Technical Advisory Group Meeting
Florida Atlantic University

Funded by the Hinkley Center for Solid and Hazardous Waste Management (HCSHWM)
and the Solid Waste Authority of Palm Beach County

DATE: **Monday, December 14, 2020**
TIME: **1:00 pm**
WHERE: **Virtual via Zoom**

MEETING AGENDA
Monday, December 14, 2020

1:00 – 1:15 pm	**Opening Address and Introduction of Participants**	**Daniel Meeroff**
1:15 – 1:25 pm	**Overview of Florida Atlantic University Studies**	**Daniel Meeroff**
1:25 – 1:55 pm	**Development of Biosensor for Odor Detection**	**Sharmily Rahman**
1:55 – 2:25 pm	**Examination of Leachate Collection System Clogging**	**Bishow Shaha**
2:25 – 2:55 pm	**Investigation of Leachate Management Solutions at the Solid Waste Authority of Palm Beach County**	**Rakib Chowdhury**
2:55 – 3:15 pm	**Open Forum**	**Participants**
3:15 pm	**Adjourn, Thank You**	**Daniel Meeroff**

Attendance: Daniel Meeroff, John Schert, Roshan Jachuck, Marc Bruner, Sharmily Rahman, Damaris Lugo – BCSD, Diuto Esiobu, Wester Henderson, Bishow Nath Shaha, Ramana Kari, Myles Clewner, Rakib Ahmed Chowdhury, Craig Ash, Paul Sgriccia, Richard Tedder - Landfill Permitting (Geosyntec), Md Fahim Salek, El Kromhout, Ram Tewari, David Gregory, Patrick Carroll, Hala Sfeir, Samuel Levin, Fred Bloetscher, Nathan Mayer, Debbie Reinhart UCF, Bryan Tindell, J McNamara, and 5 others with unidentified telephone numbers.

Minutes of Meeting

1. Opening address by D. Meeroff followed by an overview of the current status of Florida Atlantic University. Then the group members and all other participants present in the meeting introduced themselves. (1:10pm).
2. S. Rahman gave a presentation on development of a biosensor for measuring odorants in the ambient air near solid waste management facilities which is a continuation of previous researcher J. Roblyer's work. She outlined the challenges faced by landfill authorities in the absence of an objective odor measurement technique. She then introduced the research focus of using human odorant binding protein (hOBPIIa) to measure odorant levels. The mechanism is to use hOBPIIa combined with a fluorophore such as 1-AMA to create a biosensor complex which can be used to detect odorants by means of spectrofluorometry. Rahman then mentioned how the biosensor complex has been developed and described the model odorants used for conducting the experiments. The fluorescence binding assay showed that protein-fluorophore complex causes a high emission intensity at around 485 nm. The optimum concentration ratio of the protein and the 1-AMA is verified to be 1:1 as Roblyer found. Rahman showed that the peak fluorescence intensity curves of the various pure gases as well as the gas mixtures followed a general

trend of decreasing intensity with increasing concentration of gases up to a certain limit known as quantitation range. The mass of each of the gases consumed by the protein was also determined. The gas mixtures were found to decrease the peak intensity at a lower rate compared to that of its component gases. To be able to reuse the spent protein, a regeneration test was performed based on the hypothesis that passing nitrogen gas would help the protein rebind with 1-AMA but the initial experimentation indicated that protein regeneration might take a longer period of time than the current period of passing nitrogen gas. Rahman finally provided a number of recommendations based on the findings to be able to carry on the research towards its goal of implementing a handheld biosensor device. John Schert asked whether any fatty acid has been used in this research. Rahman responded that they have not used any fatty acid yet but they have plans to incorporate acetic acid in future research. John Schert asked about the typical odor threshold value of fatty acids with respect to hydrogen sulfide. Rahman responded that the odor threshold values of fatty acids are much higher than that of hydrogen sulfide. Dr. Esiobu asked the reason why the fluorescence intensity in case of the odorant mixtures did not reduce as much as that of the individual component gases. Rahman responded that in case of the mixtures there might be a competition between the odorant molecules for the binding sites of the protein and that is a likely reason behind the higher intensity value for mixtures. Dr. Esiobu then asked how an ideal handheld device will be able to identify a specific odorant as it would capture a wide fluorescence spectrum. Rahman responded that this might be obtained by comparing with standard response curves of individual odorants. However, further investigation is required in this regard.

3. B. Shaha gave a presentation on the Critical Evaluation of Leachate Clogging Potential in Gravity Collection System and Management Solutions. He first described the history of the leachate clogging issue faced by the SWA of Palm Beach County followed by a site description of the landfill where he did his research on. After that he explained the methodology of incorporating a side-by-side pipe network system in the site in order to observe the clogging formed in them. He then presented the water quality parameters of the leachate collected from different locations of the site including pH, conductivity, alkalinity, total solids, calcium, and magnesium which were all found to fall within the standard range. The LSI value indicated that the NEFCO wastewater is corrosive in nature with respect to $CaCO_3$ precipitation and also contains high concentration of microbes. Data from a cyclone separator indicated that the leachate contains about 180 lb of solids each day for a flow of 6 MGD. Shaha then discussed the three types of precipitation that were observed to form in the leachate, one among those adhering to the surface and was found to be very difficult to remove. Calcite is found to be the predominant component in the precipitation, and results indicate that the higher the initial pH of the leachate, the higher the amount of precipitation formed. Removing microbes and maintaining a consistent flow in the leachate collection system are found to reduce the quantity of adherents formed. Shaha then presented the water quality parameters and microbiological composition of the leachate collected at three depth levels of the deep injection well. He wrapped up the presentation by providing some recommendations based on the results he found. John Schert asked what would be the one take-away that Shaha would suggest in terms of managing the injection well. Shaha responded by saying that the leachate from the well is found to have some microbes creating the biofilms and added that by conducting a pretreatment analysis in the laboratory setting they would be able to suggest an idea to decrease the microbes. John Schert asked about bleeding in strong acid into the injection well at small constant amounts following Shaha's response. Dr. Meeroff responded that his research team is currently doing a microbe analysis of the leachate collected from the well and has been able to find out the specific microbe causing the biofilm

formation. He added that if the mineral clogging is dependent on the formation of the microbial biofilm then bleeding acid might not get at the root of the problem, although it might be a good preventative maintenance technique. Dr. Esiobu mentioned that using acid might reduce the population of biofilm producing microbes but also impact the beneficial microorganisms that help degrade the waste. Dr. Meeroff added that SWA does periodically inject acid with high pressure water when they find any major clogging. Ramana Kari added that they are doing routine brushing of the injection well recently which seems to be an effective preventative maintenance process, although temporary.

4. R. Chowdhury gave a presentation on Investigations of Leachate Management Solutions at the Solid Waste Authority of Palm Beach County, a follow up work of Shaha. He started by talking about a number of sources from where he has been collecting samples. Wastewater from all the mentioned sources mix into the wet well. The data of the water quality parameters showed that the Class I and groundwater samples demonstrated high turbidity likely due to lack of rainfall. The LSI value for the NEFCO wastewater is found to be in the neutral range. Chowdhury then showed a mass balance table where he estimated a range of water quality parameter values by incorporating all the samples that mixes in the wet well and the observed values of those parameters of the actual wet well sample determined in the laboratory. The dominant microorganism behind the biofilm formation in the deep injection well of the Palm Beach County is found to be *Entamoeba dispar*, and NEFCO and Class I wastewater are assumed to be the two most likely sources of *E. dispar*. The microscopic analysis did not clearly indicate the presence of *E. dispar* in any of the samples collected from the various sources, which led the team to consider doing a RT-qPCR analysis/ agarose-gel electrophoresis for detecting *E. dispar*. He then explained the process of RT-qPCR analysis which is currently in progress. A few techniques regarding well rehabilitation were mentioned as future work.
5. Dr. Meeroff thanked all of the participants, and the meeting was adjourned at 3:57 pm.

