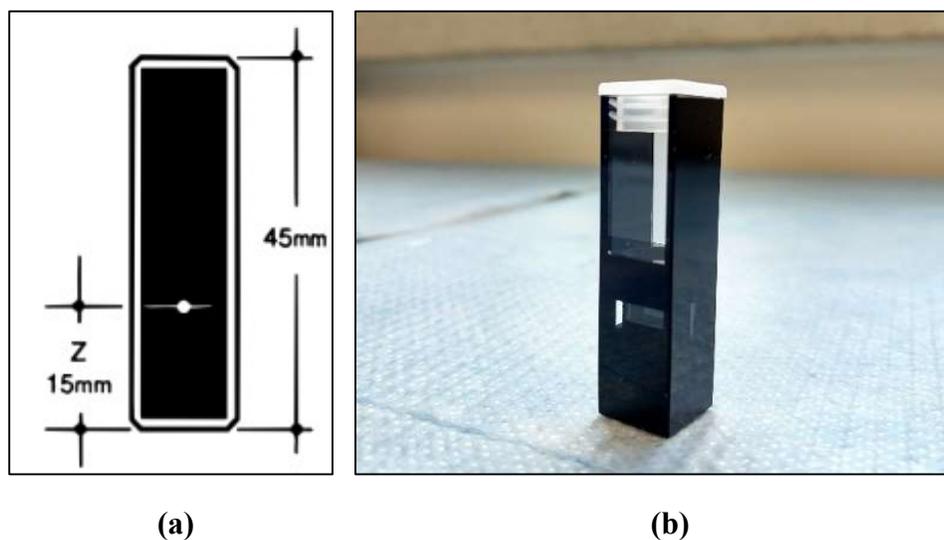


(a)

(b)

Figure 16: Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (a) and Fluorescence software screenshot (b)

Samples were collected using 100 μL capacity (nominal volume) fluorometric quartz cuvette (“Z” Dim 15 mm) with 10 mm path length (Figure 17). Slit widths were set to 5 nm in both excitation and emission stages. Between sample collection in the cuvette, care was taken to wash the cuvette properly using diluted ethanol and deionized water so that no residue remains in the cuvette that could cause cross-contamination.



(a)

(b)

Figure 17: 'Z' dimension (distance from the base to the center of the sample chamber window) of the quartz cuvette (a) and a 100 μL capacity quartz cuvette containing 100 μL of sample each time (b)

2.5 Outlet Gas Concentration Detection

As odorant gas is passed into the reaction chamber, it is assumed that all of the gas reacts with the biosensor solution, and none of the gas or biosensor escapes through the outlet valve. However, this assumption needs to be verified. For that purpose, a MiniRAE Lite photoionization detector (PID) was used to test for the presence of volatile organic and some inorganic compounds (Gastech n.d.). In this case, the device is capable of measuring in the range of 0~5,000 ppm (RAE Systems Inc. 2013). PIDs come with a water trapping filter made of PTFE (polytetrafluoroethylene) Teflon membrane with a pore size of 0.2 micron that can be used to detect the presence of gases dissolved in droplets, while in the “without-filter” condition, the detector can only detect free gas molecules. The filter captures droplets and separates dissolved gas molecules from them. These gas molecules pass into the detector afterwards so that their concentration can be measured.

For this verification experiment, a PID sensor was connected to the outlet tubing both with and without the filter to verify whether there is any gas present at the outlet of the reaction chamber. Figure 18 shows the setup of both instances of the experiment.

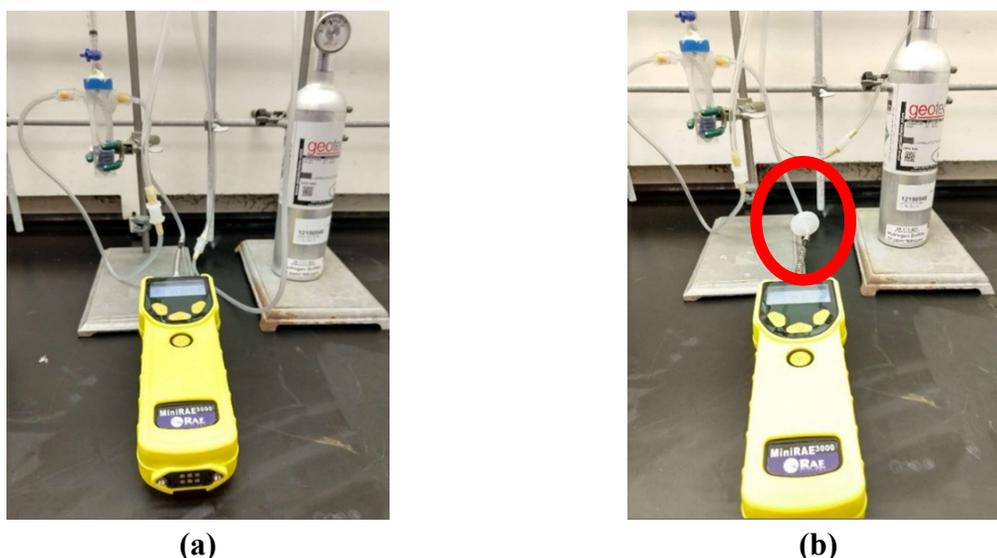


Figure 18: Experimental setup for verifying whether there is any gas present at the outlet of the reaction chamber without filter (a) and with filter circled in red (b)

2.6 pH

Some of the selected odorant gases are acidic or alkaline, and since it is known that even small changes in pH can denature proteins (O’Brien 2012), it was important to monitor for any pH changes detected during the reaction exposure time. Since the reactor chamber is closed, it was not possible to measure pH in realtime using a pH meter. However, in all the experiments, pH strips

were used to measure the pH of the samples collected at each time interval. No pH change was observed during testing. Therefore, as expected, the buffer solution was effective in keeping the pH of the biosensor solution constant.

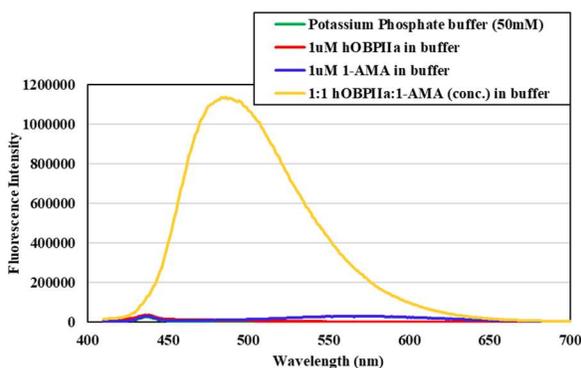
3.0 RESULTS

3.1 Fluorescence Binding Assay with Extrinsic Fluorophore (1-AMA)

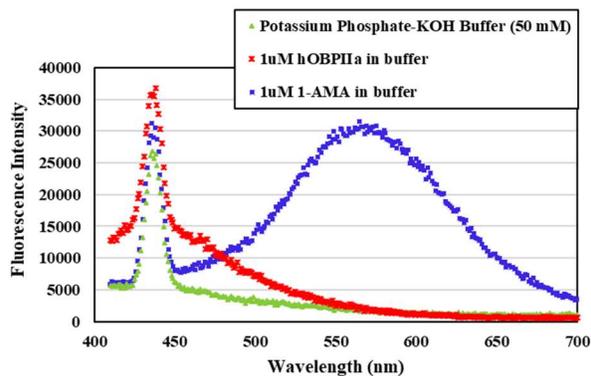
Spectrofluorometry is used to assess the binding assay between hOBPIIa and different odorant gases where 1-AMA, which forms a complex with OBPs, has an effective role in understanding the interaction between the protein and odorants. 1-AMA is a fluorophore whose quantum yield increases when it remains in a hydrophobic environment. OBPs contain a hydrophobic cavity inside their β -barrel (Briand 2002) and this explains the increased quantum yield when 1-AMA forms a complex with these proteins. 1-AMA by itself in an aqueous solution does not have the same high quantum yield (Briand 2000).

To demonstrate that each component of the biosensor does not create a positive interference with the assay, each individual component was tested for background fluorescence signature and compared to the complete biosensor molecule. Figure 19a shows the spectrofluorometric emission spectra of the four different solutions excited at 380 nm:

1. 50 mM Potassium Phosphate-KOH, pH 7.5 buffer solution only
2. 1 μ M 1-AMA only in buffer
3. 1 μ M hOBPIIa only in buffer
4. 1:1 hOBPIIa:1-AMA in buffer (complete biosensor)



(a)



(b)

Figure 19: Spectrofluorometric emission spectra for four different solutions (a) and magnified curves of the other three solutions except solution #4 (b)

For the complete biosensor consisting of hOBPIIa and 1-AMA in buffer solution (solution #4), a sharp spike of greater than 1,100,000 fluorescence intensity units is observed near 485 nm. This is 2 orders of magnitude larger than the fluorescence intensity obtained for 1-AMA solution alone in

the buffer (~30,000), with peak at 565 nm. So, a blue shift emission spectrum was observed in this case along with the increased fluorescence intensity. Figure 19b shows only the fluorescence signature for solutions 1, 2, and 3, which were not clearly distinguishable in Figure 19a due to the 2 orders of magnitude difference in intensity when compared to the complete biosensor molecule (solution #4). The resulting difference in intensity for hOBPIIa is even higher than previous results reported in Briand et al. (2000) for other OBPs e.g. rat OBP-1F (15-fold increase) and porcine OBP-1 (80-fold increase). All other combinations in the fluorescence intensity curves show small spikes in the 400-450 nm range. These peak emissions are likely due to light scattering effects. There are no other peaks near 485 nm for any of the component curves, which provides evidence that the protein-fluorophore complex (hOBPIIa:1-AMA) is responsible for the high emission intensity at 485 nm. These results also verify the findings obtained by Roblyer (2017).

Next, the optimum concentration ratio between 1-AMA and hOBPIIa was verified by conducting a saturation binding experiment of the protein and fluorophore complex. Keeping the concentration of hOBPIIa fixed at 1 μM , the concentration of 1-AMA was increased in increments of 0.1 μM , starting from 0.1 μM up to 1.5 μM . Figure 20 shows the binding curve of hOBPIIa with 1-AMA. The fluorescence intensity of the complex increases as the concentration of 1-AMA is increased gradually. The binding experiment reaches the saturation point at around 1 μM of 1-AMA. With further increase in the concentration of 1-AMA, the fluorescence intensity increase is negligible. The concentration ratio of 1-AMA to hOBPIIa found here confirms the same ratio obtained by Roblyer (2017).

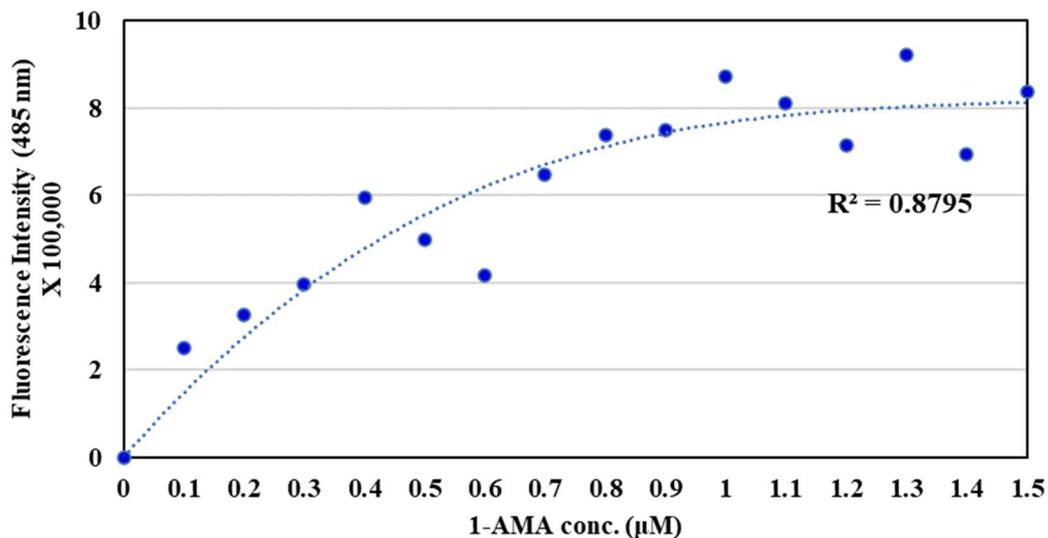


Figure 20: Binding curve of 1-AMA at different concentrations with hOBPIIa

3.2 Biosensor Sensitivity Experiments on Model Compounds

For selecting model odorants, some of the more commonly found gases in landfills were considered. Hydrogen sulfide is considered for its pungent smell and also due to its acidic nature. Ammonia is another common landfill gas and is alkaline. In landfills, mixtures of gases are usually present, and this has been replicated using a commercially available gas mixture of methane, hydrogen sulfide and carbon monoxide. Methane has been tested by itself as well so that its response can be better understood. Methyl mercaptan has also been selected, since it has a very low detection threshold and a powerful smell. All of these gases were procured in cylinders where they are balanced with either air or nitrogen.

3.2.1 Experimentation with Nitrogen (N_2)

Since many of the odorants to be tested are balanced with nitrogen in the cylinder, it is necessary to check if nitrogen gas has any effect on the biosensor solution. By passing 99.998% N_2 gas at a rate of 0.5 standard liters per minute (slpm) into the biosensor solution, it is confirmed that nitrogen gas has a negligible effect on the biosensor solution and is unable to lower the fluorescence intensity of the solution appreciably indicating that the nitrogen gas molecules do not competitively bind to the biosensor (Figure 21).

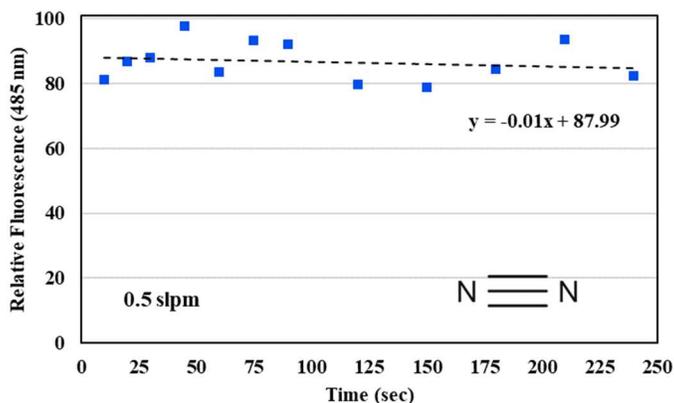


Figure 21: Graph showing peak emission intensity against time for nitrogen gas flowing at 0.5 slpm

3.2.2 Experimentation with Hydrogen Sulfide (H_2S)

One of the objectives of this research was to replicate and verify previous experimentation carried out by Roblyer (2017), where by passing hydrogen sulfide (25 ppm +/- 5% balanced with N_2), an inverse relationship was established between peak fluorescence intensity occurring at 485 nm and the amount of hydrogen sulfide. In that experiment, a total of 100 mL protein-fluorophore complex was used for a protein to fluorophore ratio 1:1 (i.e. 1 μ M hOBPIIa and 1 μ M 1-AMA), which was then exposed to 0.5 standard liters per minute (slpm) of hydrogen sulfide gas in a prototype reactor

chamber. Samples of 1 mL were extracted at a constant time interval of 30 seconds for a total range of 450 seconds for spectrofluorometric analysis. The fluorescence intensity decreases with time as shown in Figure 22a. The results established an inverse fluorescence relationship with time below 200 seconds and showed increased scatter above that time limit (Figure 22b). The decrease in intensity is due increasing mass of gas binding with the biosensor complex solution. In other words, more of the 1-AMA initially bound to the protein is substituted by the odorant gas, which decreases the fluorescence intensity.

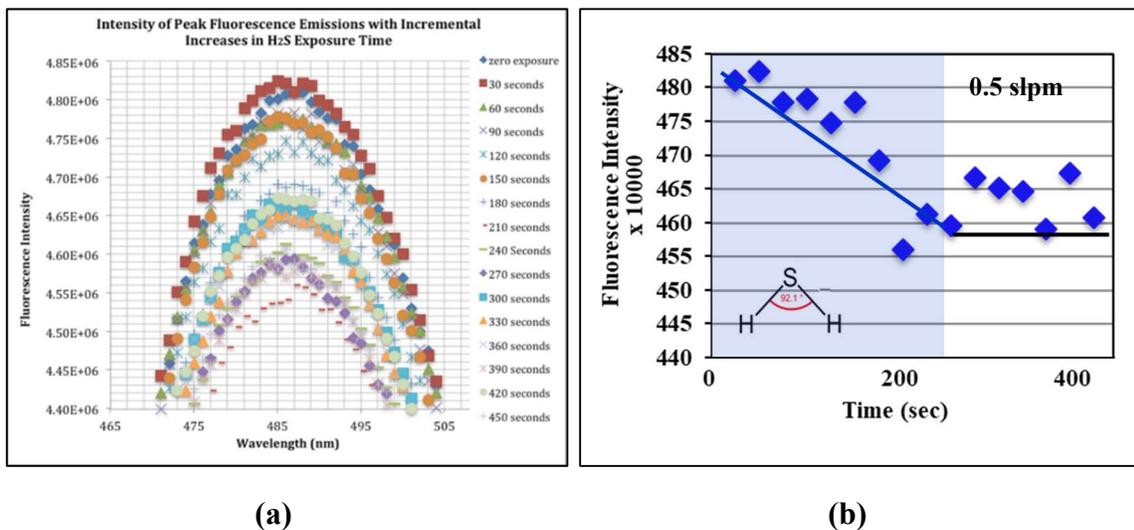


Figure 22: Spectrofluorometric emission spectra at 380 nm excitation for 0.5 slpm hydrogen sulfide found by Roblyer (2017) (a), and graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)

In the verification experiment, the concentration of the protein and 1-AMA were kept the same as the previous ratio, but due to limited protein availability and the capacity of the improved reactor chamber being only 50 mL, the total volume of the biosensor complex was adjusted to 25 mL as compared to 100 mL in the previous experiment. Therefore, it was necessary to adjust the sampling time interval to analyze the fluorescence intensity trend correctly. The overall experiment runtime was also kept shorter since it was anticipated that a shorter time would be required for the hydrogen sulfide gas to bind with the protein present in the smaller reactor chamber. Figure 23a shows the measured emission spectra where the emission peaks occurred at ~485 nm as expected for each 1 mL of sample for the biosensor complex. Also as expected, an inverse relationship between fluorescence intensity and the amount of time the biosensor was exposed to hydrogen sulfide gas, as shown in Figure 23b, was observed when plotting the peak emission obtained at each sampling time. The intensity decreases initially in a linear fashion with increasing exposure to hydrogen sulfide, as in the previous experiments reported by Roblyer (2017).

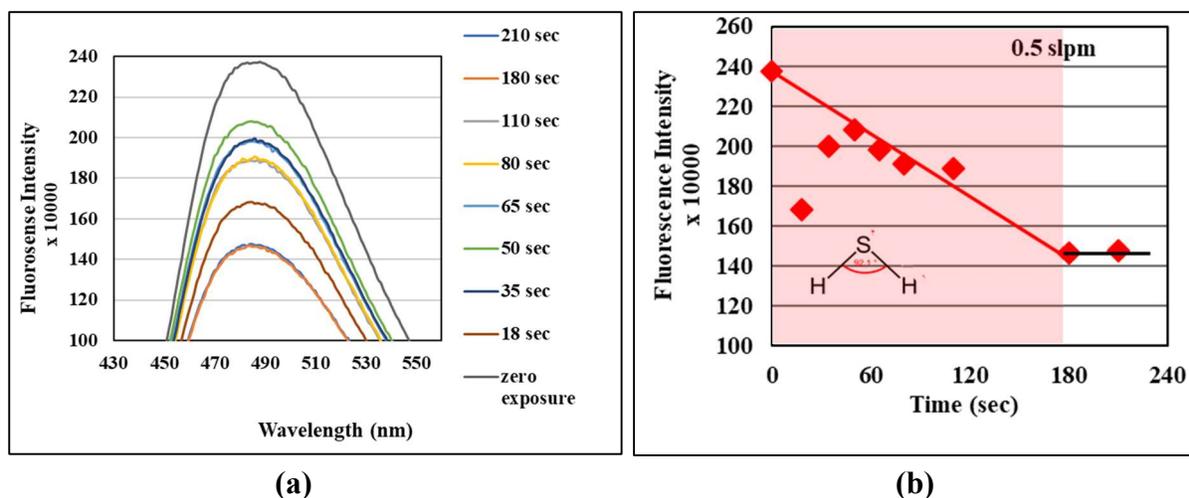


Figure 23: Spectrofluorometric emission spectra for excitation at 380 nm for the verification experiment at 0.5 slpm hydrogen sulfide for 25 mL sample (a), and graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)

The quantitation range, i.e. the time up to which the biosensor complex shows an inverse relationship between emission intensity and the odorant gas exposure time is derived by plotting linear trendlines, y-intercepted at the initial value and up to the point of any substantial decrease in fluorescence intensity. Therefore, the estimated quantitation range maximum is the point in which the initial linear trendline and the zero-slope portion of the curve intersect around approximately around 120-180 seconds. The initial intensity reading obtained after the start of the experiment is observed in this experiment to drop sharply compared to some of the readings following that point. This may just be an artifact of the analytical technique or might be caused by the protein initially binding strongly with the odorant once it starts flowing but releases some of it with time, leading to higher intensity values in the next few seconds of exposure due to mass transfer limitations. If the second case is true, then a more accurate general linear trend can be obtained by starting the analysis after one minute exposure, which seems to be the lag time to reach a stable decreasing relationship.