FAU

LAB.E.E.S. Laboratories for Engineered Environmental Solutions

mission green

Florida Atlantic University
College of Engineering & Computer Science

Technical Advisory Group Meeting

1. "Odor Biosensor"
2. "Leachate Collection System Clogging"
3. "Leachate Management Solutions"

Daniel E. Meeroff, Ph.D.
Department of Civil, Environmental & Geomatics Engineering

Daniel Meeroff, Ph.D.

- **Professor & Associate Chair, Department of Civil, Environmental & Geomatics Engineering, FAU**
  - **Director of the Laboratories for Engineered Environmental Solutions (Lab.EES) http://labees.civil.fau.edu**
  - **College Liaison, Undergraduate Research & Community Engagement**
- **Research Interests**
  - Environmental Engineering
  - Water/Wastewater Treatment Technologies
  - Utilities Resiliency
  - Water Quality & Aquatic Toxicity
  - Solid/Hazardous Waste Management
  - Water Use Efficiency and Pollution Prevention Strategies
  - Industrial Wastewater & Leachate Management
  - Air Quality Management
  - STEM Education Research

Recent Funded Research

- **USEPA Anaerobic Digestion for Food Waste Diversion**
- **FEMA Watershed Master Planning Initiative**
- **Community Foundation of Broward Technical Assistance Grant**
- **Clogging Prevention Studies at Solid Waste Authority of Palm Beach County and Lee County**
- **SARS-CoV-2 Surveillance Program**
- **NSF STEM Education Grant (LEARN Program)**

"Support for Regional Community AD for Organic Waste Diversion

FAU FLORIDA ATLANTIC UNIVERSITY

EPA United States Environmental Protection Agency

News Releases

News Releases from Region 04

EPA Announces the Selection of Florida Atlantic University to Receive $195,426 in Funding to Support Anaerobic Digestion

Nationally, 12 Organizations selected to receive $3 million in funding to support anaerobic digestion in communities

"Finding solutions to better curb food waste continues to be a top priority for the Trump administration," **said EPA Administrator Andrew Wheeler.** "This year's round of innovative community projects is focusing on ways to reduce food waste at the local and state levels and divert it from landfills."

"EPA commends Florida Atlantic University for stepping up to research solutions to reduce food loss and waste," **said EPA Region 4 Administrator Mary S. Walker.** "We look forward to the sustainable environmental results that will help create a cleaner, healthier environment."

"FAU is excited to transform food waste into sustainable energy resources at wastewater treatment facilities so that communities across the country can benefit from our work," **said Dr. Daniel E. Meeroff, Professor and Associate Chair in the Department of Civil, Environmental & Geomatics Engineering at Florida Atlantic University.**

FLORIDA ATLANTIC UNIVERSITY

Agenda

1. Introductions/Opening Remarks	*Dr. Meeroff*
2. Odor Biosensor	*Rahman*
3. Leachate Clogging	*Shaha*
4. Leachate Management Solutions	*Chowdhury*
5. User Input/Open Forum	*Everyone*

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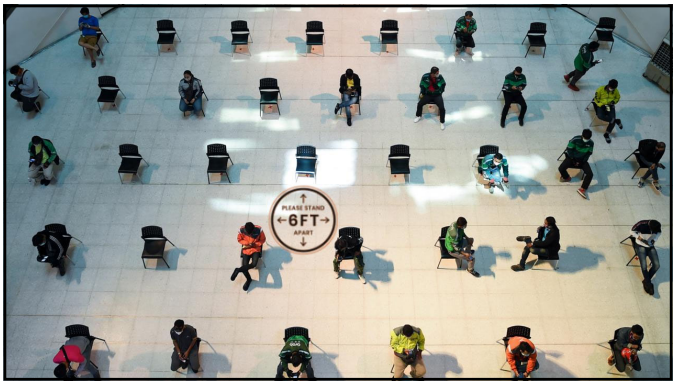
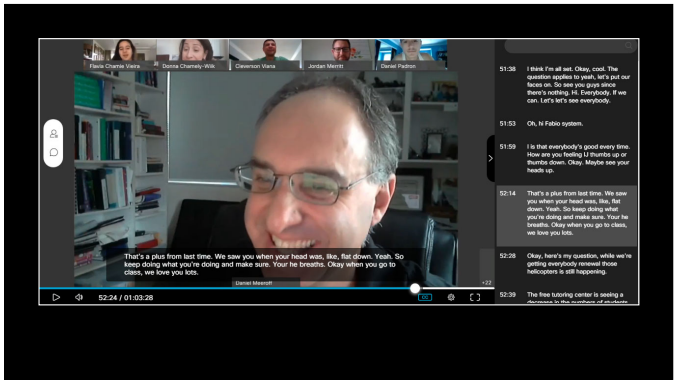
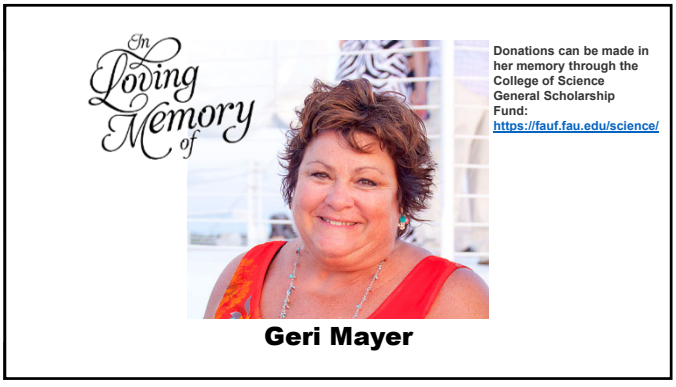
December 14, 2020





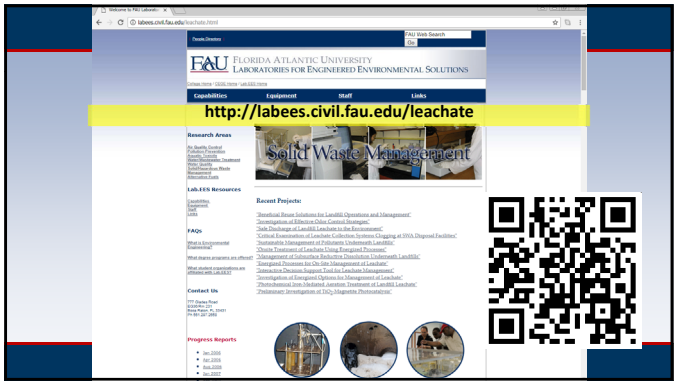


Donations can be made in her memory through the College of Science General Scholarship Fund: https://fauf.fau.edu/science/

Geri Mayer

ABET

BE CONFIDENT℠



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Reports for Comment (due Dec. 31)