A similar experiment was conducted using 10 mL of biosensor complex and keeping the same gas flow rate (0.5 slpm) to verify whether similar results can be obtained for an even smaller amount of biosensor solution. A reduced sample volume of 100 μ L was drawn at 10 second intervals for the first 30 seconds followed by a 15 second interval up to 90 seconds and then a 30 second interval for the remainder of the experiment. Figure 24a shows the intensity curves obtained at different times. The intensities are shown in relative terms, which allows for better comparison of the results. As time passes, the intensity decreases gradually as before. Figure 24b shows the graph plotted for peak emission intensity against time of gas exposure. The graph also shows the quantitation range of the biosensor solution and has been derived by plotting linear trendlines in a similar manner as mentioned for earlier experiments, but y-intercepted at 100 (since the relative intensity of all

samples at the beginning is 100). In this case, the quantitation range is around 120 seconds after which the intensity does not show a considerable change with time. A probable reason for this is that the biosensor complex becomes saturated by the hydrogen sulfide gas passed through it within the first 120 seconds, thus establishing a possible quantitation range for a gas flowrate of 0.5 slpm.

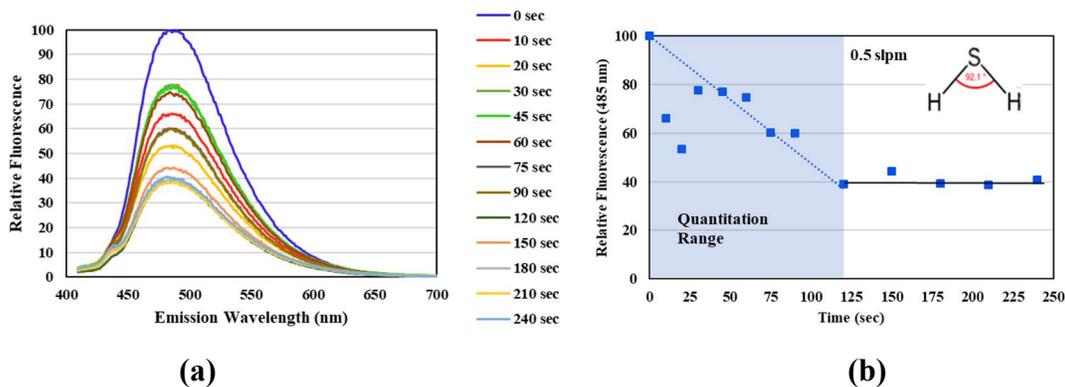


Figure 24: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.5 slpm hydrogen sulfide and 10 ml sample (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)

Adjusting the flow rate to 0.7 slpm of hydrogen sulfide gas, intensity curves were obtained at different times in a similar manner as shown in Figure 25a. As shown in Figure 25b, the biosensor complex becomes saturated in a slightly shorter time than before (approximately around 110 seconds), giving an even lower quantitation range at this higher flow rate. This is logical since the higher gas flow rate causes more of the odorant gas to bind with the biosensor complex more quickly and so, saturation occurs faster than before.

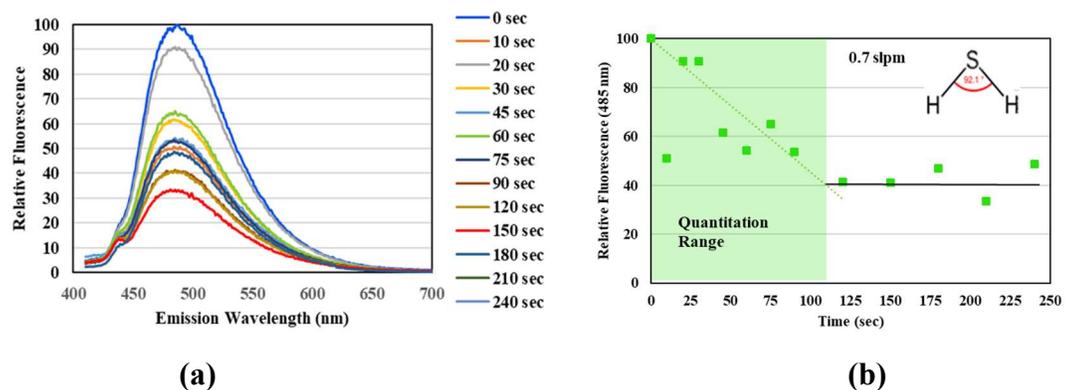


Figure 25: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.7 slpm hydrogen sulfide (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)

An even higher flow rate of 0.9 slpm was used to check whether the quantitation range falls even further. The results are shown in Figure 26a, where the biosensor complex becomes saturated in an even shorter time than the previous two cases (approximately around 90 seconds in Figure 26b). These experiments provide evidence that the process can be miniaturized to use a small amount of biosensor solution with a short response time.

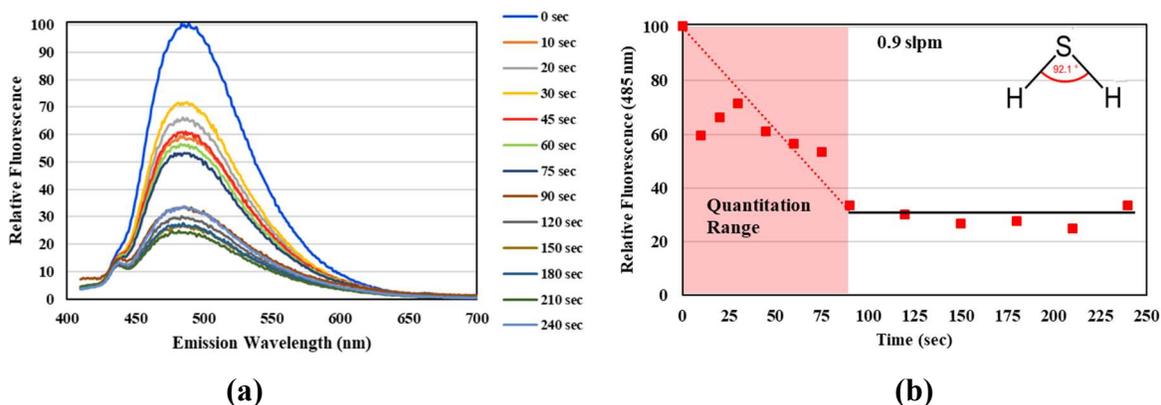


Figure 26: Spectrofluorometric emission spectra for excitation taking place at 380 nm for 0.9 slpm hydrogen sulfide (a), and a graph showing the inverse relationship between peak fluorescence intensity and exposure time for the same experiment (b)

In Figure 27a, a comparison is made between the effect of the three different flow rates for hydrogen sulfide gas. The quantitation range (or instrument response time) is highest for the lowest flow rate and lowest for the highest flow rate. Also, the decrease in intensity is faster for the higher flow rate as evident by a steeper slope. This is likely due to increased mass flux of hydrogen sulfide at higher flow rates, which results in increased binding with the biosensor complex in a given time. Although it was expected that a lower gas flow rate would facilitate the mixing of the odorant gas in the biosensor solution, possibly resulting in a shorter quantitation range, this has not been observed in this case as shown by the longer quantitation range found for lower flow rates.

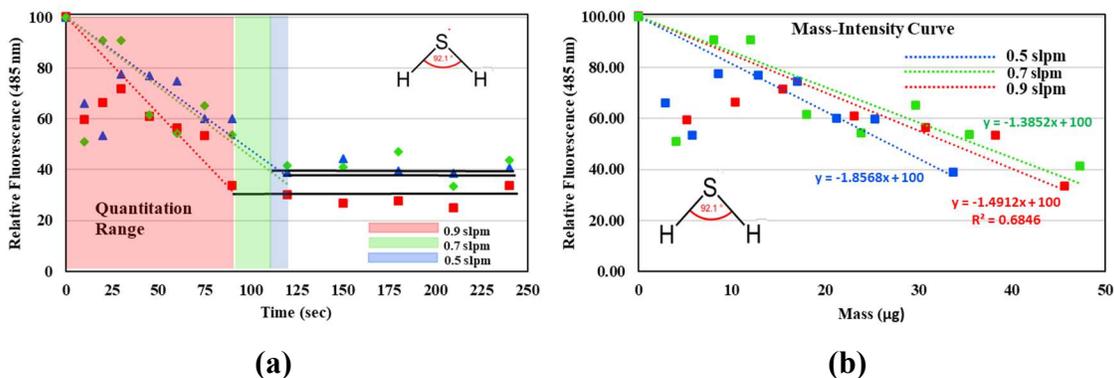


Figure 27: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for hydrogen sulfide (a), and a graph showing change in peak fluorescence intensity with mass flow of hydrogen sulfide at all three flow rates (b)

To quantify odorants measured by this technique, the flow rate of hydrogen sulfide was converted to mass flow using a modified form of the ideal gas equation. Figure 27b shows the mass flow in µg of hydrogen sulfide against decreasing intensity up to the quantitation range in each case. For all three flow rates, the change in intensity with mass is found to be relatively close to each other, signifying that a certain mass of hydrogen sulfide binds with the protein for a certain decrease in intensity. Overall, the 10 mL biosensor solution (around 180 µg of protein) had an upper limit of measuring hydrogen sulfide in the range of around 35-45 µg depending on the flow rate.

3.2.3 Experimentation with Ammonia (NH₃)

Ammonia (25 ppm +/- 5% gas balanced with N₂) was tested using the same flow rates of 0.5 slpm, 0.7 slpm, and 0.9 slpm. The peak emission curves are shown in Figure 28 where similar types of trendlines were observed as that of hydrogen sulfide gas. For the lower flow rate of 0.5 slpm, the intensity seems to decrease up to around 90 seconds before becoming stable. For 0.7 slpm, the quantitation range was around 75 seconds. Whereas, for the 0.9 slpm flow rate, no considerable change is observed after 50 seconds. Although ammonia is an alkaline odorant, none of the experiments indicated a change in pH during testing.

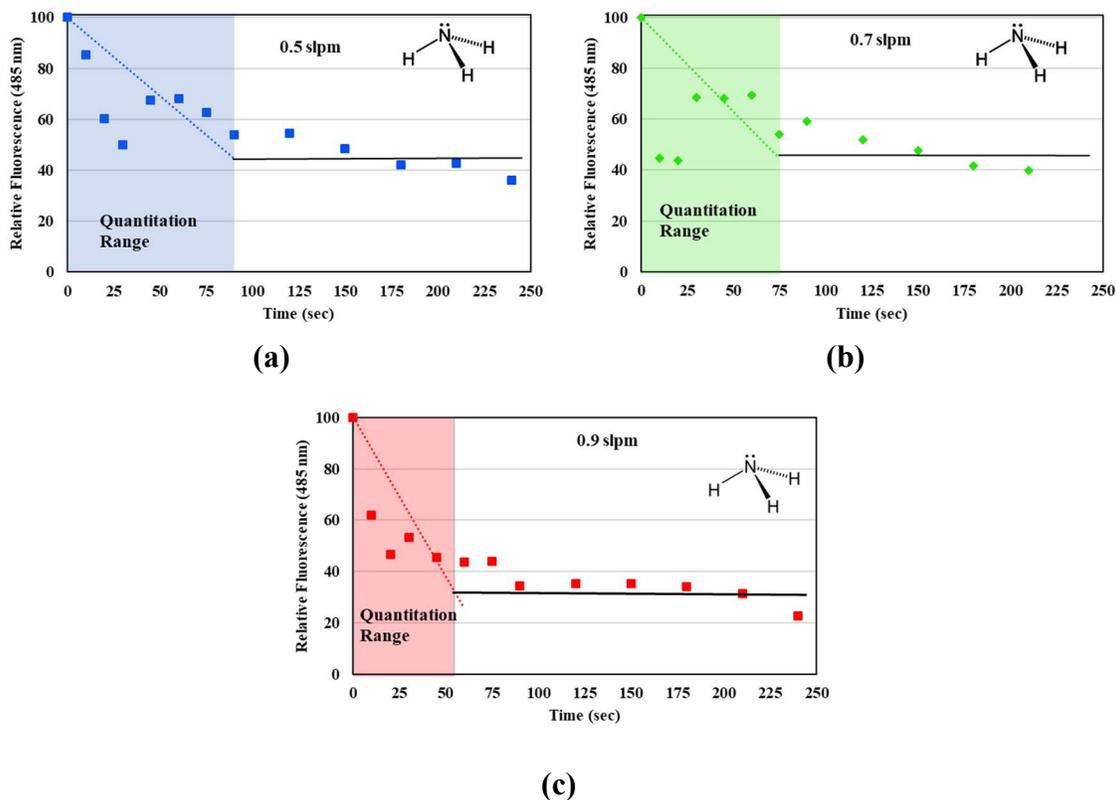


Figure 28: Graph showing peak emission intensity against time for ammonia gas flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)

Figure 29a shows the comparison of the three different flow rates for ammonia. Here again, the quantitation range is highest for the lowest flow rate and lowest for the highest flow rate. Similarly, the higher flow rate curves have a steeper slope, indicating a faster decrease in intensity.

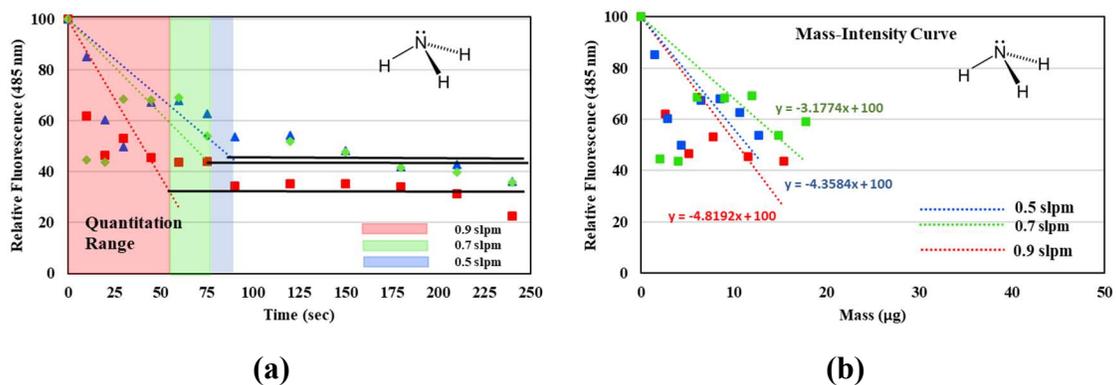


Figure 29: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for ammonia gas (a), and a graph showing change in peak fluorescence intensity with mass flow of ammonia gas at all three flow rates (b)

Figure 29b shows the mass flow of ammonia gas against decreasing intensity. Yet again, the curves show that a certain mass of ammonia gas binds with the protein for a certain decrease in intensity, just like in the case of hydrogen sulfide gas. The same amount of protein is able to measure ammonia up to around 12-18 μg depending on the flow rate.

3.2.4 Experimentation with Methyl Mercaptan (CH_3SH)

The odor threshold value for methyl mercaptan is around 25 times lower than that of hydrogen sulfide (Ruth 1986). Conducting an experiment with a flow rate of 0.5 slpm, the intensity was found to decrease up to around 95 seconds as shown in Figure 30a. For 0.7 slpm, the quantitation range is around 75 seconds, while for the higher flow rate of 0.9 slpm, the range became even shorter than the other two (around 60 seconds) as seen in Figure 30c.

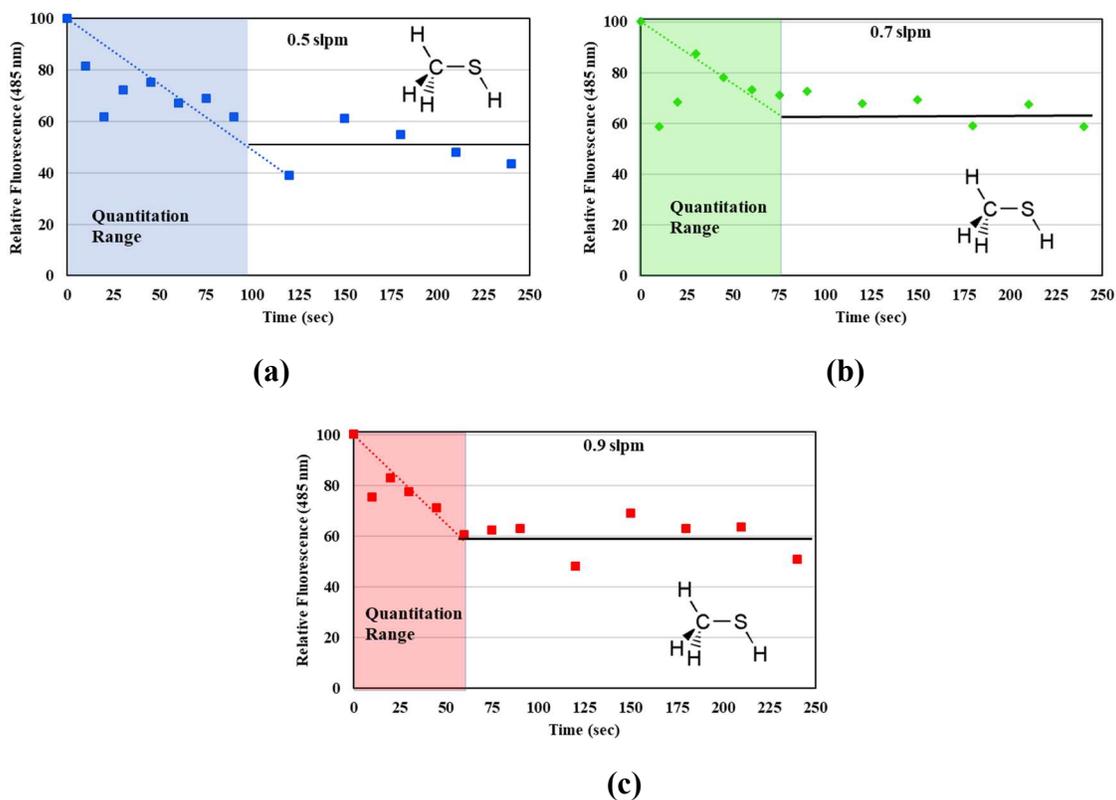


Figure 30: Graph showing peak emission intensity against time for methyl mercaptan gas flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)

The comparative peak fluorescence intensity curves of methyl mercaptan are shown in Figure 31a. The graph is different compared to that obtained for the other gases tested so far. While in the case of the other gases, the intensity decreased more at higher flow rates, this is not completely true in this experiment since the intensity for 0.5 slpm and 0.7 slpm are so similar. As a higher flow rate of methyl mercaptan is passed into the solution, the gas may escape faster from the solution in the

form of droplets due to its highly volatile nature and was thus unable to lower the fluorescence intensity by a larger amount than the lower flow rate. However, the change in quantitation range follows the expected trend, i.e. increases with an increase in flow rate. Figure 31b shows the mass flow curve for all flow rates for methyl mercaptan. Using 180 μg of protein in 10 mL solution, around 83-95 μg of methyl mercaptan can be measured depending on the flow rate.

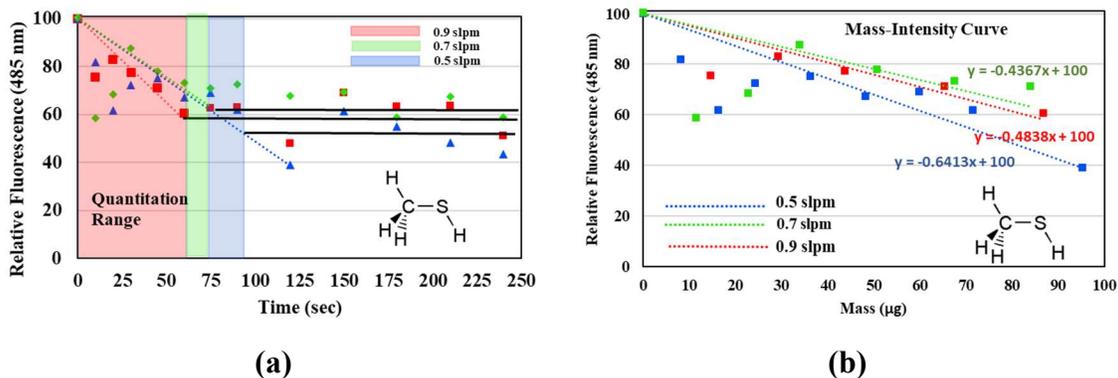


Figure 31: Comparative change in peak fluorescence intensity at 0.5 slpm, 0.7 slpm, and 0.9 slpm for methyl mercaptan gas (a), and a graph showing change in peak fluorescence intensity with mass flow of methyle mercaptan gas at all three flow rates (b)

3.2.5 Experimentation with Methane (CH_4)

A main focus of this research was to determine how the biosensor reacts to gas mixtures. The two different gas mixtures used in this study both contained methane gas, which is a common component of landfill gas. Therefore, methane (25 ppm \pm 5% gas balanced with air) was tested separately with the biosensor. Following the same procedure as in the previous experiments, methane was examined at a flow rate of 0.5 slpm since a lower flow rate seemed to delay biosensor saturation by the other individual gases tested so far. Figure 32a shows the peak emission intensities obtained at different times for this particular experiment. An inverse relationship between time and intensity is maintained for approximately 100 seconds. In Figure 32b, the mass flow of methane against decreasing intensity is shown. Here, up to 15 μg of methane has been measured within the quantitation range. Though the odor of methane cannot be perceived by humans, the biosensor still shows a reaction with the gas. This is due to methane being a purely hydrophobic compound and since odorant binding protein has affinity for hydrophobic ligands, the absence of smell of methane was not an issue in this case (Li et al. 2007; Franks 1975; Briand et al. 2000) and followed the behavior of the other individual odorants tested in this study.

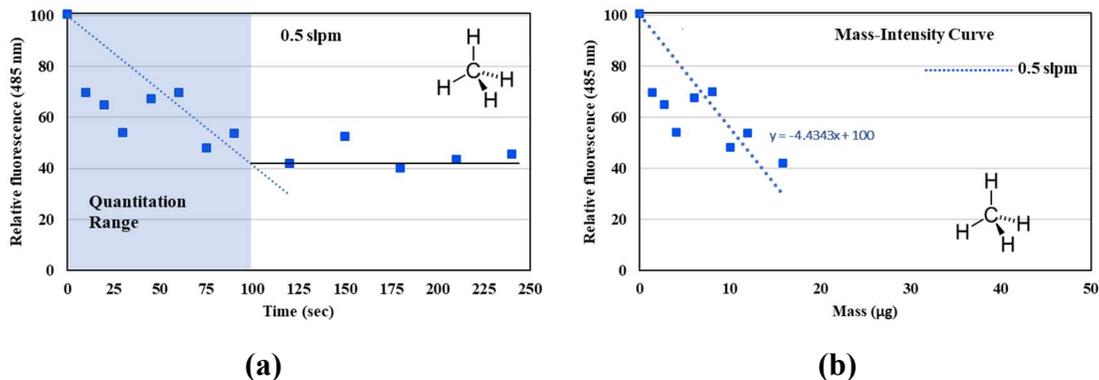


Figure 32: Graph showing peak emission intensity against time for methane gas flowing at 0.5 slpm (a), and a graph showing change in peak fluorescence intensity with mass flow of methane gas at 0.5 slpm (b)

3.2.6 Experimentation with Gas Mixture 1 ($NH_3 + CH_4$)

Since the research is focused on landfill odorants that would likely be encountered as a mixture of a number of odorant gases, the reaction of the biosensor with odorant gas mixtures was examined. Gas mixture 1 consisted of a binary mixture of NH_3 (25 ppm +/- 5%), and CH_4 (25 ppm +/- 5%) and was passed at 0.5 slpm into the reaction chamber by means of a Y-connector as mentioned in Section 2.3. Figure 33a shows the peak emission intensity against time curve while the gas is passed. The quantitation range of this mixture is around 90 seconds, which is approximately the same as that obtained for 0.5 slpm of ammonia (~90 seconds) and methane (100 seconds), at the same flow rate. Also, no change in pH is observed throughout the duration of this experiment, as was the case for all previous experiments. Figure 33b shows the emission intensity curves of the mixture as well as the individual components obtained from previous experiments when each component was measured individually. The gas mixture shows a slope (-0.38) that is less steep than either of its individual component gases (-0.59 for methane and -0.62 for ammonia). Since for gas mixtures, identification of the component gas actually combining with hOBPIIa was beyond the scope of this research, total mass of the gas mixture passed into the biosensor solution was calculated. Here, up to 13 μg of gas mixture 1 was passed into the solution until saturation was reached.

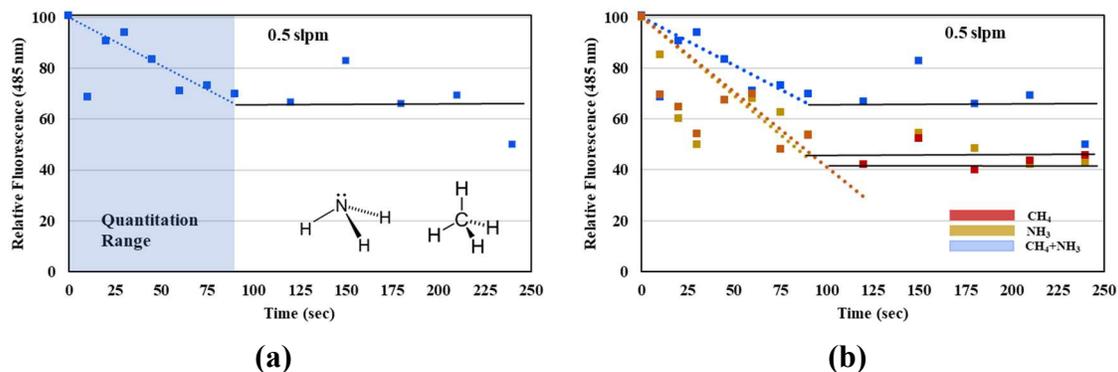


Figure 33: Graph showing peak emission intensity against time for gas mixture 1 (NH_3 25 ppm \pm 5% and CH_4 25 ppm \pm 5%) flowing at 0.5 slpm (a), and a graph showing change in peak fluorescence intensity with mass flow of gas mixture 1 along with its individual component gases at 0.5 slpm (b)

3.2.7 Experimentation with Gas Mixture 2 (H_2S + CH_4 + CO)

Gas mixture 2 consisted of H_2S (25 ppm \pm 5%), CO (50 ppm \pm 5%), and CH_4 (2.5% \pm 2%) balanced with air, all components being mixed in the same gas cylinder. Although CO is not an odorant, it was included in the experimental mixture due to the commercial availability of this combination of gas mixtures. In the case of the 0.5 slpm flow rate, the intensity decreases slowly with time, which causes the quantitation limit to be longer (approximately 115 seconds), as shown in Figure 34. This limit is shorter for 0.7 slpm (around 90 seconds) and even shorter in the case of the higher flow rate of 0.9 slpm, where the intensity becomes stable at around 70 seconds. Again, no change in pH is observed throughout the duration of the experiment with any of the three flow rates.

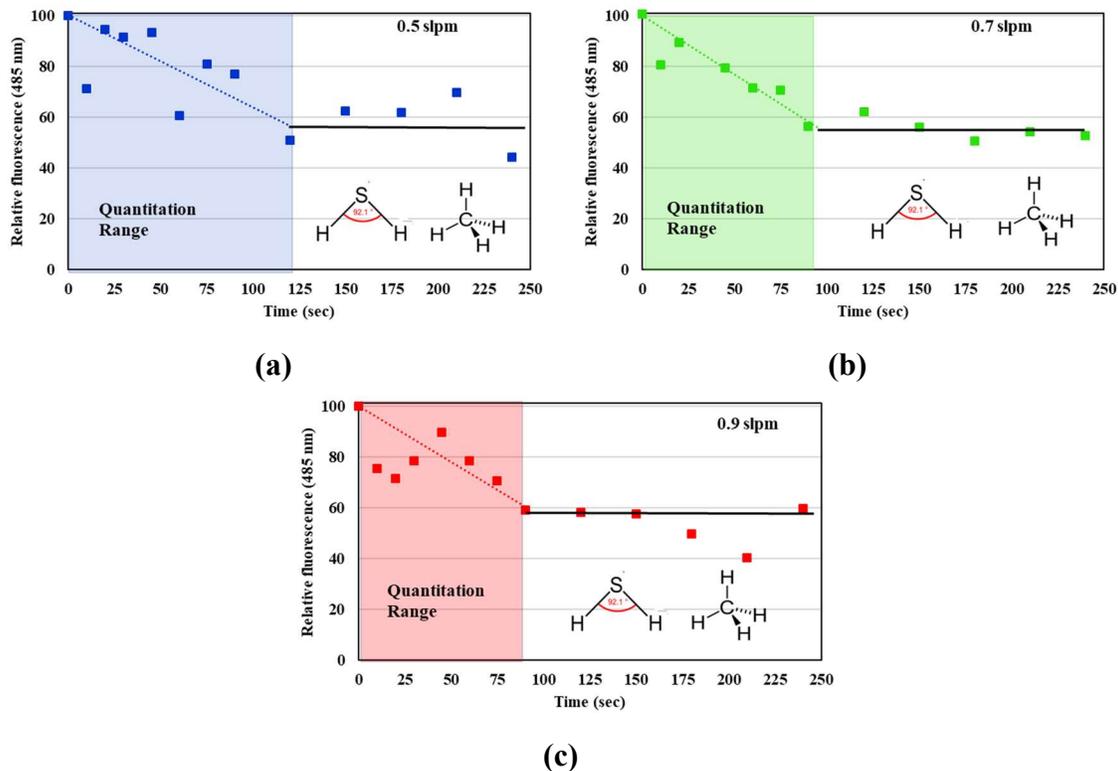


Figure 34: Graph showing peak emission intensity against time for gas mixture 2 (H₂S 25 ppm +/- 5%, CO 50 ppm +/- 5%, and CH₄ 2.5% +/- 2%) flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)

Figure 35 shows the peak emission intensity curves for all three flow rates for gas mixture 2. There is little difference observed in the quantitation ranges for the higher flow rates of 0.7 slpm and 0.9 slpm. In all three cases, the decrease in intensity is almost the same. This is because the mass of the gas passed into the solution is orders of magnitude higher (1.5×10^4 - 2.1×10^4 μg) depending on the flow rates compared to the other experiments. The larger number of gas molecules striking and interacting with each other results in a mass transfer limitation.

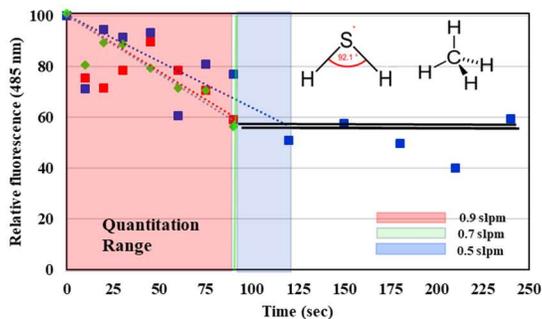


Figure 35: Graph showing peak emission intensity against time for gas mixture 2 for all three flow rates

While comparing the peak emission intensity curves of gas mixture 2 with the constituent gases for all the flow rates (Figure 36), it can be seen that the gas mixture decreases the intensity by a lower amount for all three flow rates than its constituents, just like the case previously observed with gas mixture 1.

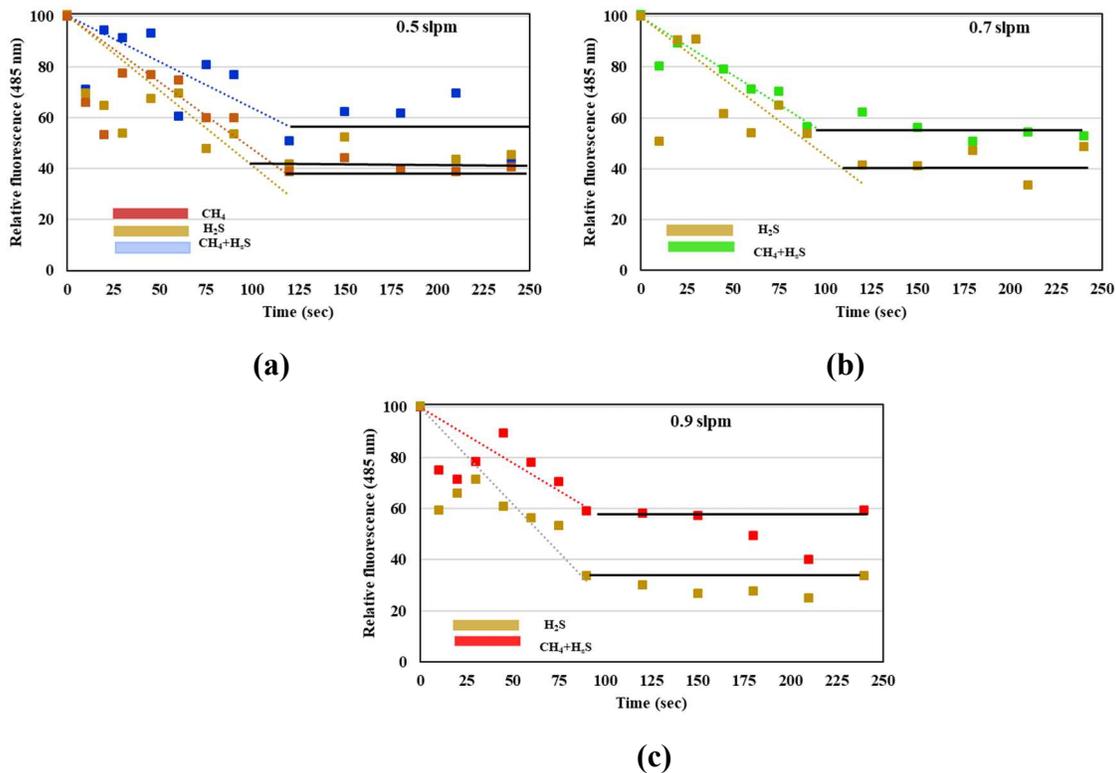


Figure 36: Graph showing peak emission intensity against time for gas mixture 2 (H₂S 25 ppm +/- 5%, CO 50 ppm +/- 5%, and CH₄ 2.5% +/- 2%) along with individual component gases (from previous experiments) flowing at 0.5 slpm (a), 0.7 slpm (b), and 0.9 slpm (c)

3.3 Interpretation of Results for Different Odorant Gases

Table 1 summarizes the mass ranges detected of the pure odorant gases and the gas mixtures up to the apparent saturation limit. For the gas mixtures, total masses for each of the flowrates that were passed into the solution were estimated and not the actual mass that combined with the protein. Whereas in all cases, the gases were in the ppm concentration range, for gas mixture 2, methane was 2.5% of the combined gas and that is why the mass value is larger compared to all other odorant gases. Since only one flowrate was used for methane and gas mixture 1, single values are tabulated in these cases.

Table 1: Mass of pure odorant gases combining with hOBPIIa and of gas mixtures passed into the solution

Odorant gas	Mass Range (μg)
Hydrogen Sulfide	35-45
Ammonia	12-18
Methyl Mercaptan	83-95
Methane	15
Gas mixture 1 (NH_3 25 ppm \pm 5% and CH_4 25 ppm \pm 5%)	13
Gas mixture 2 (H_2S 25 ppm \pm 5%, CO 50 ppm \pm 5%, and CH_4 2.5% \pm 2%)	1.5×10^4 - 2.1×10^4

The bar chart shown in Figure 37a shows the quantitation ranges of the different odorant gases tested for different flow rates, while Figure 37b represents the slopes of emission intensity curves of the same gases. Generally, for all gases, the quantitation range decreases with an increase in flow rate. Among the flow rates used in the experiments, 0.9 slpm is comparatively faster and thus saturates the biosensor complex faster. However, the logic behind using a faster flow rate is that in a field setting, the response time to obtain results may be shorter. This is possible in case of faster saturation and so, it was important to document the reaction of the biosensor using different flowrates. The general trend for slopes is also the same, i.e. a steeper slope is obtained when the flow rate is comparatively higher. For the gas mixtures, it can be seen that the slopes obtained have a lower magnitude than that of their component gases in all cases. This indicates that gas mixtures are generally less capable of decreasing the fluorescence intensity than their constituents. The reason behind this might be that the individual gases present in the mixture compete for the binding sites in hOBPIIa, which slows down the reaction.

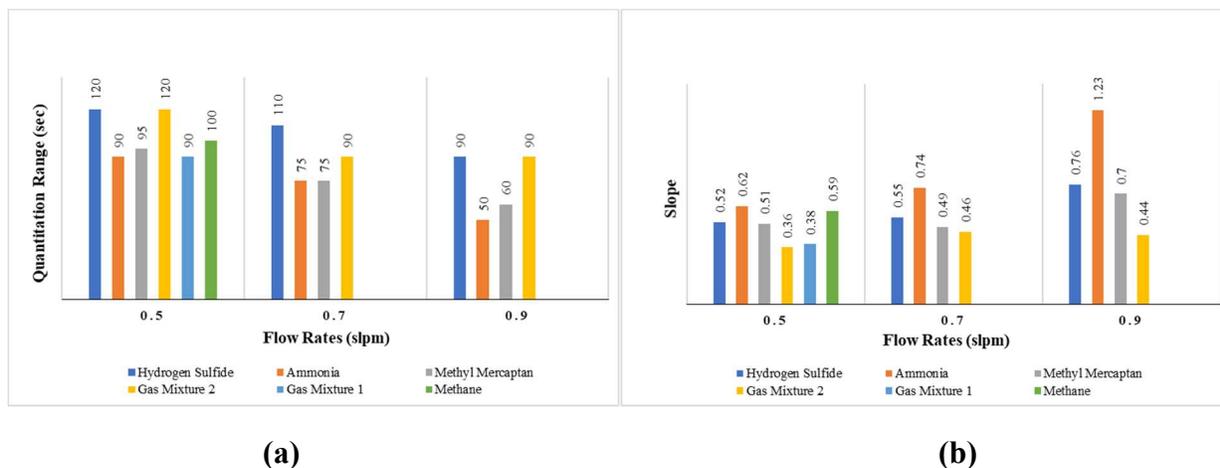


Figure 37: Quantitation range of different odorant gases (a), and slope of emission intensity curves of different odorant gases tested at different flow rates (b)

For all flow rates, the quantitation range for hydrogen sulfide is the largest followed by methyl mercaptan and ammonia as shown in Figure 37a. One possible reason might be the high solubility of ammonia, which leads to faster saturation than the other gases. The higher volatility keeps the saturation time lower for methyl mercaptan, which is a little higher than ammonia. Though the solubility of methane is lower, the saturation did not seem to be delayed for 0.5 slpm by that much. Methane is a purely hydrophobic compound, and since odorant binding protein has affinity for hydrophobic ligands, the effect of solubility was not important. In all cases, ammonia has the steepest slope, followed by hydrogen sulfide and methyl mercaptan among the pure gases. This indicates that ammonia has the highest affinity for protein among the gases, resulting in the largest decrease in fluorescence intensity compared to other gases.