- **Biosensor**
- http://labees.civil.fau.edu/FAU-Meeroff-Development%20of%20a%20biosensor%20Draft%20Final%20Report2019.pdf
- **Leachate Clogging**
- http://labees.civil.fau.edu/FAU-Meeroff-Leachate%20Biogeochemical%20Clogging%20Draft%20Final%20Report2020.pdf

Wishing you
A HAPPY
Holiday Season

Hinkley Center TAG Meeting, December 14,2020

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

Daniel E. Meeroff, Ph.D.
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Sharmily Rahman, MSCV
Department of Civil, Environmental & Geomatics Engineering
Florida Atlantic University

Prepared for
HINKLEY CENTER FOR SOLID AND HAZARDOUS WASTE MANAGEMENT

OUTLINE

01 **INTRODUCTION** — Introduction to the issues, currently faced challenges, research inspiration, and objectives

02 **METHODOLOGY** — Research approach, relevant science, and experimental tasks

03 **RESULTS & FINDINGS** — Results obtained, including graphical analysis and major findings

04 **FUTURE WORKS** — Further research to explore, recommendations, and wrapping up

INTRODUCTION

- Detecting and quantifying odor at landfills is difficult due to subjectivity
- Low-cost objective methods are not readily available
- Human odorant binding proteins can be used as biosensors to objectively measure odorants
- Odorant biosensors are a potential game-changer for dealing with odors

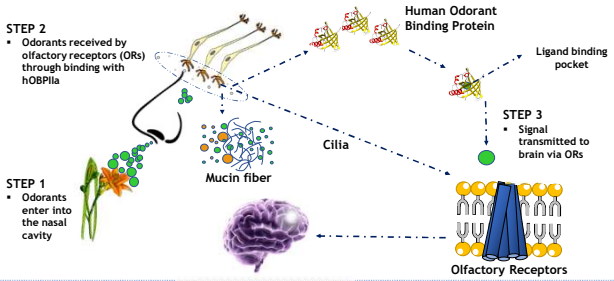
CHALLENGES

- Subjectivity and different individual perception of odors
- State-of-the-art odor measurement devices:
  - Usually work only for specific odorants
  - Unable to quantify odors
  - Unable to deal with adverse environmental or meteorological conditions (temperature, humidity, etc.)
  - Suffer low sensitivity at trace (<ppb) levels
  - Unable to deal with synergistic effects

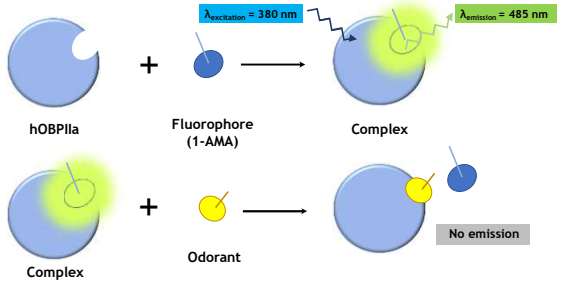
RESEARCH INSPIRATION

- Turn to biology for inspiration
- In 2013, Silva et al. used porcine Odorant Binding Proteins (pOBP) to mask the smell of cigarettes
- OBPs can bind with odorants in the μM-range
- Odor intensity is based on the number of bound receptors in OBPs
- If so, the spectroscopic tag response will be concentration-dependent

OLFACTION SCIENCE



MECHANISM



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HUMAN ODORANT BINDING PROTEIN

- Member of lipocalin super-family
- The ligand binding cavity inside the β-barrel allows binding with a large variety of hydrophobic odorants to deliver them to ORs
- Stable to temperature, organic solvents, and proteolytic digestion
- Broad binding affinity across different ligand structures and sizes
- Can be expressed in bacterial systems at low cost & easily purified

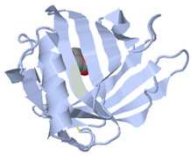

Image source: http://proteopedia.org/wiki/index.php/4run

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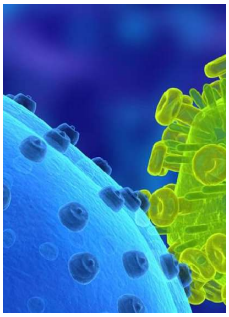

FLUOROPHORE (1-AMA)

- Widely used as a fluorophore to study interaction of lipocalin proteins
- Hydrophobic in nature
- Shows a strong fluorescence signal when forming complex with OBPs

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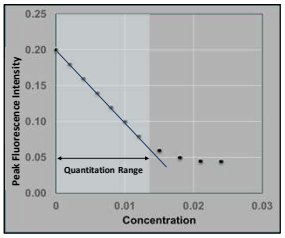
RESEARCH APPROACH

- Create a prototype biosensor complex
  - hOBPIIa + Fluorescent marker
- Expose biosensor to odorants for various times
- Measure intensity of the spectroscopic signal to determine time-dependent relationship
- The fluorescent tag response is expected to follow an inverse Beer's Law



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OBJECTIVES

Objective 1
- Develop a novel biosensor technology that uses human odorant binding protein (hOBPIIa) upon constructing a revised reactor chamber

Objective 2
- Verify the protein-fluorophore optimum ratio and expose biosensor to odorant gas (both pure and mixture)

Objective 3
- Determine spectrofluorometric response and establish quantitation range

Objective 4
- Check reaction reversibility between hOBPIIa and the odorant gases

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TASK 1: DEVELOP BIOSENSOR COMPLEX

Purified hOBPIIa + 1-AMA fluorescent tag + Buffer solution =

Biosensor complex

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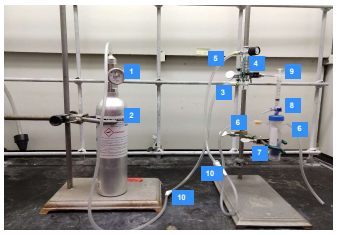
TASK 2: DEVELOP REACTOR CHAMBER

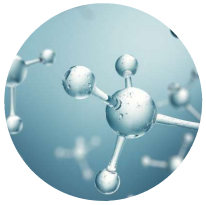


KEY

1. Regulator
2. Gas cylinder
3. Tube connected to cylinder
4. Flowmeter
5. Tube connected to chamber
6. One-way check valve
7. Reactor chamber
8. 3-way stopcock
9. Syringe
10. Tube connector

TASK 3: FLUORESCENCE BINDING ASSAY WITH MODEL COMPOUNDS

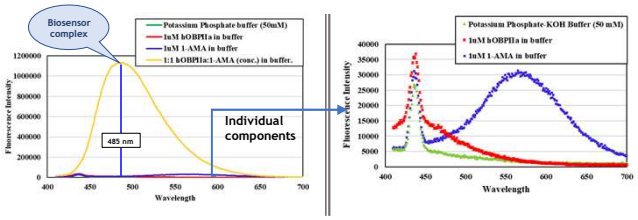
- Perform experiments on variety of odorants including acidic and basic gases
- Gas concentrations
  - H_2S, 25 ppm balanced with N_2
  - NH_3, 25 ppm balanced with N_2
  - CH_4, 25 ppm balanced with Air
  - CH_3S, 50 ppm balanced with N_2
  - Mixture 1 (NH_3, 25 ppm; CH_4 25 ppm) balanced with Air
  - Mixture 2 (H_2S, 25 ppm; CO, 50 ppm and CH_4 2.5%) balanced with Air
- Gas flow rates
  - Higher (0.9 slpm)
  - Intermediate (0.7 slpm)
  - Lower (0.5 slpm)
- Exposure times were up to 4 minutes
- Volume: 10 mL protein-fluorophore-buffer solution



TASK 3: FLUORESCENCE BINDING ASSAY WITH MODEL COMPOUNDS

- Samples collected at different time intervals for spectrofluorometry using:
  - 100 µL quartz cuvettes
  - Horiba Jobin Yvon FluoroMax-4 spectrofluorometer with Fluorescence software interface

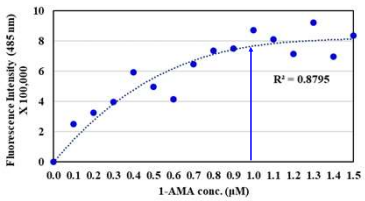
RESULTS: FLUORESCENCE BINDING ASSAY



Spectrofluorometric analysis for individual and combination of biosensor components

RESULTS: BINDING CURVE

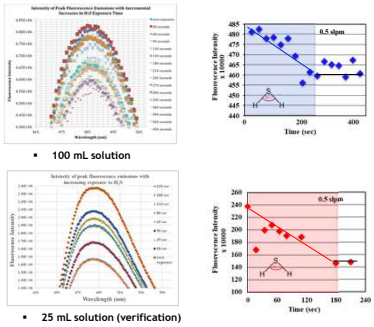
Optimum concentration of protein-fluorophore complex (1:1)



Titration curve of 1-AMA at different concentrations to hOBPIIa

RESULTS: MODEL COMPOUND (H_2S)

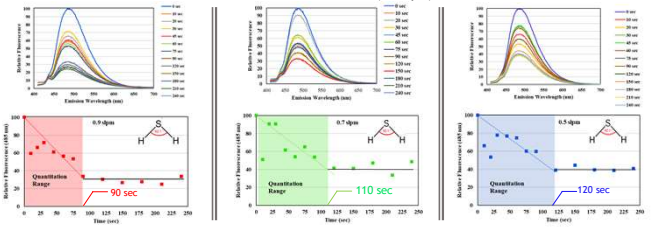
Verification experiment with 0.5 slpm H_2S



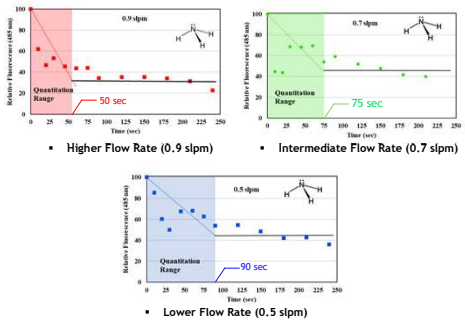
- 100 mL solution
- 25 mL solution (verification)

RESULTS: MODEL COMPOUND (H_2S)

- Higher Flow Rate (0.9 slpm)
- Intermediate Flow Rate (0.7 slpm)
- Lower Flow Rate (0.5 slpm)

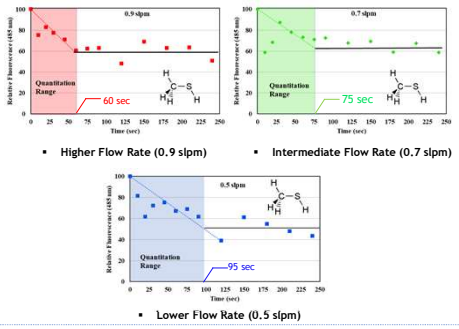


RESULTS: MODEL COMPOUND (NH_3)



- Higher Flow Rate (0.9 slpm)
- Intermediate Flow Rate (0.7 slpm)
- Lower Flow Rate (0.5 slpm)

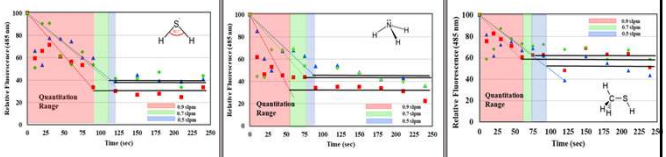
RESULTS: MODEL COMPOUND (CH_3SH)



- Higher Flow Rate (0.9 slpm)
- Intermediate Flow Rate (0.7 slpm)
- Lower Flow Rate (0.5 slpm)

RESULTS: MODEL COMPOUNDS (PURE GASES)

- H_2S
- NH_3
- CH_3SH



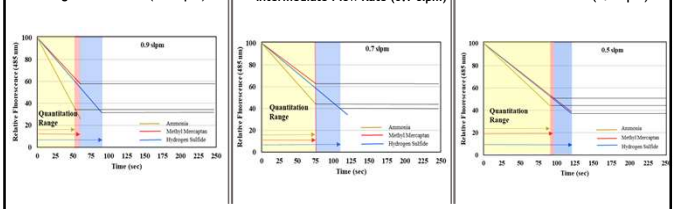
Slopes
- 0.9 slpm: -0.76
- 0.7 slpm: -0.55
- 0.5 slpm: -0.52

Slopes
- 0.9 slpm: -1.23
- 0.7 slpm: -0.74
- 0.5 slpm: -0.62

Slopes
- 0.9 slpm: -0.70
- 0.7 slpm: -0.49
- 0.5 slpm: -0.51

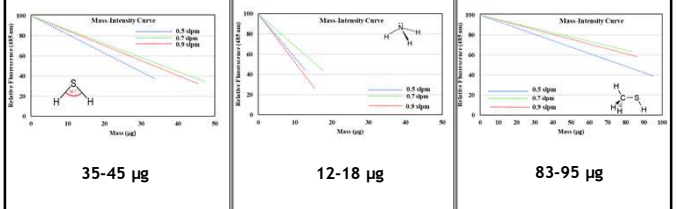
RESULTS: MODEL COMPOUNDS (PURE GASES)

- Higher Flow Rate (0.9 slpm)
- Intermediate Flow Rate (0.7 slpm)
- Lower Flow Rate (0.5 slpm)



RESULTS: MODEL COMPOUNDS (MASS vs INTENSITY)

- H_2S
- NH_3
- CH_3SH

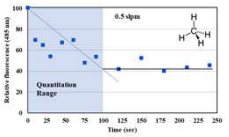


35-45 µg

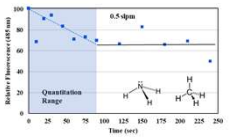
12-18 µg

83-95 µg

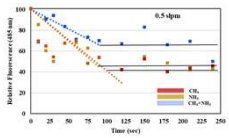
RESULTS: MODEL COMPOUND (Mixture 1: NH_3+CH_4)



- CH_4 (0.5 slpm)

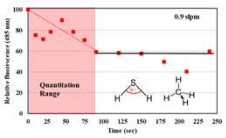


- CH_4 + NH_3 (0.5 slpm)

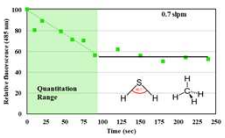


Slopes
Mixture: -0.38
NH_3: -0.62
CH_4: -0.59

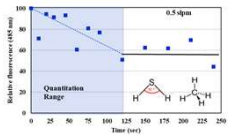
RESULTS: MODEL COMPOUND (Mixture 2: H_2S+CH_4+CO)



- Higher Flow Rate (0.9 slpm)



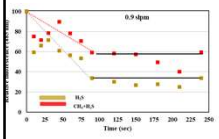
- Intermediate Flow Rate (0.7 slpm)



- Lower Flow Rate (0.5 slpm)

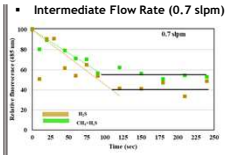
RESULTS: MODEL COMPOUND (Mixture 2) contd.