3.4 Binding Affinity of Pure Odorant Gases

Since the components of the biosensor complex used in the experiments were in the micromolar concentration range, the odorants binding within the saturation time were converted to μM . Table 2 shows the range of concentration of the odorant gas that was present up to the saturation limit.

Table 2: Detection ranges of mass and concentration of the odorants

Odorant gas	Mass Range (μg)	Concentration Range (μM)
Hydrogen Sulfide	35-45	100-130
Ammonia	12-18	70-100
Methyl Mercaptan	83-95	170-200
Methane	15	94

In all cases, the binding affinity of the gases was less than that of 1-AMA with the protein. 1-AMA binds with the protein at a concentration ratio of 1:1, indicating one binding site per monomer, which is also shown by Briand et al. (2002) in their experiments including hOBP with N-phenyl-1-naphthylamine (NPN) and 11-((5-(dimethylaminonaphthalenyl-1-sulfonyl)amino)undecanoic acid (DAUDA). However, 1 μM of protein requires a much higher concentration of odorant gas to displace 1 μM 1-AMA before the saturation limit is reached. Among the gases, ammonia has the highest affinity, while methyl mercaptan has the lowest. Although an initial supposition was that the protein will combine with each of the odorant gases in the same ratio, this is not the case. Silva et al. (2014) conducted an experiment with pOBP where the optimum ratio of protein:1-AMA was found to be around 1:2 (to be exact, 1:2.5). Using the protein:fluorophore ratio 1:2.5, they experimented with four different odorant gases. It was found that the saturation of the protein occurs at different concentrations for different odorants. The researchers explained this difference in odorant concentrations for saturation by varying binding affinities of different odorants towards

the protein. So, it will not be the case that all odorants will achieve saturation at the same concentration.

3.5 Results from Experiments with PID Sensor

For verification of the outlet gas concentration, a PID analyzer (refer to Section 2.5) was used to monitor hydrogen sulfide gas that was passed through the biosensor solution at 0.5 slpm both with and without the filter. Without the filter, the outlet concentration showed no detection for all 4 minutes of the experiment; whereas, with the filter, the concentration reading showed a non-zero value, indicating that a little amount of gas might be escaping in the form of droplets. This shows the importance of fitting a filter at the outlet to prevent the escape of odorant gas droplets liberated from the reaction chamber.

3.6 Biosensor Reversibility Experiment

An objective of this research was to test the reversibility of the odorant-protein binding. If the protein can be reused after it binds with the odorant, this will make any future device constructed using this principle much less expensive to produce. The idea is to pass an odorant gas through the biosensor solution for some time followed by purging the system with air or pure nitrogen to observe if the intensity curve returns to its original height, meaning that the biosensor complex bonding has been regenerated. Air was considered first, since this can be easily used in the field for any possible regeneration. However, the presence of carbon dioxide may affect the pH of the solution and thus nitrogen was selected as the purge gas, while hydrogen sulfide was selected as the target odorant gas. Nitrogen was already shown to not appreciably react with the biosensor complex in previous tests (Section 3.2.1).

For the reversibility test, hydrogen sulfide was passed through the biosensor solution for 4 minutes at 0.5 slpm and immediately following, the reactor was purged with nitrogen gas for another 4 minutes at the same flow rate. The intensity curve obtained is shown in Figure 38, in which the intensity curve did not return to the original level after purging. However, at the very end, there is a slight upward trend of the curve. This is promising to some extent as there is the possibility of a reversible reaction if nitrogen is passed for a longer period of time (>4 minutes).

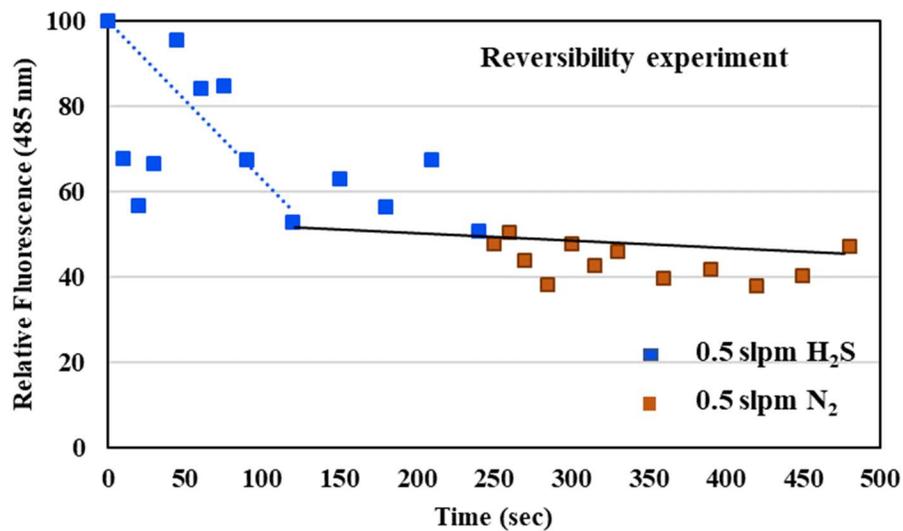


Figure 38: Graph showing peak emission intensity against time for passing hydrogen sulfide gas through the biosensor solution at 0.5 slpm (first 240 seconds) followed by nitrogen gas at the same flow rate (final 240 seconds)

4.0 CONCLUSIONS

4.1 Major Findings

This research has made several important findings regarding human odorant binding protein and its response when binding with odorant gases. An important aspect of this research was to design and test the biosensor itself. The optimum concentration ratio of hOBPIIa and 1-AMA was verified to be 1:1. Increasing the concentration of 1-AMA in the mixture above that ratio increases the fluorescence intensity only very slightly. Next, experiments using hydrogen sulfide, ammonia, methyl mercaptan, methane, and two different gas mixtures were conducted. Fluorescence response curves for a diverse range of gases including acidic and basic gases typically found in landfills were obtained. The usage of different flow rates for the gases established trends in quantitation range based on the flow rates. Generally, it was found that the biosensor complex saturates faster with an increased gas flow rate. This was shown in the form of shorter quantitation ranges for the higher flow rates for all the gases, indicating that the biosensor complex can keep working for a longer time with lower flow rates. Also, the higher flow rates led to higher decrease in fluorescence intensity in most cases, which was found in the form of steeper slopes of the fluorescence intensity curves. Ammonia has a shorter quantitation range due to its high solubility, and methyl mercaptan has a shorter quantitation range as well because of its volatility. Around 180 µg of protein in 10 mL solution was able to detect approximately 35-45 µg of hydrogen sulfide, 12-18 µg of ammonia, 83-95 µg of methyl mercaptan, and 15 µg of methane.

Additional experiments were conducted to check for reaction reversibility of the protein-odorant gas binding so that after the assay, the odorants can be purged, and the biosensor can be regenerated for another assay. Although the intensity shows no change during most of the 4-minute nitrogen purge time, there was a slight upward trend at the very end, which makes this idea promising to explore with an extended purge time range beyond 4 minutes.

4.2 Recommendations

The ultimate goal of this research is to lay the groundwork to develop a handheld biosensor device that can be deployed in the field to objectively measure odorant levels. Most of the currently available odor measurement techniques are not good predictors of actual odor intensity and thus require some degree of human subjectivity in the analysis. Other techniques are overly complex and costly (e.g. GC-MS), even though they do not always effectively characterize odor strength and concentration of field air samples. The proposed biosensor technology will be able to objectively measure odor intensity since it does not require active human participation. Also the technology can be made available at a low cost, since the OBPs can be manufactured industrially in bulk. To accomplish that, there needs to be more investigation on how the decrease in spectrofluorometric intensity for any gas can be translated to its odor intensity. This can be achieved by conducting more experiments for a variety of odorant gases and observing the change

in fluorescence across different flow rates. This will also allow a deeper understanding of the relationship between flow rates and the time taken by the biosensor complex to be saturated. Also, further investigation is needed on how the slopes of the peak emission intensity curves change with concentration of the biosensor complex and with concentration of the odorant gases. For any future application, this will help determine how long any biosensor cartridge can be used in the presence of certain types of gases.

Understanding the reaction mechanism between the gas mixtures and biosensor complex is also important so that the component gases competing for the binding sites and successfully binding can be readily identified. Although this research has used a diverse array of gases, these are bottled gases with a uniform concentration throughout. Observing the reaction of field collected samples is the ultimate objective of developing this biosensor technology. To that end, we are planning to test the technique with real field samples and compare the performance results with parallel tests using conventional field olfactometry.

The protein-odorant reaction reversibility must also be explored further. This is important because any future technology will be more easily adopted if biosensor cartridges can be reused multiple times before discarding. There was encouraging results in the reversibility experiment conducted as part of this research. Further experiments need to be carried out to check whether increasing the purging time with nitrogen gas, or with air, in the biosensor solution makes the reaction reversible. According to Pelosi et al. (2018), vertebrate OBPs have dissociation kinetics which are in the order of minutes to hours (Kotlowski et al. 2018). The binding site of bovine OBP was found to contain a gated entrance in the form of the benzene ring of a phenylalanine residue. This mechanism greatly slows down the process of dissociation of the odorant-protein binding (Pelosi et al. 2018). This suggests that OBP regeneration may occur beyond 4 minutes as was tested in this work.

Another interesting question was brought up by our industry partners regarding using non-human odorant binding proteins. For example, dogs have a highly tuned sense of smell, and therefore dog odorant binding proteins might have better characteristics for this application for example. In this project, only human odorant binding protein was used. It is possible that other proteins that are widely available such as insect, porcine, bovine, rat, or even canine OBP might have better analytical characteristics, greater binding capacity, or more rapid or finer signal response. Therefore, those proteins should be tested as well. Beyond exploring other proteins, it is also now possible with current technology to tweak protein binding sites or change the binding characteristics so as to tailor the biosensor molecule for a particular odorant. This will allow users to control the binding affinity for specific odorants and selectively detect certain odorants among a mixture of gases.

For the more immediate future, one of the challenges of using a small amount of protein for each experiment is that the sample sizes themselves need to be reduced. Although there are benefits

such as miniaturizing the reactor chamber and conserving protein mass, the smaller samples required quartz cuvettes for testing the fluorescence intensity using the spectrofluorometer since plastic disposable cuvettes need a larger sample size. Another way to overcome this problem is to use a higher concentration of protein, which will enable a small sample to be used easily in a flow-through cuvette. In this advanced configuration, the cuvette itself becomes the reaction chamber, which is more like how the field device is envisioned to operate. There are other advantages to using a flow-through cuvette as a reaction chamber such as eliminating the need of removing subsamples from the reaction zone. This will ensure a constant mass of biosensor protein in the reaction chamber at all times. Also, in the current process, no matter which type of cuvette is used (quartz or disposable), gas bubbles or tiny pockets of air create a false positive or false negative signal. This error can be eliminated if a flow-through cuvette is used. Figure 39 illustrates a proposed experimental setup using a flow-through cuvette.

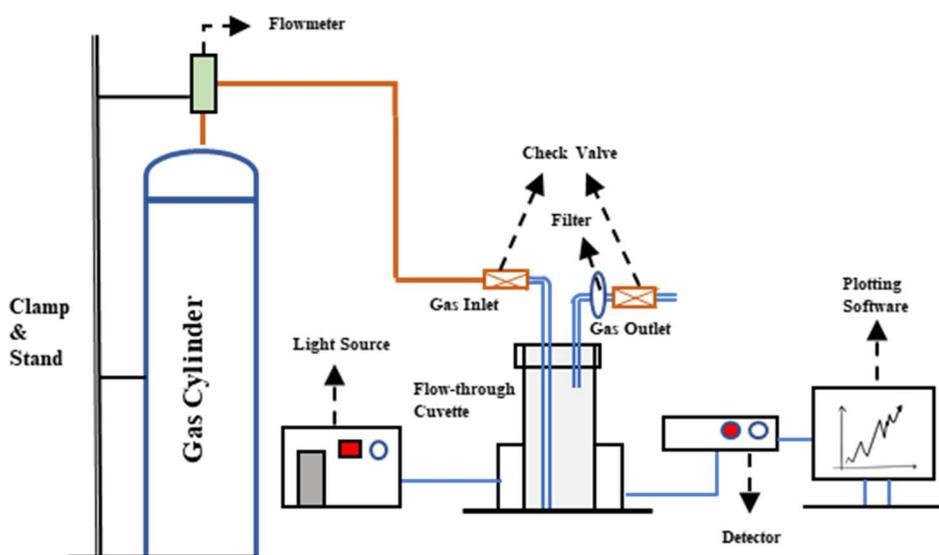


Figure 39: Schematic diagram for experimentation with a flow-through cuvette

BIBLIOGRAPHY

- Aatamila, M., Verkasalo, P. K., Korhonen, M. J., Suominen, A. L., Hirvonen, M. R., Viluksela, M. K., & Nevalainen, A. (2011). Odour annoyance and physical symptoms among residents living near waste treatment centres. *Environmental Research*, 111(1), 164-170. <https://doi.org/10.1016/j.envres.2010.11.008>
- Abdurachim, K., & Ellis, H. R. (2006). Detection of protein-protein interactions in the alkanesulfonate monooxygenase system from *Escherichia coli*. *Journal of bacteriology*, 188(23), 8153-8159.
- Albani, J. R., Bretesche, L., Vogelaer, J., & Kmiecik, D. (2015). Energy Transfer Studies between Trp Residues of Three Lipocalin Proteins Family, α 1-Acid Glycoprotein, (Orosomuroid), β -Lactoglobulin and Porcine Odorant Binding Protein and the Fluorescent Probe, 1-Aminoanthracene (1-AMA). *Journal of fluorescence*, 25(1), 167-172.
- Allen, M. R., Braithwaite, A., & Hills, C. C. (1997). Trace organic compounds in landfill gas at seven UK waste disposal sites. *Environmental Science & Technology*, 31(4), 1054-1061. <https://doi.org/10.1021/es9605634>
- Agency for Toxic Substances and Disease Registry (ATSDR). (1992). TOXICOLOGICAL PROFILE FOR METHYL MERCAPTAN. Retrieved from <https://www.atsdr.cdc.gov/toxprofiles/tp139.pdf>
- Agency for Toxic Substances and Disease Registry (ATSDR). (2001). Landfill Gas Primer - An Overview for Environmental Health Professionals. Retrieved from <https://www.atsdr.cdc.gov/HAC/landfill/html/ch3a.html>
- Agency for Toxic Substances and Disease Registry (ATSDR). (2014). Toxic Substances Portal – Methyl Mercaptan. Retrieved from <https://www.atsdr.cdc.gov/MMG/MMG.asp?id=221&tid=40>
- Agency for Toxic Substances and Disease Registry (ATSDR). (2015). Toxic Substances Portal - Ammonia. Retrieved from <https://www.atsdr.cdc.gov/PHS/PHS.asp?id=9&tid=2>
- Agency for Toxic Substances and Disease Registry (ATSDR). (2016). Landfill Gas Safety and Health Issues. Retrieved from Agency for Toxic Substances & Disease Registry: <https://www.atsdr.cdc.gov/hac/landfill/html/ch3.html>
- Bai, Z., Dong, Y., Wang, Z., & Zhu, T. (2006). Emission of ammonia from indoor concrete wall and assessment of human exposure. *Environment international*, 32(3), 303-311.
- Baker, B. P. (1982). Land Values Surrounding Waste Disposal Facilities (No. 639-2016-42174).
- Baltrėnas, P., Andrulevičius, L., & Zuokaitė, E. (2013). Application of Dynamic Olfactometry to Determine Odor Concentrations in Ambient Air. *Polish Journal of Environmental Studies*, 22(2).

- Bax, C., Sironi, S., & Capelli, L. (2020). How Can Odors Be Measured? An Overview of Methods and Their Applications. *Atmosphere*, 11(1), 92.
- Belgiorno, V., Naddeo, V. & Zarra, T. (2013). *Odour Impact Assessment Handbook*. A John Wiley & Sons, Ltd., Publication
- Bernhard, B. (2014, August 12). More odor lawsuits filed against Bridgeton Landfill owner. Retrieved from https://www.stltoday.com/lifestyles/health-med-fit/health/more-odor-lawsuits-filed-against-bridgeton-landfill-owner/article_c059212f-0385-5124-a3bf-fc67e9aa6c99.html
- Bertucci, J. J., Sawyer, B., Calvano, J., Tata, P., Zenz, D. R., & Lue-Hing, C. (1994). The application of odor measurement technologies to large-scale odor evaluation studies. *Proc. Odor and Volatile Organic Compound Emission Control for Municipal and Industrial Wastewater Treatment Facilities*, Jacksonville, FL, 3-37.
- Blizzard, N. (2018, December 3). NEW DETAILS: \$4.1 million settlement approved in Dayton landfill odor lawsuit. Retrieved from <https://www.daytondailynews.com/news/new-details-million-settlement-approved-dayton-landfill-odor-lawsuit/r4HZKBL7rGD9M4ctJkaD7M/>
- Bokowa, A. H. (2011). The effect of sampling on the measured odour concentration. *Chemical Engineering Transactions*, 23, 43-48. <https://doi.org/10.3303/CET1123008>
- Boudreau, M. D., Taylor, H. W., Baker, D. G., & Means, J. C. (2006). Dietary exposure to 2-aminoanthracene induces morphological and immunocytochemical changes in pancreatic tissues of Fisher-344 rats. *Toxicological Sciences*, 93(1), 50-61. <https://doi.org/10.1093/toxsci/kfl033>
- Brattoli, M., De Gennaro, G., De Pinto, V., Demarinis Loiotile, A., Lovascio, S., & Penza, M. (2011). Odour detection methods: Olfactometry and chemical sensors. *Sensors*, 11(5), 5290-5322. <https://doi.org/10.3390/s110505290>
- Briand, L., Eloit, C., Nespoulous, C., Bézirard, V., Huet, J. C., Henry, C., ... & Pernollet, J. C. (2002). Evidence of an odorant-binding protein in the human olfactory mucus: location, structural characterization, and odorant-binding properties. *Biochemistry*, 41(23), 7241-7252. <https://doi.org/10.1021/bi015916c>
- Briand, L., Nespoulous, C., Perez, V., Rémy, J. J., Huet, J. C., & Pernollet, J. C. (2000). Ligand-binding properties and structural characterization of a novel rat odorant-binding protein variant. *European journal of biochemistry*, 267(10), 3079-3089. <https://doi.org/10.1046/j.1432-1033.2000.01340.x>
- Bruno, P., Caselli, M., De Gennaro, G., Solito, M., & Tutino, M. (2007). Monitoring of odor compounds produced by solid waste treatment plants with diffusive samplers. *Waste Management*, 27(4), 539-544. <https://doi.org/10.1016/j.wasman.2006.03.006>

- CalRecycle. (2019). Odor Characteristics of Compostable Materials. Retrieved from <https://www.calrecycle.ca.gov/swfacilities/compostables/odor/characteris>
- Carannante I., Marasco A. (2018) Olfactory Sensory Neurons to Odor Stimuli: Mathematical Modeling of the Response.
- Carlson, C. A., Lloyd, J. A., Dean, S. L., Walker, N. R., & Edmiston, P. L. (2006). Sensor for fluorene based on the incorporation of an environmentally sensitive fluorophore proximal to a molecularly imprinted binding site. *Analytical chemistry*, 78(11), 3537-3542. <https://doi.org/10.1021/ac051375b>
- Charlier, L., Cabrol-Bass, D., & Golebiowski, J. (2009). How does human odorant binding protein bind odorants? The case of aldehydes studied by molecular dynamics. *Comptes Rendus Chimie*, 12(8), 905-910.
- Chen, S. J., Hsieh, L. T., Hwang, W. I., Xu, H. C., & Kao, J. H. (2003). Abatement of odor emissions from landfills using natural effective microorganism enzyme. *Aerosol Air Qual. Res*, 3(1), 87-99.
- Chiriac, R., Carre, J., Perrodin, Y., Fine, L., & Letoffe, J. M. (2007). Characterisation of VOCs emitted by open cells receiving municipal solid waste. *Journal of hazardous materials*, 149(2), 249-263. <https://doi.org/10.1016/j.jhazmat.2007.07.094>
- Choi, S. H., Lee, J. W., & Sim, S. J. (2004). Enhancement of the sensitivity of surface plasmon resonance (SPR) immunosensor for the detection of anti-GAD antibody by changing the pH for streptavidin immobilization. *Enzyme and microbial technology*, 35(6-7), 683-687.
- Curren, J., Hallis, S. A., Snyder, C. C. L., & Suffet, I. M. H. (2016). Identification and quantification of nuisance odors at a trash transfer station. *Waste management*, 58, 52-61. <https://doi.org/10.1016/j.wasman.2016.09.021>
- Davoli, E. (2004). I recenti sviluppi nella caratterizzazione dell'inquinamento olfattivo. Tutto sugli odori, *Rapporti GSISR*.
- Davoli, E., Gangai, M. L., Morselli, L., & Tonelli, D. (2003). Characterisation of odorants emissions from landfills by SPME and GC/MS. *Chemosphere*, 51(5), 357-368. [https://doi.org/10.1016/S0045-6535\(02\)00845-7](https://doi.org/10.1016/S0045-6535(02)00845-7)
- De Feo, G., De Gisi, S., & Williams, I. D. (2013). Public perception of odour and environmental pollution attributed to MSW treatment and disposal facilities: A case study. *Waste management*, 33(4), 974-987.
- Dincer, F., Odabasi, M., & Muezzinoglu, A. (2006). Chemical characterization of odorous gases at a landfill site by gas chromatography–mass spectrometry. *Journal of chromatography A*, 1122(1-2), 222-229.