- Higher Flow Rate (0.9 slpm)



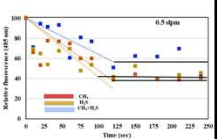
Slopes
Mixture: -0.44
H_2S: -0.76

- Intermediate Flow Rate (0.7 slpm)



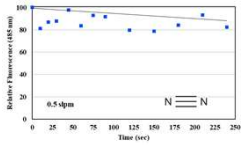
Slopes
Mixture: -0.46
H_2S: -0.55

- Lower Flow Rate (0.5 slpm)



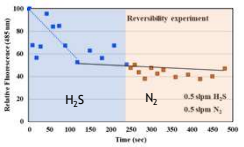
Slopes
Mixture: -0.36
H_2S: -0.52
CH_4: -0.59

RESULTS: Reaction Reversibility



- N_2 (0.5 slpm)

- N_2 has no mentionable interaction with the biosensor

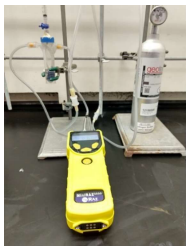


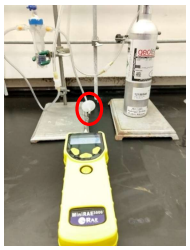
- 1st 4 mins H_2S (0.5 slpm)
- Final 4 mins N_2 (0.5 slpm)

- Regeneration Check

RESULTS: PID ANALYZER

Without Filter





With Filter

MAJOR FINDINGS

- Verification of the optimum concentration ratio of hOBPIIa and 1-AMA (1:1)
- Fluorescence response curves obtained for a diverse range of gases
- The biosensor complex saturates faster with an increased gas flow rate
- No mentionable pH change observed during the experiments

MAJOR FINDINGS

- Depending on the gas flow rates the fluorescence response curve obtained from the experiments can be used to quantify:
  - 35-45 µg of hydrogen sulfide (H_2S)
  - 12-18 µg of ammonia (NH_3)
  - 83-95 µg of methyl mercaptan (CH_3SH)
  - 15 µg of methane (CH_4)

FUTURE WORK

- More experiments should be conducted for a larger range of common odorants
- Develop models for quantifying different odorants
  - Test more odorant mixtures starting with 3-component mixtures to determine if the slope can be used to quantify specific odors in mixtures
  - Eventually test field samples to better understand real life scenarios
- Use flow-through cuvettes
- Find out a way to make the protein-odorant reaction reversible
- Eventually design a handheld device for objectively measuring odors in the field using hOBPIIa

Critical Evaluation of Leachate Clogging Potential in Gravity Collection System and Management Solutions

By

Bishow Shaha, PhD, EI

Under Supervision of-

Daniel E. Meeroff, Ph.D (PI)

Table of Contents

01 **Introduction**
Background, Site description & Research inspiration

02 **Methodology**
Research approach, Experimental setup, Data analysis methods

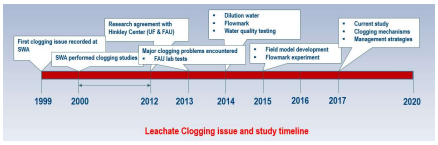
03 **Results & Discussion**
Results obtained including graphical analysis and major findings

04 **Conclusion & Recommendation**
Summary of findings & Recommendations

Introduction

- **Leachate**
  - Liquid percolates through the landfill
  - Generated from rainfall & wet organic waste
- **Clogs/Precipitates**
  - Solids form in the leachate collection system

Background



Leachate Clogging issue and study timeline

Site Description

- **Class I Landfill**
- **160 Acres**
- **16 Cells**
  - 4 closed, 11 active, and 1 new
- **Waste types**
  - MSW
  - Bottom & Fly Ash
  - RDF residues
  - Miscellaneous
- **Leachate disposal**
  - Deep well injection
  - NEFCO, DIW, REF 1&2, Class III +Dyer, PS A & B

Inspiration/Rationale of Study

Clogging present since 1999

Literature & research

Need better understanding of mechanisms

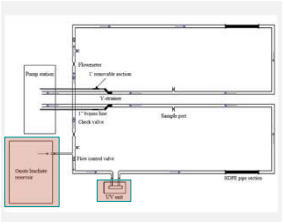
Several management strategies

Mechanisms & measures

- To better understand formation mechanisms
- To investigate the triggers/factors of precipitation
- To recommend preventative measures based on the findings

Methods

UV disinfection (Field Set up)



Storage Tank

1 ft removable section (1" dia)

UV unit retrofit

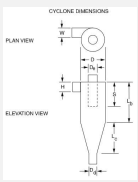
UV unit

Methods

pH adjustment setup

1. Gas cylinder
2. Adjustable valve
3. 2L plastic bottle
4. Sample port
5. Gas release valve

Cyclone Separator

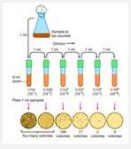


Methods

Parameters	Method
pH	SM4500-H+ B, Electrometric Method
Temperature	SM2550B
Conductivity	SM2510B
Calcium hardness	Hach Method 8204
Alkalinity	Hach Method 8203
Solids (TDS, TSS)	TDS: SM2540C/EPA Method 160.2 TSS: SM2540D/EPA Method 106.4
Sulfides	Method 8131, Equivalent to SM4500-S2-D
Sulfates	SulfaVer 4 method equivalent to SM10248

Methods

Bacteriological Analysis



- To confirm disinfection
- To investigate the microbial community present

Saturation Index (LSI)

Equations:

$$LSI = pH - pH_s$$

$$pH_s = pK_2 - pK_s + p[Ca^{2+}] + p[HCO_3^-] + 5pf_m$$

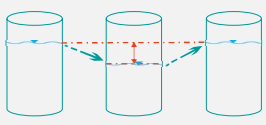
- LSI = 0; Neutral (Equilibrium)
- LSI < 0; Undersaturated (Corrosive)
- LSI > 0; Supersaturated (Scale/ Precipitate forming)

Methods

Heat Sterilization & Precipitation

- Boiled in Oven at 150°C, 30 min.
- Autoclaved at 121°C, 30 min.

Alternative Submerge & Dry Condition



- Addition of leachate
- Addition of groundwater
- 15-20 days for each set of test

Results and Data Analysis

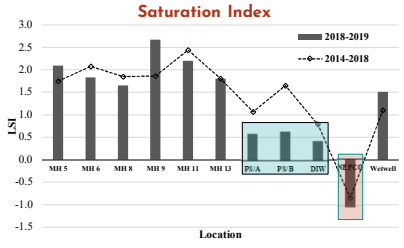
Leachate Characteristics

Parameters	Literature reviewed	Range n = 135 samples (2017-2019)
pH	2.0 – 11.3	6.3 - 7.3
Conductivity (µS/cm)	5.2 – 95,000	3,600 - 58,300
Total Solids (mg/L)	10.1 – 133,000	3,100 - 34,800
Calcium (mg/L as $CaCO_3$)	10 – 7,200	800 - 4,100
Magnesium (mg/L as $CaCO_3$)	30 – 15,000	100 - 1,000
Alkalinity (mg/L as $CaCO_3$)	610 – 11,000	900 - 4,400

Major Observation: Leachate water quality parameters are consistent with literature review