- Du Preez, M., & Lottering, T. (2009). Determining the negative effect on house values of proximity to a landfill site by means of an application of the hedonic pricing method. *South African Journal of Economic and Management Sciences*, 12(2), 256-262.
- El-Fadel, M., Findikakis, A. N., & Leckie, J. O. (1997). Environmental impacts of solid waste landfilling. *Journal of environmental management*, 50(1), 1-25.
- Ekathimerini. (2019, December 19). Corfu police arrest four over landfill vandalism. Retrieved from <http://www.ekathimerini.com/238193/article/ekathimerini/news/corfu-police-arrest-four-over-landfill-vandalism>
- El-Fadel, M., Findikakis, A. N., & Leckie, J. O. (1997). Environmental impacts of solid waste landfilling. *Journal of environmental management*, 50(1), 1-25. <https://doi.org/10.1006/jema.1995.0131>
- Elliott, P., Richardson, S., Abellan, J. J., Thomson, A., De Hoogh, C., Jarup, L., & Briggs, D. J. (2009). Geographic density of landfill sites and risk of congenital anomalies in England: authors' response. *Occupational and environmental medicine*, 66(2), 140-140.
- "Energy and Environmental Affairs." (2016). Mass.gov. Retrieved from <http://www.mass.gov/eea/docs/dep/recycle/laws/lfgasapp.pdf>.
- Environmental Protection Agency. (2000). Biosolids and Residuals Management Fact Sheet Odor Control in Biosolids Management. Retrieved from <https://www.epa.gov/sites/production/files/2018-11/documents/order-control-biosolids-management-factsheet.pdf>
- Environmental Protection Agency. (2001). Odour Impacts and Odour Emission Control Measures for Intensive Agriculture. Final Report. Retrieved from <https://www.epa.ie/pubs/reports/research/air/Odour%20Impacts%20Final.pdf>
- Epstein, E. (2011). *Industrial composting: environmental engineering and facilities management*. CRC Press.
- Fang, J. J., Yang, N., Cen, D. Y., Shao, L. M., & He, P. J. (2012). Odor compounds from different sources of landfill: characterization and source identification. *Waste Management*, 32(7), 1401-1410. <https://doi.org/10.1016/j.wasman.2012.02.013>
- Flower, D. R. (1995). Multiple molecular recognition properties of the lipocalin protein family. *Journal of Molecular Recognition*, 8(3), 185-195. <https://doi.org/10.1002/jmr.300080304>
- Flyger, H., Lewin, E., Thomsen, E. L., Fenger, J., Lyck, E., & Gryning, S. E. (1977). Physical and chemical processes of sulphur dioxide in the plume from an oil-fired power station.
- Franks, F. (1975). The hydrophobic interaction. In *Water a comprehensive treatise* (pp. 1-94).

- Springer, Boston, MA. <https://doi.org/10.1073/pnas.0610945104>
- Freeman, T., & Cudmore, R. (2002). Review of odour management in New Zealand. New Zeal. Minist. Environ. Air Qual. Tech. Rep, 24.
- Ganni, M., Garibotti, M., Scaloni, A., Pucci, P., & Pelosi, P. (1997). Microheterogeneity of odorant-binding proteins in the porcupine revealed by N-terminal sequencing and mass spectrometry. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 117(2), 287-291.
- Gardner, J. W., Bartlett, P. N. (1999). "Electronic Noses: Principles and Applications".
- Gardner, J. W., Bartlett, P. N., Dodd, G. H., & Shurmer, H. V. (1988). Pattern recognition in the Warwick electronic nose.
- Gastech (n.d.). MiniRAE Lite. Retrieved from <https://gastech.com/products/gas-detectors-portable/single-gas/minirae-lite>
- Gonçalves, F., Silva, C., Ribeiro, A., & Cavaco-Paulo, A. (2018). 1-Aminoanthracene Transduction into Liposomes Driven by Odorant-Binding Protein Proximity. *ACS applied materials & interfaces*, 10(32), 27531-27539. Gonçalves, F., Silva, C., Ribeiro, A., & Cavaco-Paulo, A. (2018). 1-Aminoanthracene Transduction into Liposomes Driven by Odorant-Binding Protein Proximity. *ACS applied materials & interfaces*, 10(32), 27531-27539.
- Gostelow, P., Parsons, S. A., & Stuetz, R. M. (2001). Odour measurements for sewage treatment works. *Water Research*, 35(3), 579-597. [https://doi.org/10.1016/S0043-1354\(00\)00313-4](https://doi.org/10.1016/S0043-1354(00)00313-4)
- Heaney, C. D., Wing, S., Campbell, R. L., Caldwell, D., Hopkins, B., Richardson, D., & Yeatts, K. (2011). Relation between malodor, ambient hydrogen sulfide, and health in a community bordering a landfill. *Environmental research*, 111(6), 847-852. <https://doi.org/10.1016/j.envres.2011.05.021>
- Henry, C. G., Meyer, G. E., Schulte, D. D., Stowell, R. R., Parkhurst, A. M., & Sheffield, R. E. (2011). Mask scentometer for assessing ambient odors. *Transactions of the ASABE*, 54(2), 609-615.
- Heydel, J. M., Coelho, A., Thiebaud, N., Legendre, A., Bon, A. M. L., Faure, P., ... & Briand, L. (2013). Odorant-binding proteins and xenobiotic metabolizing enzymes: implications in olfactory perireceptor events. *The Anatomical Record*, 296(9), 1333-1345. <https://doi.org/10.1002/ar.22735>
- Hirsch, A. R. (2009). Parkinsonism: the hyposmia and phantosmia connection. *Archives of neurology*, 66(4), 538-542. doi:10.1001/archneurol.2009.38
- Hite, D., Chern, W., Hitzhusen, F., & Randall, A. (2001). Property-value impacts of an environmental disamenity: the case of landfills. *The Journal of Real Estate Finance and*

- Economics, 22(2-3), 185-202.
- Howard, J. (2001). Nuisance flies around a landfill: patterns of abundance and distribution. *Waste management & research*, 19(4), 308-313.
- Iwasaki, Y. (2004). Olfactory measurement of odor (new version). Japan: Japan Association on Odor Environment, 145-152.
- Iwasaki, Y. (2003). The history of odor measurement in Japan and triangle odor bag method. *Odor measurement review*, 37-47.
- Jasper, S. (2013). The Nervous System. Retrieved from <http://www.zo.utexas.edu/faculty/sjasper/bio301L/nervous.html>
- Jiang, J. K. (1996). Concentration measurement by dynamic olfactometer. *Water environment & technology*, 8(6), 55-59.
- Jin, P. C. (2015). "Characteristics of Gas Emissions in Landfill Site in Recent Years." *International Journal of Environmental Science and Development*, 6(5).
- Kang, C. D., Lee, S. W., Park, T. H., & Sim, S. J. (2006). Performance enhancement of real-time detection of protozoan parasite, *Cryptosporidium* oocyst by a modified surface plasmon resonance (SPR) biosensor. *Enzyme and microbial technology*, 39(3), 387-390. <https://doi.org/10.1016/j.enzmictec.2005.11.039>
- Kazakov, A. S., Markov, D. I., Gusev, N. B., & Levitsky, D. I. (2009). Thermally induced structural changes of intrinsically disordered small heat shock protein Hsp22. *Biophysical chemistry*, 145(2-3), 79-85.
- Kim, K.-H. H., Choi, Y., Jeon, E., and Sunwoo, Y. (2005b). "Characterization of malodorous sulfur compounds in landfill gas." *Atmospheric Environment*, Pergamon, 39(6), 1103–1112.
- Kim, S. U., Kim, Y. J., Yea, C. H., Min, J., & Choi, J. W. (2008). Fabrication of functional biomolecular layer using recombinant technique for the bioelectronic device. *Korean Journal of Chemical Engineering*, 25(5), 1115-1119.
- Kim, T. H., Lee, S. H., Lee, J., Song, H. S., Oh, E. H., Park, T. H., & Hong, S. (2009). Single-carbon-atomic-resolution detection of odorant molecules using a human olfactory receptor-based bioelectronic nose. *Advanced Materials*, 21(1), 91-94. <https://doi.org/10.1002/adma.200801435>
- Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A., & Christensen, T. H. (2002). Present and long-term composition of MSW landfill leachate: a review. *Critical reviews in environmental science and technology*, 32(4), 297-336. <https://doi.org/10.1080/10643380290813462>

- Kmiecik, D., & Albani, J. R. (2010). Effect of 1-aminoanthracene (1-AMA) binding on the structure of three lipocalin proteins, the dimeric β lactoglobulin, the dimeric odorant binding protein and the monomeric α 1-acid glycoprotein. *Fluorescence spectra and lifetimes studies. Journal of fluorescence*, 20(5), 973-983.
- Kobayashi, H., Ogawa, M., Alford, R., Choyke, P. L., & Urano, Y. (2009). New strategies for fluorescent probe design in medical diagnostic imaging. *Chemical reviews*, 110(5), 2620-2640.
- Ko, H. J., Lee, S. H., Oh, E. H., & Park, T. H. (2010). Specificity of odorant-binding proteins: a factor influencing the sensitivity of olfactory receptor-based biosensors. *Bioprocess and biosystems engineering*, 33(1), 55.
- Ko, H. J., & Park, T. H. (2008). Enhancement of odorant detection sensitivity by the expression of odorant-binding protein. *Biosensors and Bioelectronics*, 23(7), 1017-1023. <https://doi.org/10.1016/j.bios.2007.10.008>
- Ko, J. H., Xu, Q., & Jang, Y. C. (2015). Emissions and control of hydrogen sulfide at landfills: a review. *Critical Reviews in Environmental Science and Technology*, 45(19), 2043-2083.
- Kotlowski, C.; Larisika, M.; Guerin, P.M.; Kleber, C.; Kröber, T.; Mastrogiacomo, R.; Nowak, C.; Schutz, S.; Schwaighofer, A.; Knoll, W. Fine discrimination of volatile compounds by graphene-immobilized odorant-binding proteins. *Sens. Actuators B Chem.* 2018, 256, 564–572.
- Lacazette, E., Gachon, A. M., & Pitiot, G. (2000). A novel human odorant-binding protein gene family resulting from genomic duplicons at 9q34: differential expression in the oral and genital spheres. *Human Molecular Genetics*, 9(2), 289-301. <https://doi.org/10.1093/hmg/9.2.289>
- R. E., Mukhtar, S., Carey, J. B., & Ullman, J. L. (2004). A review of literature concerning odors, ammonia, and dust from broiler production facilities: 1. Odor concentrations and emissions. *Journal of Applied Poultry Research*, 13(3), 500-508. <https://doi.org/10.1093/japr/13.3.500>
- Lacey, R. E., Mukhtar, S., Carey, J. B., & Ullman, J. L. (2004). A review of literature concerning odors, ammonia, and dust from broiler production facilities: 1. Odor concentrations and emissions. *Journal of Applied Poultry Research*, 13(3), 500-508.
- Laister, G., Stretch, D. D., & Strachan, L. (2002). Managing landfill odour using dispersion modelling and community feedback. *Proc. of Wastecon 2002*.
- Laor, Y., Parker, D., & Pagé, T. (2014). Measurement, prediction, and monitoring of odors in the environment: a critical review. *Reviews in Chemical Engineering*, 30(2), 139-166. <https://doi.org/10.1515/revce-2013-0026>
- Leal, J., Smyth, H. D., & Ghosh, D. (2017). Physicochemical properties of mucus and their impact

- on transmucosal drug delivery. *International journal of pharmaceutics*, 532(1), 555-572. <https://doi.org/10.1016/j.ijpharm.2017.09.018>
- Lebrero, R., Bouchy, L., Stuetz, R., & Muñoz, R. (2011). Odor assessment and management in wastewater treatment plants: a review. *Critical Reviews in Environmental Science and Technology*, 41(10), 915-950. <https://doi.org/10.1080/10643380903300000>
- Lechner, M., Wojnar, P., & Bernhard, R. (2001). Human tear lipocalin acts as an oxidative-stress-induced scavenger of potentially harmful lipid peroxidation products in a cell culture system. *Biochemical Journal*, 356(1), 129-135.
- Lee, S. H., Jun, S. B., Ko, H. J., Kim, S. J., & Park, T. H. (2009). Cell-based olfactory biosensor using microfabricated planar electrode. *Biosensors and Bioelectronics*, 24(8), 2659-2664. <https://doi.org/10.1016/j.bios.2009.01.035>
- Lehning, M., Shonnard, D. R., Chang, D. P., & Bell, R. L. (1994). An inversion algorithm for determining area-source emissions from downwind concentration measurements. *Air & waste*, 44(10), 1204-1213.
- Li, J. L., Car, R., Tang, C., & Wingreen, N. S. (2007). Hydrophobic interaction and hydrogen-bond network for a methane pair in liquid water. *Proceedings of the National Academy of Sciences*, 104(8), 2626-2630.
- Li, J., Zou, K., Li, W., Wang, G., & Yang, W. (2019). Olfactory Characterization of Typical Odorous Pollutants Part I: Relationship Between the Hedonic Tone and Odor Concentration. *Atmosphere*, 10(9), 524. <https://doi.org/10.3390/atmos10090524>
- Liu, C., Furusawa, Y., & Hayashi, K. (2013). Development of a fluorescent imaging sensor for the detection of human body sweat odor. *Sensors and Actuators B: Chemical*, 183, 117-123.
- Lu, Y., Li, H., Zhuang, S., Zhang, D., Zhang, Q., Zhou, J., ... & Wang, P. (2014). Olfactory biosensor using odorant-binding proteins from honeybee: Ligands of floral odors and pheromones detection by electrochemical impedance. *Sensors and Actuators B: Chemical*, 193, 420-427. <https://doi.org/10.1016/j.snb.2013.11.045>
- Martuzzi, M., Mitis, F., & Forastiere, F. (2010). Inequalities, inequities, environmental justice in waste management and health. *European Journal of Public Health*, 20(1), 21-26. <https://doi.org/10.1093/eurpub/ckp216>
- Mei, B., Kennedy, M. W., Beauchamp, J., Komuniecki, P. R., & Komuniecki, R. (1997). Secretion of a novel developmentally regulated fatty acid-binding protein into the perivitelline fluid of the parasitic nematode, *Ascaris suum*. *Journal of Biological Chemistry*, 272(15), 9933-9941.
- Mel Suffet, I. H., Decottignies, V., Senante, E., & Bruchet, A. (2009). Sensory assessment and characterization of odor nuisance emissions during the composting of wastewater biosolids. *Water Environment Research*, 81(7), 670-679.

- Mikhailopulo, K. I., Serchenya, T. S., Kiseleva, E. P., Chernov, Y. G., Tsvetkova, T. M., Kovganko, N. V., & Sviridov, O. V. (2008). Interaction of molecules of the neonicotinoid imidacloprid and its structural analogs with human serum albumin. *Journal of Applied Spectroscopy*, 75(6), 857.
- Millery, J., Briand, L., Bézirard, V., Blon, F., Fenech, C., Richard-Parpaillon, L., ... & Gascuel, J. (2005). Specific expression of olfactory binding protein in the aerial olfactory cavity of adult and developing *Xenopus*. *European Journal of Neuroscience*, 22(6), 1389-1399. <https://doi.org/10.1111/j.1460-9568.2005.04337.x>
- Ministry of the Environment. (1995). Offensive Odor Control Law. Retrieved from <https://www.env.go.jp/en/laws/air/odor/index.html>
- Mirmohseni, A., Shojaei, M., & Farbodi, M. (2008). Application of a quartz crystal nanobalance to the molecularly imprinted recognition of phenylalanine in solution. *Biotechnology and Bioprocess Engineering*, 13(5), 592-597.
- Murphy, C., Doty, R. L., & Duncan, H. J. (2003). Clinical disorders of olfaction. In *Handbook of olfaction and gustation* (pp. 822-849). CRC Press.
- Nagata, Y., & Takeuchi, N. (2003). Measurement of odor threshold by triangle odor bag method. *Odor measurement review*, 118, 118-127.
- Nelson, A. C., Genereux, J., & Genereux, M. (1992). Price effects of landfills on house values. *Land economics*, 359-365. DOI: 10.2307/3146693
- Nicell, J. A. (2009). Assessment and regulation of odour impacts. *Atmospheric Environment*, 43(1), 196-206. <https://doi.org/10.1016/j.atmosenv.2008.09.033>
- Nicolas, J., Craffe, F., & Romain, A. C. (2006). Estimation of odor emission rate from landfill areas using the sniffing team method. *Waste Management*, 26(11), 1259-1269. <https://doi.org/10.1016/j.wasman.2005.10.013>
- Njoku, P. O., Edokpayi, J. N., & Odiyo, J. O. (2019). Health and environmental risks of residents living close to a landfill: A case study of Thohoyandou Landfill, Limpopo Province, South Africa. *International journal of environmental research and public health*, 16(12), 2125. <https://doi.org/10.3390/ijerph16122125>
- Norton, J. M., Wing, S., Lipscomb, H. J., Kaufman, J. S., Marshall, S. W., & Cravey, A. J. (2007). Race, wealth, and solid waste facilities in North Carolina. *Environmental Health Perspectives*, 115(9), 1344-1350. <https://doi.org/10.1289/ehp.10161>
- O'Brien, E. P., Brooks, B. R., & Thirumalai, D. (2012). Effects of pH on proteins: predictions for ensemble and single-molecule pulling experiments. *Journal of the American Chemical Society*, 134(2), 979-987.

Occupational Safety and Health Administration (OSHA). (2005). "OSHA Fact Sheet". Retrieved from

https://www.osha.gov/OshDoc/data_Hurricane_Facts/hydrogen_sulfide_fact.pdf

OSHA. How Smelly is Ammonia?

Retrieved from <https://www.osha.gov/sites/default/files/2019-03/fs5-howsmelly.pdf>

Palmiotto, M., Fattore, E., Paiano, V., Celeste, G., Colombo, A., & Davoli, E. (2014). Influence of a municipal solid waste landfill in the surrounding environment: Toxicological risk and odor nuisance effects. *Environment international*, 68, 16-24. <https://doi.org/10.1016/j.envint.2014.03.004>

Paolini, S., Tanfani, F., Fini, C., Bertoli, E., & Pelosi, P. (1999). Porcine odorant-binding protein: structural stability and ligand affinities measured by Fourier-transform infrared spectroscopy and fluorescence spectroscopy. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, 1431(1), 179-188. [https://doi.org/10.1016/S0167-4838\(99\)00037-0](https://doi.org/10.1016/S0167-4838(99)00037-0)

Paraskaki, I., & Lazaridis, M. (2005). Quantification of landfill emissions to air: a case study of the Ano Liosia landfill site in the greater Athens area. *Waste management & research*, 23(3), 199-208. <https://doi.org/10.1177/0734242X05054756>

Parker, T., Dottridge, J., & Kelly, S. (2002). Investigation of the composition and emissions of trace components in landfill gas. Environment Agency, R&D Technical Report P1-438/TR.

Paxéus, N. (2000). Organic compounds in municipal landfill leachates. *Water Science and Technology*, 42(7-8), 323-333. <https://doi.org/10.2166/wst.2000.0585>

Pearce, T. C., Schiffman, S. S., Nagle, H. T., & Gardner, J. W. (Eds.). (2006). *Handbook of machine olfaction: electronic nose technology*. John Wiley & Sons.

Pelosi, P. (2001). The role of perireceptor events in vertebrate olfaction. *Cellular and Molecular Life Sciences CMLS*, 58(4), 503-509. <https://doi.org/10.1007/PL00000875>

Pelosi, P., Baldaccini, N. E., & Pisanelli, A. M. (1982). Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. *Biochemical Journal*, 201(1), 245-248. <https://doi.org/10.1042/bj2010245>

Pelosi, P., Mastrogiacomo, R., Iovinella, I., Tuccori, E., & Persaud, K. C. (2014). Structure and biotechnological applications of odorant-binding proteins. *Applied microbiology and biotechnology*, 98(1), 61-70.

Pelosi, P., Zhu, J., & Knoll, W. (2018). Odorant-binding proteins as sensing elements for odour monitoring. *Sensors*, 18(10), 3248.

RAE Systems Inc. (2013). *The PID Handbook* (3rd ed.). Retrieved from

https://www.raesystems.com/sites/default/files/content/resources/pid_handbook_1002-02.pdf

- Ramoni, R., Bellucci, S., Gryczynski, I., Gryczynski, Z., Grolli, S., Staiano, M., ... & Conti, V. (2007). The protein scaffold of the lipocalin odorant-binding protein is suitable for the design of new biosensors for the detection of explosive components. *Journal of Physics: Condensed Matter*, 19(39), 395012.
- Ready, R. (2010). Do landfills always depress nearby property values?. *Journal of Real Estate Research*, 32(3), 321-339.
- Reinhart, D. R. (1993). A review of recent studies on the sources of hazardous compounds emitted from solid waste landfills: a US experience. *Waste Management & Research*, 11(3), 257-268. <https://doi.org/10.1006/wmre.1993.1025>
- Ritzkowski, M., Heyer, K. U., & Stegmann, R. (2006). Fundamental processes and implications during in situ aeration of old landfills. *Waste Management*, 26(4), 356-372. <https://doi.org/10.1016/j.wasman.2005.11.009>
- Roblyer, J. G. (2017). Development of a Biosensor to Detect Landfill Odors Using Human Odorant Binding Protein (Doctoral dissertation, Florida Atlantic University).
- Roebuck, D. U. N. C. A. N., Strecht, D., & Strachan, L. I. N. D. S. A. Y. (2004). Investigating odour sources and odour emission rates from landfills through direct communication with residents. *Proc. of Wastecon 2004*.
- Ruth, J. H. (1986). Odor thresholds and irritation levels of several chemical substances: a review. *American Industrial Hygiene Association Journal*, 47(3), A-142. <https://doi.org/10.1080/15298668691389595>
- Sakabe, M., Asanuma, D., Kamiya, M., Iwatate, R. J., Hanaoka, K., Terai, T., ... & Urano, Y. (2012). Rational design of highly sensitive fluorescence probes for protease and glycosidase based on precisely controlled spirocyclization. *Journal of the American Chemical Society*, 135(1), 409-414.
- Sakawi, Z., Sharifah, S. A., Jaafar, O., & Mahmud, M. (2011). Community perception of odor pollution from the landfill. *Research Journal of Environmental and Earth Sciences*, 3(2), 142-145.
- Saladin, K. S. (2004). *Anatomy & physiology: the unity of form and function*.
- Salamone, A. (2019, March 11). Second lawsuit alleges 'offensive odors' at Plainfield Township landfill. Retrieved from <https://www.mcall.com/business/mc-biz-grand-central-second-lawsuit-20190311-story.html>
- Sarkar, U., & Hobbs, S. E. (2002). Odour from municipal solid waste (MSW) landfills: A study

- on the analysis of perception. *Environment International*, 27(8), 655-662.
[https://doi.org/10.1016/S0160-4120\(01\)00125-8](https://doi.org/10.1016/S0160-4120(01)00125-8)
- Sarkar, U., Longhurst, P. J., & Hobbs, S. E. (2003). Community modelling: a tool for correlating estimates of exposure with perception of odour from municipal solid waste (MSW) landfills. *Journal of environmental management*, 68(2), 133-140.
[https://doi.org/10.1016/S0301-4797\(03\)00027-6](https://doi.org/10.1016/S0301-4797(03)00027-6)
- Sattler, M., & Devanathan, S. (2007). Which meteorological conditions produce worst-case odors from area sources?. *Journal of the Air & Waste Management Association*, 57(11), 1296-1306.
<https://doi.org/10.3155/1047-3289.57.11.1296>
- Scheutz, C., Kjeldsen, P., Bogner, J. E., De Visscher, A., Gebert, J., Hilger, H. A., Huber-Humer, M., and Spokas, K. (2009). "Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions." ISSN 0734-242X *Waste Management & Research*, 27, 409-455.
- Schiefner, A., Freier, R., Eichinger, A., & Skerra, A. (2015). Crystal structure of the human odorant binding protein, OBP IIa. *Proteins: Structure, Function, and Bioinformatics*, 83(6), 1180-1184. <https://doi.org/10.1002/prot.24797>
- Schiffman, S. S. (1998). Livestock odors: implications for human health and well-being. *Journal of animal Science*, 76(5), 1343-1355.
- Schiffman, S. S., Miller, E. A. S., Suggs, M. S., & Graham, B. G. (1995). The effect of environmental odors emanating from commercial swine operations on the mood of nearby residents. *Brain research bulletin*, 37(4), 369-375. [https://doi.org/10.1016/0361-9230\(95\)00015-1](https://doi.org/10.1016/0361-9230(95)00015-1)
- Schiffman, S. S., Walker, J. M., Dalton, P., Lorig, T. S., Raymer, J. H., Shusterman, D., & Williams, C. M. (2000). Potential health effects of odor from animal operations, wastewater treatment, and recycling of byproducts. *Journal of Agromedicine*, 7(1), 7-81.
https://doi.org/10.1300/J096v07n01_02
- Sela, L., & Sobel, N. (2010). Human olfaction: a constant state of change-blindness. *Experimental Brain Research*, 205(1), 13-29.
- Sheffield, R., Thompson, M., Dye, B., & Parker, D. (2004). Evaluation of field-based odor assessment methods. *Proceedings of the Water Environment Federation*, 2004(3), 870-879.
- Shen, T. T., Nelson, T. P., & Schmidt, C. E. (1990). Assessment and control of VOC emissions from waste disposal facilities. *Critical Reviews in Environmental Science and Technology*, 20(1), 43-76.
- Shigeoka, K., Nakatsuji, Y., Ogawa, K., Iwasaki, Y., & Ueno, H. (2009). The established method

- of three regulation standards in Japan. *Odours and VOCs: Measurement, Regulation and Control Techniques*, 31, 179.
- Shults, M. D., & Imperiali, B. (2003). Versatile fluorescence probes of protein kinase activity. *Journal of the American Chemical Society*, 125(47), 14248-14249. <https://doi.org/10.1021/ja0380502>
- Silva, C., Matamá, T., Azoia, N. G., Mansilha, C., Casal, M., & Cavaco-Paulo, A. (2014). Odorant binding proteins: a biotechnological tool for odour control. *Applied microbiology and biotechnology*, 98(8), 3629-3638.
- Sneath, R. W. (2001). *Olfactometry and the CEN Standard EN13725. Odours in Wastewater Treatment: Measurement, Modelling and Control*. Eds Stuetz R. and Frechen BF, IWA Publishing.
- Song, H. M., & Lee, C. S. (2008). Simple fabrication of functionalized surface with polyethylene glycol microstructure and glycidyl methacrylate moiety for the selective immobilization of proteins and cells. *Korean Journal of Chemical Engineering*, 25(6), 1467-1472.
- Spinelli, S., Ramoni, R., Grolli, S., Bonicel, J., Cambillau, C., & Tegoni, M. (1998). The structure of the monomeric porcine odorant binding protein sheds light on the domain swapping mechanism. *Biochemistry*, 37(22), 7913-7918.
- Sreejith, R. K., Yadav, V. N., Varshney, N. K., Berwal, S. K., Suresh, C. G., Gaikwad, S. M., & Pal, J. K. (2009). Conformational characterization of human eukaryotic initiation factor 2 α : A single tryptophan protein. *Biochemical and biophysical research communications*, 390(2), 273-279.
- Stangor, C. (2012). *Beginning Physiology v.1*. Creative Commons by-nc-sa 3.0.
- St Croix Sensory, I. (2005). *A review of the Science and Technology of Odor Measurement*.
- Stuetz, R. M., & Frechen, F. B. (Eds.). (2001). *Odours in wastewater treatment*. IWA publishing.
- Takuwa, Y., Matsumoto, T., Oshita, K., Takaoka, M., Morisawa, S., & Takeda, N. (2009). Characterization of trace constituents in landfill gas and a comparison of sites in Asia. *Journal of Material Cycles and Waste Management*, 11(4), 305.
- Tcatchoff, L., Nespoulous, C., Pernollet, J. C., & Briand, L. (2006). A single lysyl residue defines the binding specificity of a human odorant-binding protein for aldehydes. *FEBS letters*, 580(8), 2102-2108.
- Tegoni, M., Pelosi, P., Vincent, F., Spinelli, S., Campanacci, V., Grolli, S., ... & Cambillau, C. (2000). Mammalian odorant binding proteins. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, 1482(1-2), 229-240. [https://doi.org/10.1016/S0167-4838\(00\)00167-9](https://doi.org/10.1016/S0167-4838(00)00167-9)

- Thu, K., Donham, K., Ziegenhorn, R., Reynolds, S., Thorne, P. S., Subramanian, P., ... & Stookesberry, J. (1997). A control study of the physical and mental health of residents living near a large-scale swine operation. *Journal of agricultural safety and health*, 3(1), 13-26. doi: 10.13031/2013.17747
- Turk, A., Haring, R. C., & Okey, R. W. (1972). Odor control technology. *Environmental Science & Technology*, 6(7), 602-607.
- Van Harreveld, A. P., Heeres, P., & Harssema, H. (1999). A review of 20 years of standardization of odor concentration measurement by dynamic olfactometry in Europe. *Journal of the Air & Waste Management Association*, 49(6), 705-715. <https://doi.org/10.1080/10473289.1999.11499900>
- Vidovic, M. (2017). *Meteorological Conditions Affecting the Dispersion of Landfill Odor Complaints*. Florida Atlantic University.
- Walker, J. M. (1991). *Fundamentals of odor control*. BioCycle (USA).
- Wei, Y., Brandazza, A., & Pelosi, P. (2008). Binding of polycyclic aromatic hydrocarbons to mutants of odorant-binding protein: a first step towards biosensors for environmental monitoring. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1784(4), 666-671. <https://doi.org/10.1016/j.bbapap.2008.01.012>
- Wenjing, L., Zhenhan, D., Dong, L., Jimenez, L. M. C., Yanjun, L., Hanwen, G., & Hongtao, W. (2015). Characterization of odor emission on the working face of landfill and establishing of odorous compounds index. *Waste Management*, 42, 74-81. <https://doi.org/10.1016/j.wasman.2015.04.030>
- Whitson, K. B., & Whitson, S. R. (2014). Human odorant binding protein 2a has two affinity states and is capable of binding some uremic toxins. *Biochemistry and Analytical Biochemistry*, 3(2), 1.
- Wing, S., & Wolf, S. (2000). Intensive livestock operations, health, and quality of life among eastern North Carolina residents. *Environmental health perspectives*, 108(3), 233-238. <https://doi.org/10.1289/ehp.00108233>
- Wood, J. A., & Porter, M. L. (1987). Hazardous pollutants in class II landfills. *Japca*, 37(5), 609-615. <https://doi.org/10.1080/08940630.1987.10466250>
- Xia, F. F., Zhang, H. T., Wei, X. M., Su, Y., and He, R. (2015). "Characterization of H₂S removal and microbial community in landfill cover soils." *Environmental Science and Pollution Research*, 22(23), 18906–18917.
- Xing, B., Khanamiryan, A., & Rao, J. (2005). Cell-permeable near-infrared fluorogenic substrates for imaging β -lactamase activity. *Journal of the American Chemical Society*, 127(12), 4158-

4159.

Xu, P. (2005). A *Drosophila* OBP required for pheromone signaling. *Science*, 310(5749), 798-799.

Yazdani, R. (2015). Evaluation of the Landfill Odor Problem at the Sunshine Canyon Landfill.

Ying, D., Chuanyu, C., Bin, H., Yueen, X., Xuejuan, Z., Yingxu, C., & Weixiang, W. (2012). Characterization and control of odorous gases at a landfill site: A case study in Hangzhou, China. *Waste management*, 32(2), 317-326. <https://doi.org/10.1016/j.wasman.2011.07.016>

Yoon, H., Lee, S. H., Kwon, O. S., Song, H. S., Oh, E. H., Park, T. H., & Jang, J. (2009). Polypyrrole nanotubes conjugated with human olfactory receptors: high-performance transducers for FET-type bioelectronic noses. *Angewandte Chemie International Edition*, 48(15), 2755-2758. <https://doi.org/10.1002/anie.200805171>

Yuwono, A. S., & Lammers, P. S. (2004). Odor pollution in the environment and the detection instrumentation. *Agricultural Engineering International: CIGR Journal*.

Zarra, T. (2007). Procedures for detection and modelling of odours impact from sanitary environmental engineering plants (Doctoral dissertation, PhD Thesis, University of Salerno, Salerno, Italy).

Zarra, T., Naddeo, V., & Belgiorno, V. (2009). A novel tool for estimating the odour emissions of composting plants in air pollution management. *Global Nest Journal*, 11(4), 477-486.

Zhang, Q. (2001). Odour emissions from confined swine production facilities. In *Proc. National Conference "Livestock Options for the Future"* (pp. 25-27).

Zhuang, Y. D., Chiang, P. Y., Wang, C. W., & Tan, K. T. (2013). Environment-sensitive fluorescent turn-on probes targeting hydrophobic ligand-binding domains for selective protein detection. *Angewandte Chemie International Edition*, 52(31), 8124-8128.

APPENDIX A: ADDITIONAL LITERATURE REVIEW

Landfill Odorants and Possible Sources

Landfilling has been a common form of ultimate solid waste disposal in many countries of the world and so naturally, a landfill facilities are considered a common source of odor emissions related to the solid waste industry (El-Fadel et al. 1997). Of the various types of wastes disposed in the landfill, there are many that have their own characteristic smell e.g. household garbage, wastewater treatment biosolids, landfill gas (LFG), and others.

Rancid, sulfur and fragrant odors are most commonly perceived near MSW in landfills. In 2016, Curren et al. reported an intense rancid odor emission from MSW in the vicinity of the observation deck and tipping floor of a transfer station. Acetaldehyde, acetic acid, and butyric acid were mainly responsible for contributing to the emission of rancid and sour odor. They also found that sulfur odors (originating mostly from methyl mercaptan rather than hydrogen sulfide) are generated from MSW at off-site locations (Curren et al. 2016).

Biosolids disposal in landfills is another source of nuisance odor emissions. As the microorganisms decompose the amino acids and carbohydrates in the biosolids, various odorous compounds are released from them including ammonia, amines, mercaptans, fatty acids and sulfur (Walker 1991). Depending on the types of biosolids and the related processing mechanism, odors may vary between landfills (EPA 2000). For example, sulfur odors mainly generate from anaerobic wastewater residuals and air dried biosolids whereas alkaline biosolids can cause volatile emissions in landfills (EPA 2000, Bertucci et al. 1994).

The anerobic decomposition of waste leads to the formation of landfill gas (LFG), which is another common cause of odor emissions in landfills (Ritzkowski et al. 2006). During vaporization from solid/liquid phase, some chemical compounds can also create a pungent smell (Turk et al. 1972). Depending on the type of waste, age, water content, temperature, pH, alkalinity, etc., the characteristics of the LFG emissions varies from landfill site to landfill site (Yazdani 2015). Table A1_ contains the main components found in landfill gas.

Table A1: Components of landfill gas and their concentrations (Takuwa et al. 2009)

Compound	Typical concentration
Methane (CH ₄)	30%–60%
Carbon dioxide (CO ₂)	20%–50%
Oxygen (O ₂)	<2%
Nitrogen (N ₂)	<10%
Water (H ₂ O)	Saturated
Trace compounds	< 4000×10 ⁻⁶ mol/mol

Around 90% of the landfill gas (LFG) consists of a combination of methane (30%-60%) and carbon dioxide (20%-50%), which is odorless. Thus, only a small percentage of trace components (VOCs, HAPs, etc.) contribute to the odors in existing in landfills (El-Fadel et al. 1997; Davoli et al. 2003; Fang et al. 2012). -Table A2 characterizes some of gaseous components typically detected in the air at landfill facilities, along with their probable release mechanisms.

Table A2: Components of landfill gas along with their sources and possible release mechanism (Parker et al. 2002)

Trace component	Probable source	Probable release mechanism
Hydrogen	Organics acids	Anaerobic microbial respiration
	Metals	Corrosion
Hydrogen sulfide	Sulfate wastes	Anaerobic microbial respiration
Vinyl chloride	Chlorinated solvents	Anaerobic microbial respiration
Simple alkanes & alkenes	Organic wastes	Anaerobic microbial respiration
Organic acids	Organic wastes	Anaerobic microbial respiration
Mercaptans	Organic material	Anaerobic microbial metabolism
		Anaerobic microbial respiration
Alcohols & ketones	Organic wastes	Microbial metabolism
	Solvents	Evaporation & gas stripping Aerosols
Aldehydes	Organic wastes	Microbial metabolism
Limonene	Plant material	Anaerobic microbial respiration
Ammonia	Organic wastes	Anaerobic microbial respiration
Amines	Organic wastes	Microbial metabolism
		Aerosols
Esters	Organic acids & alcohols	Chemical reaction or microbial action
		Aerosols
Chlorinated hydrocarbons	Solvents & paints in waste	Evaporation, gas stripping
Simple aromatic hydrocarbons	Solvents & paints in waste	Evaporation, gas stripping
Chloro and chlorofluorohydrocarbons	Foams & propellants in waste	Out-gassing
Mercury	Inorganic waste	Microbial methylation

Trace component	Probable source	Probable release mechanism
		Evaporation
		Dust

While tracing VOCs in landfill gas at seven UK waste disposal sites, Allen et al. (1997) identified over 140 trace components to be present in the LFG, of which 90 were observed in all landfill sites (Allen et al. 1997). The trace components were categorized into six main groups (Parker et al. 2002):

- Alkanes, 302 - 1543 mg/m³
- Aromatic compounds, 94 - 1905 mg/m³
- Cycloalkanes, 8 - 487 mg/m³
- Terpenes, 35 – 652 mg/m³
- Alcohols and ketones, 2 – 2069 mg/m³
- Halogenated compounds, 327 – 1239 mg/m³

The molecular structure of the odorant compounds largely varies, although most of them are non-ionic hydrophobic organic compounds having a molecular weight of less than 300 (Schiffman et al. 2000). Reactive inorganic gases such as ammonia (NH₃) and hydrogen sulfide (H₂S), which are also responsible for contributing offensive odor in landfills, do not belong to any specific functional group or classification, like organic odorants. While conducting a study on different sources of odor in a landfill in Turkey, Dincer et al. (2016) found that aldehydes and ketones were most responsible for creating odor among the 53 compounds detected. Most odors at landfills result from sulfur compounds such as hydrogen sulfide, dimethyl sulfide, methanethiol, propanethiol, nitrogen compounds such as ammonia and amines, hydrocarbons such as benzene, phenols, terpenes, styrene, toluene, xylene, acetone, methanol, n-butanone, n-butylaldehyde, volatile fatty acids, etc. (Parker et al. 2002; Fang et al. 2012). Most people find the odor emitted from hydrogen sulfide, mercaptans, amines, and nitrogenous heterocyclic compounds to be offensive. A considerable amount of VOC (e.g. toluene) is also found to be emitted from the exhaust of unloading, spreading and compaction vehicles and trucks operating in and around landfill sites (Chiriac et al. 2007).