Results and Data Analysis

Saturation Index



Major Observation

- LSI remains similar except for P/S A and P/S B
- NEFCO water is a potential alternative for dilution purposes
- NEFCO water has high TSS (400-500 mg/L)

Results and Data Analysis

Solid separation (Cyclone Separator)

ID/time	Turbidity NTU		TSS mg/L	
	Single pass	Batch	Single pass	Batch
0	18.1	18.1	80	80
5	13.9	nr	80	80
10	12.1	12.3	20	25
15	12.2	16.2	40	75
% Removal @ 10 min	33%	32%	75%	69%
% Removal @ 15 min	33%	10%	50%	6%

*nr = not recorded

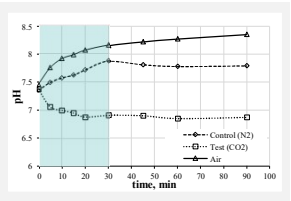
- Cyclone separator removed up to 75% TSS in 10 min.
- Accumulation of solids at the bottom increased the TSS in 15 min. sample (not enough solids storage in lab scale cyclone)

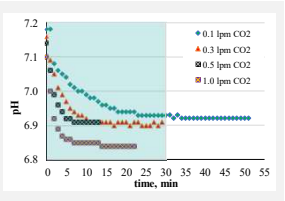
Major Observation

- Solids present are adhesive to surfaces
- 180 lb/d solids need to be handled (6 MG flow, 500 mg/L TSS, 70% removal)

Results and Data Analysis

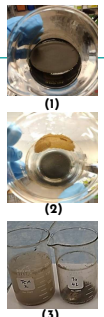
pH Adjustment and Precipitation





- pH changes within 1st 30 minutes of exposure

Results and Data Analysis

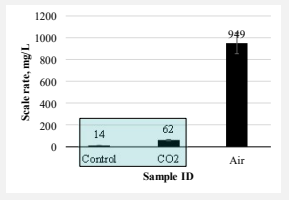


Precipitation Types

1. **Hard floating scale**
   - Thin hard layer above the leachate
   - Floats in leachate surface
2. **Non adherent loose precipitates at the bottom**
   - Non adherent to the surface
   - Easy to remove
3. **Adherent scale attached to the surface**
   - Most difficult to remove

Results and Data Analysis

pH Adjustment and Precipitation



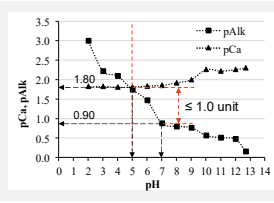
Components

- Major Components: (10 samples)
  - Calcite ($CaCO_3$)
  - Halite (NaCl)
- Conforms with historical precipitate composition analysis

- Initial pH enhances precipitation

Results and Data Analysis

pH Adjustment and Precipitation



$$LSI = pH - pH_s$$

$$pH_s = pK_2 - pK_s + \underbrace{p[Ca^{2+}] + p[HCO_3^-]}_{\text{SUM}} + 5pf_m$$

- pH change from 7.0 to 5.0 increases the "SUM" by 1.0 unit approx.
- Helps to achieve neutral LSI (-0.4 ≤ LSI ≤ +0.4)

Results and Data Analysis

Microbial Activity and Precipitation

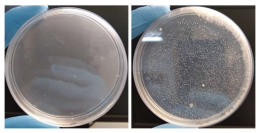
Disinfection

- **UV radiation**

Sample ID	HPC/mL
Control	24,000
UV_30 (30 minutes)	20,000
UV_60 (60 minutes)	22,000
UV_90 (90 minutes)	<20,000

- **UV does not achieve required disinfection**

- **Boiling @150°C / Autoclave @ 121°C**

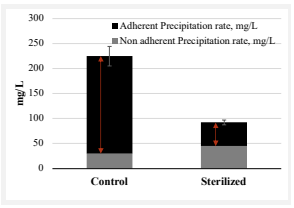


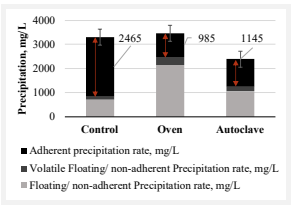
Autoclaved (48-72 hrs) Oven boiled (48-72 hrs)

- **Autoclaving at 121°C, 30 min achieves consistent disinfection**

Results and Data Analysis

Microbial Activity and Precipitation

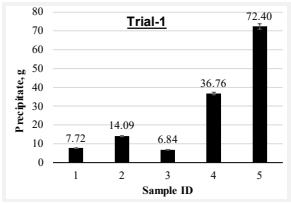


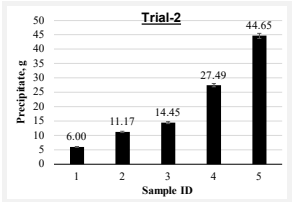


- **Sterilized sample reduces adherent precipitation**

Results and Data Analysis

Alternate Submerged/Dry Condition and Precipitation





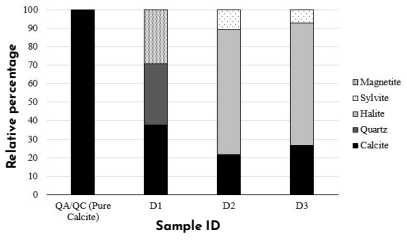
- **Alternate submerged and dry condition enhances surface adhesion of precipitates**

Impacts on Deep Injection Well

Description	Water quality parameters	Unit	Sample D1 (0-61 m)	D2 (62-457 m)	D3 (458-884 m)
Field parameters*	pH	standard unit	7.98	7.10	7.10
	Sp. Conductance	mS/cm	0.6	9.8	18.8
	Conductivity	mS/cm	0.6	9.9	20.2
	TDS (field)	mg/L	350	6,340	12,210
	Water temperature	°C	25.8	26.0	28.9
	Turbidity	NTU	113	169	162
	Dissolved oxygen	mg/L	2.2	<1.0	<1.0
	ORP	mV	-38	-404	-415
Laboratory parameters*	TDS (gravimetric)	mg/L	390	5,700	11,930
	Calcium (Ca)	mg/L as $CaCO_3$	250	1,125	1,600
	Alkalinity	mg/L as $CaCO_3$	150	2,175	2,125
	Chloride (Cl^-)	mg/L	350	2,375	5,575
Saturation Indices	pH	standard unit	7.2	5.8	5.7
	LSI	unitless	0.8	1.4	1.4
	RI	unitless	6.4	4.4	4.3

*All field parameters presented are the average of three readings recorded during sampling
*All laboratory parameters presented are the average of the sample and the duplicate collected at each depth range

Impacts on Deep Injection Well



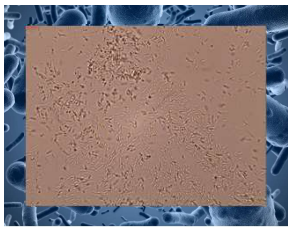
Summary

Sample ID	Compounds
QA/QC (pure calcite)	$CaCO_3$ = 100%
D1 "Surface zone" (0-61 m) 0-200 ft	Calcite, $CaCO_3$ = 37.7% Quartz, SiO_2 =33.1% Magnetite, Fe_3O_4 = 29.2%
D2 "Intermediate zone" (62-457 m) 200-1500 ft	Halite, NaCl = 67.7% Calcite, $CaCO_3$ =21.8% Sylvite, KCl = 10.6%
D3 "Injection Zone" (458-884 m) 1500-2900 ft	Halite, NaCl = 66.5% Calcite, $CaCO_3$ =26.6% Sylvite, KCl = 6.9%