Odor Characteristics

Various socio-economic and cultural factors, previous experience with odor, personal sensitivity, surrounding environment, etc. have an impact on an individual's perception of odor annoyance. Scientists are dedicating considerable resources to discover a relationship between odor perception and the molecular structure of the odorants (Belgiorno et al. 2013). The following subsections discuss odor perception as well as different odor measurement techniques used in attempting to characterize odors. There are various classifications of odor characteristics, but the four most

common are: detectability, intensity, quality, and hedonic tone (EPA, 2001). Descriptions of all four dimensions are provided in the following paragraphs.

Detectability is related to the odor threshold, which refers to the minimum concentration of an odorant detected by the human nose (CalRecycle 2019). Detectability varies from individual to individual depending on many factors (Pearce et al. 2006), ~~such as those described in the first paragraph of Section 1.3.~~ Intensity refers to strength of the odor sensation at a concentration level that has already exceeded the perceptibility threshold i.e. the detection limit (Pearce et al. 2006). In case of some odorants such as hydrogen sulfide, the perceived intensity may be high even at a very low concentrations and these odorants are typically unpleasant in nature (Belgiorno et al. 2013). A 6-point scale is often used to measure the odor intensity starting from 0 to 5 with 0 being no odor and 5 being very strong odor (Lacey et al. 2004). Several mathematical functions have been established to correlate the interdependency between odor concentration and odor intensity. In the case of Steven's law, the relationship follows an exponential function (Figure A1 left); whereas in the Weber-Fechner equation, the relationship is logarithmic (Figure A1 right) (Curren et al. 2013; Stuetz and Frechen 2001).

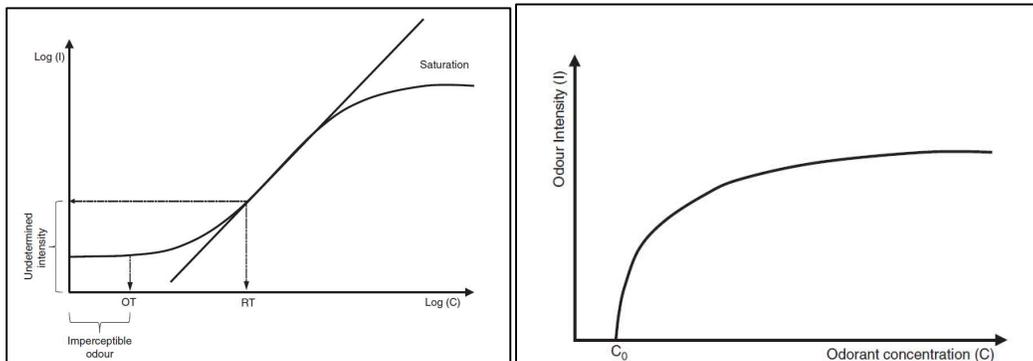


Figure A1: Relationship between odor concentration and intensity according to Stevens (left). Weber-Fechner model of change in odorant concentration vs odor intensity (right) (Belgiorno et al. 2013)

Odor quality is a descriptor of an odorant i.e. what the substance smells like (e.g. fruity, pungent, rancid etc.), which helps to describe the characteristics of the odor (Yuwono and Lammers 2004). Figure A2 shows an example of an odor wheel.

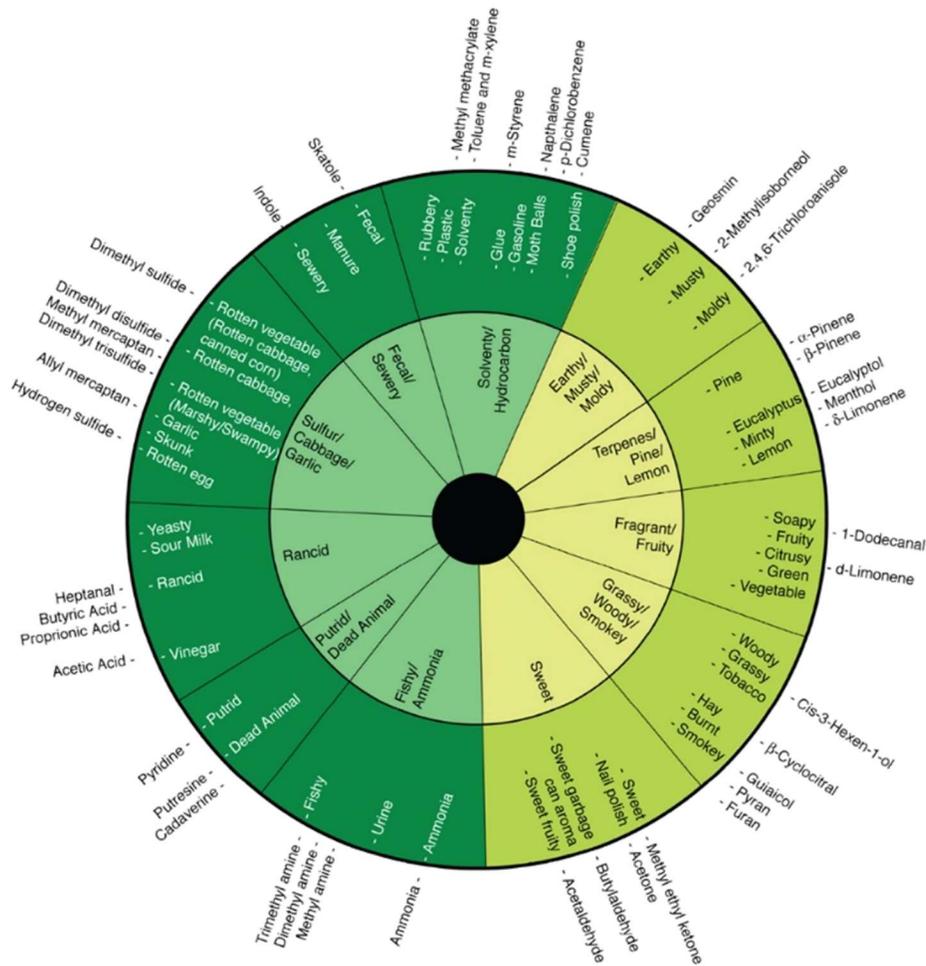


Figure A2: Example of an odor wheel describing odorant qualities from solid waste management operations (Mel Suffet et al. 2009).

The American Society for Testing and Materials (ASTM) established 146 descriptors that are most commonly used in describing odors (Schiffman et al. 2000). Table A3 presents standard odor descriptors of some common odorants typically observed in landfills.

Table A3: Description of odor associated with various landfill gas components (ATSDR 2016)

Component	Odor description
Hydrogen Sulfide	Strong rotten egg
Ammonia	Pungent acidic or suffocating
Benzene	Paint thinner-like
Dichloroethylene	Sweet, ether-like, slightly acrid
Dichloromethane	Sweet, chloroform-like
Ethylbenzene	Aromatic, benzene-like
Toluene	Aromatic, benzene-like
Trichloroethylene	Sweet, chloroform-like
Tetrachloroethylene	Sweet, ether-like or chloroform-like
Vinyl chloride	Faintly sweet

Hedonic tone refers to relative pleasantness (like) or unpleasantness (dislike) of an odor. This is a very subjective term. A smell that is pleasant to one person may not be agreeable to another person (CalRecycle 2019) and depends on an individual’s perception of the nature of the odor (Pearce et al. 2006). A 9-point scale (Table A4) is used to describe hedonic tone ranging from extremely unpleasant (low negative score, “-4”) to extremely pleasant odor (high positive score, “+4”) (Li et al. 2019).

Table A4: Verbal descriptions associated with each point of the hedonic scale of odors (Li et al. 2019)

Hedonic Tone	Verbal Description
-4	Extremely unpleasant
-3	Moderate unpleasant
-2	Unpleasant
-1	Slightly unpleasant
0	Neutral
1	Slightly pleasant
2	Pleasant
3	Moderate pleasant
4	Extremely pleasant

Since human perception of odors is highly subjective and may vary widely among individuals, there are other dimensioning procedures. Five interactive components commonly known as FIDOL are widely accepted in many countries such as Australia, New Zealand etc. (Freeman and

Cudmore 2002; Nicell 2009) to facilitate odor investigation. FIDOL stands for frequency (F), intensity (I), duration (D), offensiveness (O), and location (L), and these five parameters are used to characterize odor annoyance, its impact, and its adverse effect on humans (Nicell 2009).

Odor Threshold

Odorants can be detected at different concentrations based on an individual's perception of odor, which normally varies from person to person. Odor detection threshold (ODT) is the minimum concentration of any odorant that can be detected by the olfactory system of 50% of the human test population (Yuwono and Lammers 2004). Although the relationship between the molecular weight of the odorants and the odor detection threshold is not clear, a study conducted by Nagata and Takeuchi in 2003 found that the threshold decreases with increase in odorant molecular weight, where the molecular weights have a range of 12-170 (Figure A3 left). The tendency is more apparent in the case of homologous chemical compounds such as alcohol, aldehyde, mercaptan, ketone and hydrocarbon.

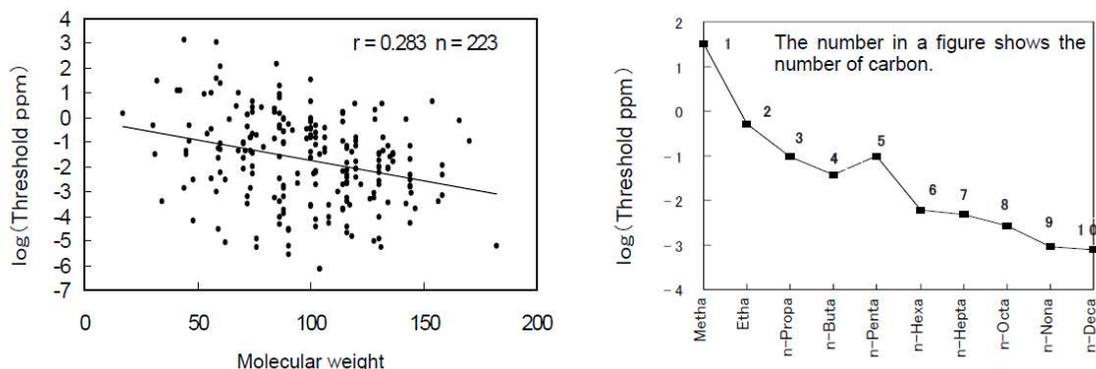


Figure A3: Graph showing the general trend of decreasing odor threshold against increasing molecular weight (left). The odor threshold decreases for an increase in the number of carbon atoms in aliphatic alcohols (right) (Nagata and Takeuchi 2003)

In 1986, Ruth reported odor threshold values for possible trace components found in landfill gas (Table A5).

Table A5: Odor descriptions of various gases found in landfills along with their detection limits for humans (Ruth 1986)

Compounds	Odor (Description)	Detection Limits	
		$\mu\text{g}/\text{m}^3$	$\times 10^{-9}$ mol/mol
Sulfur compounds			
Hydrogen sulfide	rotten eggs	0.7	0.5
Carbon disulfide	disagreeable, sweet	24.0	7.7
Dimethyl sulfide	rotten cabbage	2.5	1.0
Dimethyl disulfide	rotten cabbage	0.1	0.026
Dimethyl trisulfide	rotten cabbage	6.2	1.2
Methyl mercaptan	rotten cabbage	0.04	0.02
Ethyl mercaptan	rotten cabbage	0.032	0.01
Allyl mercaptan	garlic coffee	0.2	0.1
Propyl mercaptan	unpleasant	0.2	0.1
Amyl mercaptan	putrid	0.1	0.02
Benzyl mercaptan	unpleasant	1.6	0.3
Thiophenol	putrid garlic	1.2	0.3
Sulphur dioxide	irritating	1175.0	449.3
Carbon oxysulfide	pungent	NA	NA
Nitrogen Compounds			
Ammonia	pungent, sharp	26.6	38.3
Aminomethane	fishy, pungent	25.2	19.5
Dimethylamine	fishy, amine	84.6	46.0
Trimethylamine	fishy, pungent	0.1	0.046
Skatole	feces, chocolate	0.00004	0.00001
Volatile Fatty Acids			
Formic	biting	45.0	24.0
Acetic	vinegar	2500.0	1019.1
Propionic	rancid, pungent	84.0	27.8
Butyric	rancid	1.0	0.3
Valeric	unpleasant	2.6	0.6
Ketones			
Acetone	sweet, minty	1100.0	463.9
Butanone	sweet, minty	737.0	250.4
2-Pentanone	sweet	28000.0	7967.5
Acetaldehyde	green sweet	0.2	0.1
Methanol	alcohol	13000	9953.1
Ethanol	alcohol	342	342
Phenol	medicinal	178	46

It is worth mentioning that the lower the detection limit of an odorant, the higher the potential importance of that odorant since it can be perceived by humans even if present in small amounts (Parker et al. 2002). Due to this fact, odorants such as sulfur compounds, nitrogen compounds and oxygenated compounds often receive more attention and interest among researchers (Wenjing et al. 2015). Additionally, what may seem to be an unpleasant odor to a person may not be unpleasant at all to another person; that is why the reported threshold values for odorous compounds in the literatures are only an estimate (ATSDR 2016). Depending on the value of the threshold detection limit, Parker et al. (2002) provided an odor ranking plan (Table A6).

Table A6: Odor ranking plan based on detection concentration for humans (Parker et al. 2002)

Odor Ranking	1	2	3	4	5
Detection Concentration Range (μgm^{-3})	>1000	100-1000	10 - 100	10 – 1	<1

Based on the odor ranking and physical property ranking (1 if mobility is lower than benzene and 2 if mobility is higher than benzene), an odor importance ranking has been provided for 12 common odorants (Table A7) having the greatest potential to cause odor in landfills (Parker et al. 2002).

Table A7: Ranking of odor importance in landfills based on physical and odor rankings (Parker et al. 2002)

Chemical Name	Chemical Group	Physical Ranking	Odor Ranking	Odor Importance
1 Hydrogen sulfide	Organo Sulfur Compounds	2	5	10
2 Methanethiol	Organo Sulfur Compounds	2	5	10
3 Carbon disulfide	Organo Sulfur Compounds	2	3	6
4 Propanethiol	Organo Sulfur Compounds	1	5	5
5 Butyric acid	Carboxylic acids	1	5	5
6 Dimethyl disulfide	Organo Sulfur Compounds	1	5	5
7 Ethanal	Aldehyde	1	5	5

Chemical Name	Chemical Group	Physical Ranking	Odor Ranking	Odor Importance
8 Ethanethiol	Organo Sulfur Compounds	1	5	5
9 Butanethiol	Organo Sulfur Compounds	1	4	4
10 Pentene	Alkenes	2	2	4
11 Dimethyl sulfide	Organo Sulfur Compounds	1	4	4
12 Ethyl butyrate	Ester	1	4	4

Among the 12 compounds listed in Table 7, the organo-sulfur compounds tend to dominate in landfill settings because they are associated with older waste where the emission contains relatively low amounts of carboxylic acids, aldehydes, and esters. Odorants having a low threshold value can trigger the human sense of smell even at low concentrations. For example, if only three drops of ethyl mercaptan are added to an Olympic-sized swimming pool, its presence can be detected by humans (Sela & Sobel 2010). The odorant having the lowest detection threshold is isoamyl mercaptan, having a threshold value of 0.77 ppt (Nagata & Takeuchi 2003). When people are repeatedly exposed to an odorant, it may cause an artificial increase in threshold value, in some cases (referred to as “nose blind”).

Odor Unit (OU)

An odor unit (OU) or threshold odor number is used to express odor concentration. The European Odor Unit (OU_E), which is commonly used in European countries, refers to the concentration of any odorant that when evaporated to 1 m³ of neutral gas (odor-free air) produces the same response when 123 µg of *n*-butanol disperses into 1 m³ of neutral gas (Zhang 2001). 1 OU_E is basically the concentration of any odorant equivalent to its threshold concentration (Lacey et al. 2004). This unit is more convenient for using in calculations than for normal purposes since ppmv or ppbv (volume per volume) is more common for expressing odor concentration (Yuwono & Lammers 2004). However, the strength of the odor or offensiveness cannot be perceived by means of odor unit since the unit only conveys the meaning of how many times the concentration of the odor is greater than its threshold concentration (Laor et al. 2014). As a result, two facilities emitting the same unit of odor concentration might not be perceived equally by the receptors as to the offensiveness they illicit. The odorants having lower detection threshold might be perceived much more negatively than the other although both may have the same unit of concentration.

Factors Affecting Dispersion of Odorants

As soon as odor is released from a source, transport into the atmosphere happens readily due to their highly fugitive nature. Various environmental factors including climate, topography, wind speed and direction, temporal variation, humidity, source strength etc. affect the dispersion and dilution of odorants along with their impact on humans (Sakawi et al. 2011). According to a survey conducted in Malaysia by Sakawi et al. in 2010, 92.6% of respondents living within a 2 km radius of an open landfill concluded that nuisance odor in the vicinity of the landfill is largely affected by meteorological factors of which 40.6% identified wind, rain and hot weather to be the most important factors (Figure A4).

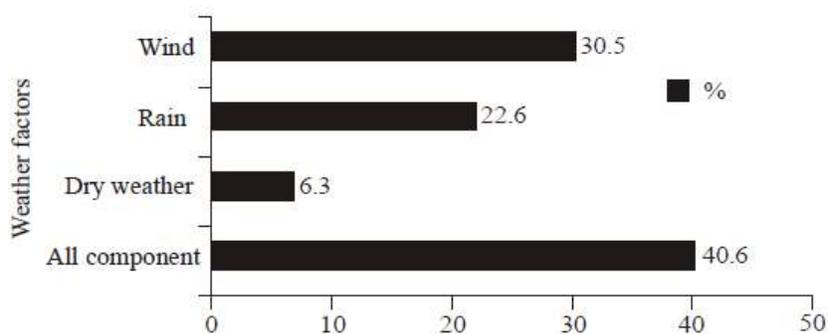


Figure A4: Survey results showing percentage of people identifying each meteorological factor as the most important factor in odor dispersion/concentration (Sakawi et al. 2011)

High vapor pressure and low solubility of VOC compounds facilitate their emission during summer (Paraskaki and Lazaridis 2005; Reinhart 1993; Wood and Porter 1987). However, unstable weather conditions on warm/hot days disperse odorants more quickly, generally leading to a lower number of odor complaints (Energy and Environmental Affairs 2017; Epstein 2011). However, there were also cases when the number of odor complaints increased due to increased heat (Vidovic 2017). In winter, the cold weather does not promote emission, but dispersion also remains lower, keeping the odorants nearer to the source for a longer time, and giving rise to a stronger smell at the source of emission (Sattler and Devanathan 2012). Research conducted on two urban landfills in South Florida also shows that odor complaints increase in the dry season i.e. during winter and spring (November – April) compared to the wet season during the summer months (Vidovic 2017).

Odor Measurement Techniques

The task of objectively quantifying odor emissions has turned out to be a challenge. Without proper quantification, it is not possible to design effective regulations to curb odor pollution, since there is no way to set legal/binding odor emission limits. Currently, measurement techniques vary from place to place. Such techniques range from odor detecting panels to electronic noses to analytical

atmospheric dispersion models for downwind receptors (Laor et al. 2014). The sections below will address the advantages and limitations of the current state-of-the-art in odor measurement techniques.