Impacts on Deep Injection Well

Microbiological Composition

- **No algae**
- **Highest microbial community at surface zone**
- **Methanogenic archaea**
- **Sulfate-reducing**
- ***Entoamoeba dispar*** (Biofilm-producing protozoa)



X400 under microscope

Major Findings

1. Explore the mechanism of clog formation

- Calcite ($CaCO_3$) is the predominant component of the clogs
- Chemical precipitation happens due to supersaturation of calcite in leachate
- Microbial activity produce EPS and forms biofilms in the leachate collection system
- Microbial activity induces calcite precipitation by increasing local pH
- Microbial EPS attached to the precipitate enhances adhesiveness
- Precipitates/microcrystals trapped inside the biofilm matrix act as skeleton and form bio-rock/clog with time

Major Findings

2. Investigate the triggers/factors of precipitation

- Impacts of pH adjustment, disinfection, and flow regime were studied

pH

- pH plays an important role in calcite precipitation
- Reduction in 1 unit pH does not reduce LSI by 1 unit
- Addition of CO_2 reduces the pH
- Addition of air or inert gas increases the pH
- Aeration, turbulence and stagnation increase pH
- Higher initial pH results in higher precipitation and vice versa

Major Findings

2. Investigate the triggers/factors of precipitation

Disinfection

- *Entoamoeba dispar* is primarily responsible for the biofilm in the deep injection well
- UV disinfection does not provide desired disinfection of leachate
- Autoclave was the preferred method of disinfection
- Disinfection of leachate reduces the quantity of adherent scale
- Therefore, it was concluded that chemical precipitation of calcite is not as concerning if microbial activities are not present or limited

Major Findings

2. Investigate the triggers/factors of precipitation

Flow regime

- Flow regime is interconnected with pH and precipitation potential of leachate
- Precipitation potential (LSI) was consistent throughout the study period
- Alternating submerged and dry conditions enhance precipitation and attachment to the surface
- NEFCO water has the potential to replace groundwater in dilution purposes if a proper solid separation and handling method is used as pretreatment

Major Findings

3. Identify and recommend potential preventative measures

- Based on the findings, it is evident that chemical precipitation in the presence of microbial fauna will always be present in a leachate collection system
- Diluting leachate with groundwater or other alternatives (NEFCO) helps to reduce precipitation by providing more flow in the gravity system
- Reducing the pH directly reduces the precipitation potential of leachate and clog formation
- Biofilm formation in the leachate collection system takes time and can be more readily removed at early stage

Challenges

- **The main limitation of this study is the variabilities in daily operation between sampling events**
- **Variability in leachate characteristics over time should be controlled for in future studies**
- **XRD/XRF analysis and the free available database does not have the capability to identify inorganic components or rare minor constituents**
- **In addition, further replication is necessary to confirm the findings**

Recommendations

- **Assess any operational changes and their impacts on clogging before implementation**
  1. Laboratory or field experiments
  2. Performance analysis and evaluation
- **Dilution of leachate was found to be an effective preventative measure with proper use**
  1. Optimization of flow is required
  2. Alternative sources need to be identified because of the limitation of ground water permit
- **Development of a monitoring schedule to reduce severe clogging in critical areas**
- **Frequent cleaning and flushing**

Recommendations

- **The performance issues in the leachate collection system directly impacts the deep injection well**
- **Pretreatment and well rehabilitation can be employed to control biofouling of injection well**
  1. Aeration, acidification, and/or disinfection
  2. Based on site specific characteristics
  3. Select and optimize disinfectant, doses, contact time etc.
- **Careful consideration regarding the outcome and future impacts is necessary**

Recommendations for further studies

- **Develop a leachate flow network in the lab**
  1. To test for flow regime
  2. To test for clogging formation rate
  3. To test for pH adjustment
  4. To test for dilution alternative and so on.
- **Further elemental analysis of clogs to identify inorganic portion of the clog**
- **Conduct laboratory and field test to develop pretreatment and well rehabilitation strategies to control biofouling of injection well**

Thanks

Does anyone have any questions?

Florida Atlantic University
College of Engineering & Computer Science

Investigations of Leachate Management Solutions at the Solid Waste Authority of Palm Beach County

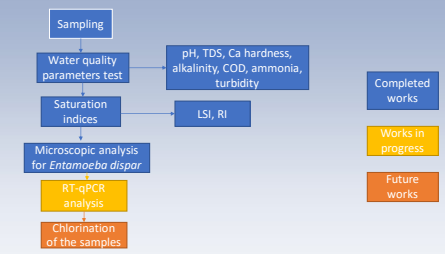
Daniel Meeroff, Ph.D.
Rakib Ahmed Chowdhury, MSCV
Florida Atlantic University

December 2020

Outline

- ❑Current works
- ❑Results
- ❑Future works

- **Current works**

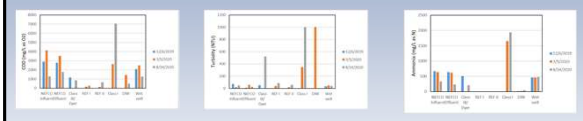


Current Work: Sampling

Sampling Date	NEFCO influent	NEFCO effluent	Class III Dyer	Class I	REF I	REF II	DIW	Wetwell
12/06/2019	*	*	*				*	*
03/03/2020	*	*		*	*	*	*	*
09/24/2020	*	*	*	*	*	*	*	*

Current Work: Water Quality Parameters

Current Work: Water Quality Parameters



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Current Work: Water Quality Parameters

DIW sample collected on 12/06/2019 and 08/24/2020

DIW sample collected on 03/05/2020

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Current Work: Water Quality Parameters

- Precipitation data (Weather Underground, 2020)
- Rainfall station:
  - Palm Beach International Airport Station, 26.69°N and 80.09°W (Weather Underground, 2020)

Time Period	Total Precipitation prior to 12/06/2019 (in)	Total Precipitation prior to 03/05/2020 (in)	Total Precipitation prior to 08/24/2020 (in)
Two months	8.61	4.36	21.48
One month	3.21	3.31	12.48
One week	0.01	0.0	3.27
Three days	0.0	0.0	0.29
Two days	0.0	0.0	0.29

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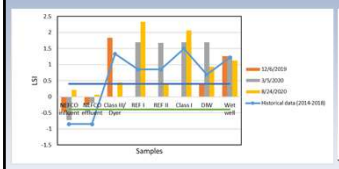
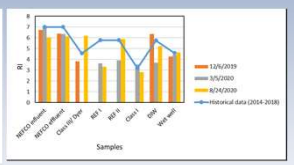
Current Work: Saturation Indices





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Current Work: Saturation Indices

Sample	TDS (mg/L)	Water Temperature (oC)	Ca hardness (mg/L)	Alkalinity (mg/L)	PHs	pH	LSI	RI
NEFCO in	830.00	33.80	450.00	800.00	6.26	6.47	0.21	6.04
NEFCO Eff	635.00	34.20	500.00	800.00	6.19	6.25	0.06	6.13
Class III/ Dyer	3345.00	30.40	110.00	1800.00	6.64	7.09	0.45	6.19
Class I	24390.00	32.10	3000.00	4400.00	4.87	6.93	2.06	2.81
REF I	3200.00	36.30	1800.00	800.00	5.67	8.00	2.33	3.33
REF II	2055.00	29.80	850.00	500.00	6.30	6.71	0.41	5.88
DIW	600.00	27.90	650.00	850.00	6.17	7.10	0.93	5.23
Wet well	1640.00	35.50	1000.00	1100.00	5.77	6.89	1.12	4.65
Avg.	5007.86	32.07	1051.43	1421.43	6.01	6.94	0.92	5.06
Max	24390.00	36.30	3000.00	4400.00	6.64	8.00	2.33	6.19
Min	600.00	27.90	110.00	500.00	4.87	6.25	0.06	2.81