Human Assessment

The most common form of odor measurement comes in the form of a panel of humans selected to apply their trained (but subjective) olfactory senses to detect the presence of odorants in ambient air. This type of odor assessment can be conducted in two ways: dynamic or static. In dynamic olfactometry, a device known as an olfactometer is used to blow a stream of air mixed with an odorant towards the human nose. The olfactometer can be used to control the concentration of the odorant using an odorless gas to control the dilution. Such a process can be used to test gaseous samples collected in Tedlar bags or other cylinders as shown in Figure A5 (Schiffman et al. 2000). In fact, this process has been standardized in the US as ASTM E679-19 (ASTM 2019), in Europe as EN13725 (CEN 2003), and in Australia and New Zealand as AS/NZS 4323.3:2001 (Laor et al. 2014). For measuring odor with this method, a human panel is formed, consisting of people with a “normal” sense of odor perception. For reference, members are selected according to their sensitivity to *n*-butanol in the range of 20×10^{-9} - 80×10^{-9} mol/mol and a specific standard deviation (Laor et al. 2014). It is worth stating that the human perception threshold for *n*-butanol is 40×10^{-9} mol/mol or 123 ug/m^3 -air (van Harreveld et al. 1999). The panel is then exposed to a stream of odorless air. The concentration of the odorant gas in the air is slowly increased (i.e. the dilution of the odorant is slowly decreased) until all members of the panel can distinguish the smell of odorant in the stream of air (St. Croix Sensory 2005; Baltrėnas et al. 2012). The concentration of the odorant can then be determined by various statistical and averaging methods (Laor et al. 2014). During the process, the panel may also be asked to provide other information such as rating the odor on other characteristics including quality (odor character), intensity (odor strength), and irritation intensity (Schiffman et al. 2015).

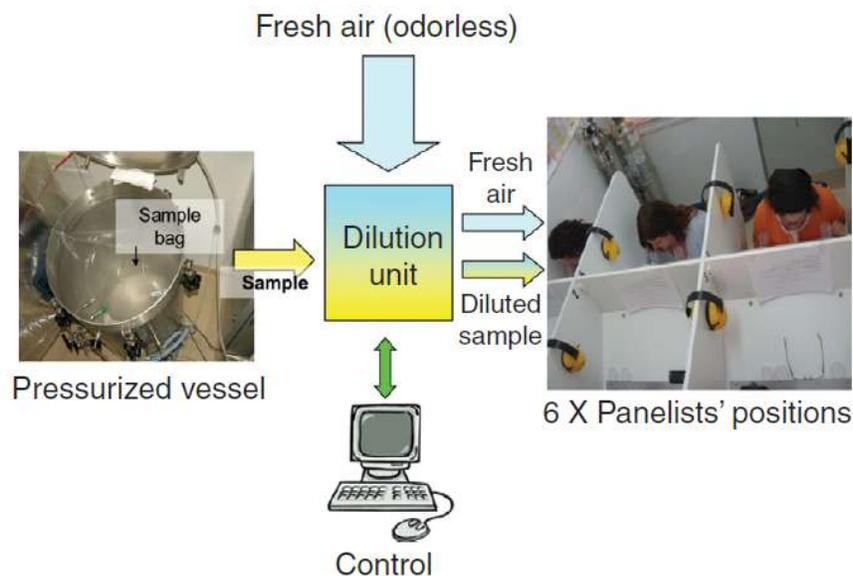


Figure A5: Block diagram showing the process of lab olfactometry and the components associated with it (Laor et al. 2014)

There are some obvious limitations in dynamic olfactometry. Since human panelists are used in this technique, some unaccounted-for measure of subjectivity is introduced, which is not the norm for scientific measurements (Belgiorno et al. 2013). The subjectivity comes from the physiological differences in the smelling capabilities of different people. Odor measurement by human assessment is also a costly procedure and is difficult to recreate or repeat (Yuwono, & Lammers 2004). Different measurements may be obtained at different times, even keeping all factors constant and so, this is not a method that can scale well with time. With a different panel, the result may vary even further. There is also the impracticality of conducting this procedure in the case of poisonous gases (Yuwono, & Lammers 2004). Cases of olfactory fatigue also cannot be ruled out and is a barrier to objective results. To reduce the subjectivity, multiple panelists may be used to test the same odorant and obtain results. In practice, the team of panelists may just be two people, or even one in some cases (Nicolas et al., 2006). Although this process provides data on odor concentration, it does not provide information on the magnitude of the disturbance experienced by the population or the effective contribution of individual components of the complete odorant mixture (Jiang 1996; Sneath 2001). Moreover, if a mixture of odors is present, nothing allows the constituent odors to be identified separately.

In static olfactometry, the odorant is sampled from a bottle that contains either the odorant emitting substance itself or, for example, cotton pieces containing the odorant emitting substance. Another method for measuring odor is known as the triangular odor bag method. This is the recommended odor measurement method for factories and livestock in Japan (Offensive Odor Control Law, 1995; Iwasaki 2004). The triangular odor bag method is an air dilution process in which odor concentration and index are measured (Belgiorno et al. 2013). Odor concentration is the dilution

ratio when air with odorant in it is mixed with odorless air such that the odor cannot be detected. The odor index is the logarithm of odor concentration of air times 10. In this method, a panel of 6 people gets three bags, one that has an odor in it and the other two containing a non-odorous gas (Shigeoka et al. 2009). The panelists are then asked to pick the odorous bag. The odorous gas is diluted until it becomes indistinguishable from the other two bags. The odor index can then be calculated. The drawback of this process is that it is complex and requires six human panelists (Iwasaki et al. 2003).

Field Olfactometry

While laboratory olfactometry depends mostly on human assessment, field olfactometry depends on static olfactometry and is accomplished using a device known as a field olfactometer (Figure A6) (Laor et al. 2014). This device works by adjusting the ratio of odorous (non-filtered) and non-odorous (filtered) air. The Dilution to Threshold (D/T) ratio is the number of dilutions to the threshold level of an odorant and is determined under field conditions (Laor et al. 2014). Different types of field olfactometers include the Nasal Ranger[®], the Barneby box, and the mask scentometer (Newby & McGinley 2004, Sheffield et al. 2004, Henry et al. 2011b).



Figure A6: Instruments used in field olfactometry (Laor et al. 2014)

Field olfactometers are an economically viable option, and there is also the advantage that readings are taken in the field instead of in a lab (Brattoli et al. 2011). However, limitations of field olfactometers such as odor fatigue caused by a scentometer due to the difficulty of not exposing the device to the odorous environment before the actual readings are taken have restricted their applications (Henry et al. 2011b; Bokowa 2010b). There are also no options for dilutions, and the sniffers cannot be rated against their ability to detect a known reference concentration. The quantification process itself is a difficult task due to factors such as shifts in the odor plume, variations in wind speed and direction, and the presence of background or competing odors (Schiffman et al. 2000).

Atmospheric Dispersion Modeling

Atmospheric dispersion models are an analytical method of calculating the concentration of odorants at a certain distance and height downwind from the emission source. Inputs to these dispersion models include source odor concentrations, which have been determined by other methods such as human assessment or electronic nose measurements. So, the accuracy of this process depends very much on the accuracy of the source concentration measurement. Tracer gases such as SF₆ or objects such as helium balloons can be used to monitor the dispersion of odorants (Flyer et al. 1977; Lehning et al. 1994). Dispersion models predict the number of dilutions required to reach the threshold concentration at a specific distance from the source. So, there is no way to accurately predict odor annoyance unless the relationship between odor concentration and perceived offensiveness is quantified objectively (Laor et al. 2014). There is also the shortcoming that such models are odorant specific and non-quantitative. Community perceptions of odors are usually carried out using such models (Sarkar and Hobbs 2002; Sarkar et al. 2003; Roebuck et al. 2004; Laister et al. 2002).

Gas Chromatography/Mass Spectrometry (GC/MS)

A method of identifying odorants is gas chromatography (GC), which is a separation technique, paired with mass spectrometry (MS), which is used as a detection technique. An advantage of GC/MS is that very small concentrations of specific constituents can be detected and quantified. For this technique, the odor is collected beforehand and then passed through a chromatographic column where the target constituents in the sample get selectively adsorbed, retained, and then released as a pulse as the column is purged with an inert carrier gas (Yuwono & Lammers 2004). The mass spectrometer detector smashes the sample and analyzes the fragments as a fingerprint to identify the original compound that was separated from the sample by comparing the fragments to a reference library of known compounds. The detector is sensitive to the constituents in the sample and not the carrier gas.

There are a few disadvantages to using this method. Essentially GC/MS is a method of measuring the concentration of specific compounds in the air sample. At times, the intensity of odors does not match with the concentration of the compound. It may be that a very small amount of the compound is present, but the odor intensity is quite high. Thus, GC/MS is not able to give information about the actual intensity of the odor or synergistic effects (Davoli, 2004; Zarra et al., 2007b; Belgiorno et al. 2013). There is no direct correlation between the level of odor potential and the concentration of the odor emitting substances. The sampling techniques also influence the results to some extent (Gostelow et al., 2001), but the effect can be reduced by means of portable GC/MS analyzers (Zarra et al., 2008b; Zarra et al., 2008c). The technique is limited to analyzing compounds that can be successfully separated in a gas chromatographic column and that are referenced in published libraries of known odorants. Another issue is the relative costliness of the

technique as well as the higher skills required for using this method for analysis (Gardner and Bartlett 1999).

Electronic Nose

The electronic nose (E-nose) is an instrument that can quantify odors by means of an array of electrochemical sensors (Gardner and Bartlett 1999; Yuwono & Lammers 2004) that can detect simple and complex odors using pattern recognition systems. An odor stimulus generates specific signals known as fingerprint (or smell print) for the odorant. In this way, a database of different fingerprints can be built, each corresponding to a specific odorant that can then be used to detect an unknown odorant as shown in Figure A7 (Yuwono & Lammers 2004).

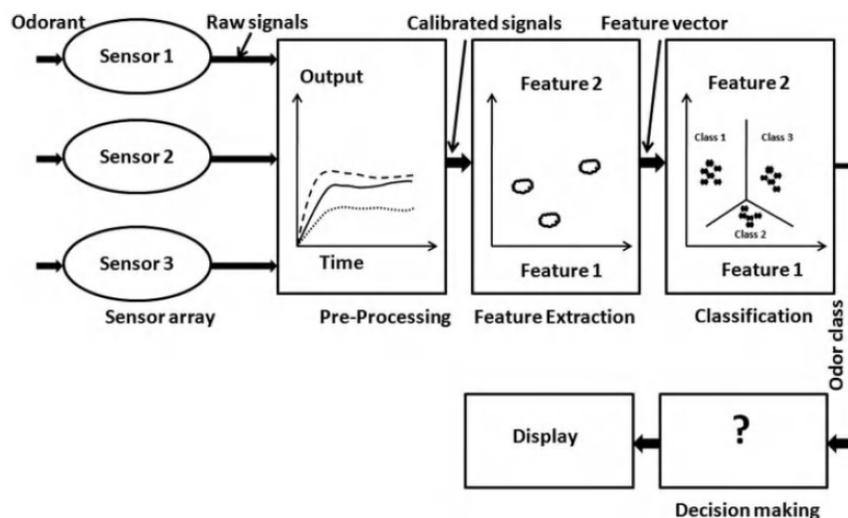


Figure A7: Block diagram showing the various components of an e-nose system (Belgiorno et al. 2013)

There are three components to an e-nose (Schiffman et al. 2000). The principal component is a number of gas sensors capable of detecting the presence of VOCs. Such sensors can be in the form of metal oxides conducting polymers or MOSFETs (metal oxide semiconductor field effect transistor sensors). The second component is known as the sample handler whose purpose is to transfer the odorant from the collection device to the gas sensors. The final component is the signal processing system that acts like the “brain” of the whole device. Signal processing can be done using pattern recognition, which in turn is performed using Artificial Neural Networks (ANNs), principal component analysis (PCA), cluster analysis, and discriminant function analysis (DFA) (Schiffman et al. 2000). As the output, the e-nose can return the actual odorants present as well as their concentrations.

Recently, e-noses have been used along with real-time dispersion modeling for regulation of odor emission sources (Laor et al. 2014). An added advantage of e-noses is their portability and their

usability in places where humans do not have to be present. However, they can only detect odorants having concentrations in the range of 10^{-9} mol/mol, while the actual human nose can detect concentrations one order of magnitude lower (Schiffman et al. 2000). The system must also undergo a training phase before it can be deployed to accurately detect odorant concentrations (Brattoli et al. 2011).

Chemical Sensor

A chemical sensor is a device that responds to a particular chemical in a way that can be used to confirm the presence and concentration of the chemical (Figure A8) (Yuwono & Lammers 2004). This definition also includes biosensors (Cattrall, 1997). Another definition is provided by Göpel and Schierbaum (1991) where it is stated that chemical and biochemical sensors are devices capable of converting chemical states (e.g. concentrations, partial pressures, or activities of particles) into electrical impulses (Yuwono & Lammers 2004). The same authors classified such sensors into categories based on the specific property used for the detection such as potential, voltages, conductivity and capacity, mass, heat, optical constant, etc. Generally, chemical sensors are composed of a chemically active substance along with a transducer. The substance detects the presence of the chemical, while the transducer converts the change induced into an electrical impulse (Gardner et al. 1988). In most cases, sensors are specific to one compound, and are not particularly useful in detecting complex odors in the field.

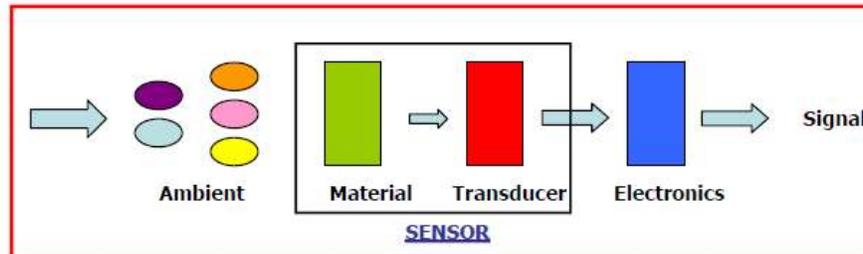


Figure A8: Block diagram representing the components in a solid state chemical sensor (Brattoli et al. 2011)

Limitations of Current Odor Measurement Techniques

A number of odor detection techniques have been discussed in the preceding paragraphs. Each of them has its own shortcomings. No one technique can perform all forms of analysis. In human assessment techniques, subjectivity is automatically introduced since the process is dependent upon human odor detection, calibration, and input. In addition, humans have large differences in odor perception (Belgiorno et al. 2013). These issues combine to make odor characterization an expensive procedure that generate results that are generally not reproducible (Yuwono & Lammers 2004). The triangular odor bag method discussed requires a panel of 6 members (Iwasaki 2003) and so, is expensive as well. The procedures are also complex, making it difficult to use for odor

quantification. In field olfactometry, scentometers are used which are susceptible to odor fatigue (Henry et al. 2011b; Bokowa 2010b), and the results obtained are affected by environmental factors such as variations in wind speed and direction as well as presence of background odors (Schiffman et al. 2000). Atmospheric dispersion models, while allowing analytical methods of quantifying odors, depend on accurate measurement of odor concentration at the source. Gas chromatography/mass spectrometry is another method that has been discussed that measures the concentration of odorants rather than odor intensity, which often do not correlate with each other (Gardner and Bartlett 1999; Davoli, 2004; Zarra et al., 2007b; Belgiorno et al. 2013). The method is fairly expensive to perform as well. Finally, electronic noses are less sensitive than human noses (Schiffman et al. 2000) and require a training phase before deployment (Brattoli et al. 2011).

There has been ongoing research that focuses on rapid identification of odors at low concentrations (Liu et al. 2013; Sakaran et al. 2012). However, these only work for specific odorants and are not quantitative. An example of such an odorant-specific chemical sensor is that found by Carlson et al. (2006), which can detect fluorine in concentrations of 10×10^{-12} mol/mol but was unable to work with its analogues such as naphthalene, fluoranthene, and anthracene. Also, the binding experiment was not reversible, which makes the sensor into a one-time use instrument. Liu et al. (2013) proposed a method for testing for the presence of different components of body odor, but their work does not include a way to quantify the odorants.

APPENDIX B: EXPERIMENTAL DATA

A. Titration test between 1 μ M hOBPIIa across varying concentrations of 1-AMA

Concentration 1-AMA	Fluorescence Intensity
0	0
0.1	2.51E+05
0.2	3.25E+05
0.3	3.97E+05
0.4	5.94E+05
0.5	4.98E+05
0.6	4.16E+05
0.7	6.47E+05
0.8	7.37E+05
0.9	7.49E+05
1.0	8.71E+05
1.1	8.10E+05
1.2	7.14E+05
1.3	9.22E+05
1.4	6.95E+05
1.5	8.37E+05

B. Relative fluorescence intensity obtained at different time intervals across all flow rates for nitrogen (N₂)

Time (s)	Relative Fluorescence Intensity
	0.5 slpm
0	100
10	81
20	87
30	88
45	98
60	83
75	93
90	92
120	80
150	79
180	84
210	93
240	82

C. Relative fluorescence intensity obtained at different time intervals across all flow rates for hydrogen sulfide (H₂S)

Time (s)	Relative Fluorescence Intensity		
	0.5 slpm	0.7 slpm	0.9 slpm
0	100	100	100
10	66	51	60
20	53	91	66
30	78	91	72
45	77	62	61
60	75	54	57
75	60	65	53
90	60	54	34
120	39	41	30
150	44	41	27
180	39	47	28
210	39	34	25
240	41	49	34

D. Relative fluorescence intensity obtained at different time intervals across all flow rates for ammonia (NH₃)

Time (s)	Relative Fluorescence Intensity		
	0.5 slpm	0.7 slpm	0.9 slpm
0	100	100	100
10	85	45	62
20	60	44	47
30	50	69	53
45	67	68	46
60	68	69	44
75	63	54	44
90	54	59	34
120	54	52	35
150	48	48	35
180	42	42	34
210	43	40	31
240	36	36	23

E. Relative fluorescence intensity obtained at different time intervals across all flow rates for methyl mercaptan (CH₃SH)

Time (s)	Relative Fluorescence Intensity		
	0.5 slpm	0.7 slpm	0.9 slpm
0	100	100	100
10	82	59	76
20	62	68	83
30	72	88	77
45	75	78	71
60	67	73	61
75	69	71	63
90	62	73	63
120	39	68	48
150	61	69	69
180	55	59	63
210	48	67	64
240	44	59	51

F. Relative fluorescence intensity obtained at different time intervals for methane (CH₄)

Time (s)	Relative Fluorescence Intensity
	0.5 slpm
0	100
10	70
20	65
30	54
45	68
60	70
75	48
90	54
120	42
150	52
180	40
210	44
240	46

G. Relative fluorescence intensity obtained at different time intervals for mixture 1 (NH₃ + CH₄)

Time (s)	Relative Fluorescence Intensity
	0.5 slpm
0	100
10	69
20	91
30	94
45	84
60	71
75	73
90	70
120	67
150	83
180	66
210	69
240	50

H. Relative fluorescence intensity obtained at different time intervals across all flow rates for mixture 2 (H₂S + CH₄ + CO)

Time (s)	Relative Fluorescence Intensity		
	0.5 slpm	0.7 slpm	0.9 slpm
0	100	100	100
10	71	81	75
20	94	90	72
30	91	89	79
45	93	79	90
60	61	72	78
75	81	71	71
90	77	57	59
120	51	62	58
150	63	56	58
180	62	51	50
210	70	54	40
240	44	53	60

I. Relative fluorescence intensity obtained at different time intervals for reaction reversibility experiment

Time (s)	Relative Fluorescence Intensity	Time (s)	Relative Fluorescence Intensity
	0.5 slpm H ₂ S		0.5 slpm N ₂
0	100	250	48
10	68	260	50
20	57	270	44
30	67	285	38
45	95	300	48
60	84	315	43
75	85	330	46
90	68	360	40
120	53	390	42
150	63	420	38
180	56	450	40
210	68	480	47
240	51	-	-

APPENDIX C: PUBLICATIONS

Conference Proceedings

- Rahman, S., Meeroff, D.E. (2020). Development of a Biosensor for Objectively Quantifying Odorants. *Odor and Air Pollutants 2020, Cincinnati, OH, March 2020*.

Conference Presentations

- Rahman, S. (2020). Development of a Biosensor for Objectively Quantifying Odorants. *Odor and Air Pollutants 2020, Cincinnati, OH, March 2020*.

Poster Presentations

- Biosensor Development to Quantify Nuisance Odors. 11th Annual Graduate and Professional Student Association (GPSA) Research Day, Florida Atlantic University, FL, April 2020.