Observed values

Estimated values

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Current Work: Water Quality Summary

- **Turbidity was high (>1000 NTU) over detection limit for DIW and class I collected on March 05 and September 24 respectively. Probable corresponding reasons are the lack of rainfall resulting in lower dilution and larger amount of mineral precipitation in leachate.**
- **COD and LSI were relatively higher for the DIW sample collected on 03/05/2020**
- **Both NEFCO influent and effluent showed neutral behavior in terms of precipitation potential for the samples collected on 08/24/2020**
- **Otherwise, water quality was similar to the historical data**

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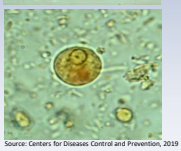
Current Work: Microbiology

- Presence of mineral precipitation and biofilms were detected in the deep injection well in 2019 (Meeroff et al. 2019)
- The dominant microorganism in the biofilm in the deep injection well is identified as *Entamoeba dispar* (Meeroff et al. 2019)
  - Morphologically identical to *Entamoeba histolytica*
  - This protozoan forms a protective cyst
  - Known biofilm colonizer
- Typically found in human feces, septic tanks, and sewers
  - Potential major sources are biosolids and diapers



Source: Centers for Diseases Control and Prevention, 2019

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Current Work: Microbiological Detection Methods

Techniques	Cost	Sensitivity
Microscopic analysis	↓	↓
ELISA		
RT-qPCR analysis/ Agarose-gel electrophoresis		

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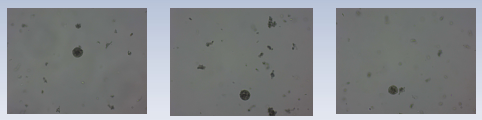
Current Work: Microscopic Analyses

- *Entamoeba histolytica (control sample)*



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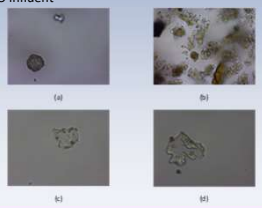
Current Work: Microscopic Analyses

- NEFCO influent



Sampling on (a) and (b) 12/06/19;
(c) And (d) 03/05/20

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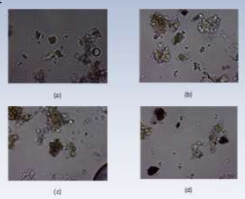
Current Work: Microscopic Analyses

- NEFCO effluent



Sampling on (a) and (b) 12/06/19;
(c) And (d) 03/05/20

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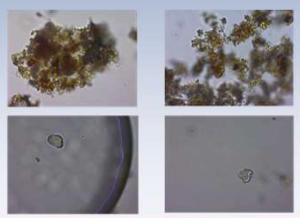
Current Work: Microscopic Analyses

- Class III/ Dyer



Sampling on
12/06/19

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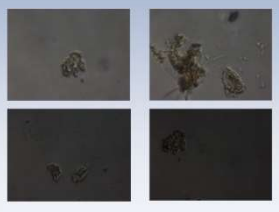
Current works

Microscopic analysis

- Class I



Sampling on
03/05/20

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Current Work: Microscopic Analyses

- REF I

Sampling on
03/05/2020

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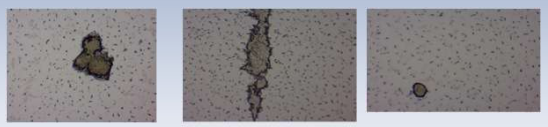
Current Work: Microscopic Analyses

- REF II



Sampling on
03/05/2020

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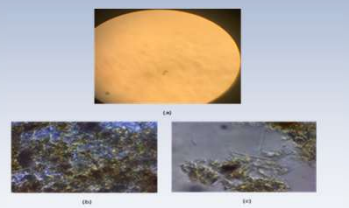
Current Work: Microscopic Analyses

- DIW



Sampling on (a)
12/06/19; (b) and (c)
03/05/20

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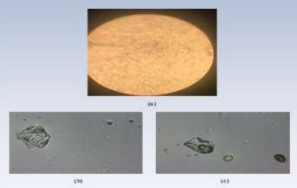
Current Work: Microscopic Analyses

- Wet well



Sampling on (a)
12/06/19; (b) and (c)
03/05/20

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Current Work: Microscopic Analyses Summary

- No clear identification of *Entamoeba dispar* from any of the collected samples using morphological techniques
- Excessive amount of biological flocs and salt crystals were detected in each of the samples
- Higher amount of flocs and suspended solids were found in the DIW sample collected in the March 5, 2020 compared to other sampling events

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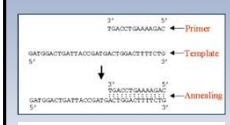
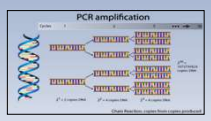
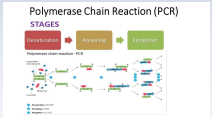
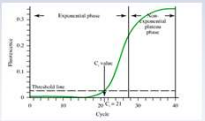
Work in Progress: RT-qPCR analysis









(Sources: Karki, 2017; ISO 22000 Resource Center, 2015; dnasoftware, 2020)

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Work in Progress: Agarose-gel electrophoresis

- A gel is produced with agarose, TE buffer and ethidium bromide
- Extracted nucleic acids (DNA) are loaded in the prepared gel
- Phosphate backbone of DNA is negatively charged. Hence, the nucleic acids migrate to positive sides (anode) during the electrophoresis
- Depends on the principle of different mass/ charge ratio for DNA
- The length of *E. dispar* is 752 bp (Hamzah et al., 2006)

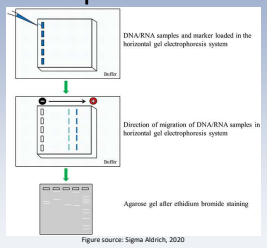

Figure source: Sigma Aldrich, 2020

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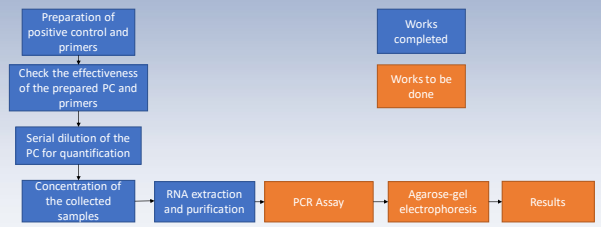
Work in Progress: RT-qPCR analysis



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Work in Progress: RT-qPCR analysis

❑NCBI gene bank association number "Z49256"

❑1949 bp long

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Work in Progress: RT-qPCR analysis

❑**Primers are derived from the study of Hamzah et al., (2006)**

Forward primer: 5'-ATG CAC GAG AGC GAA AGC AT-3'
(G_c= 50%; T_m= 60^0 C; Length: 20 bp)

Reverse primers: 5'-CAC CAC TTA CTA TCC CTA CC-3'.
(G_c= 50%; T_m= 52^0 C; Length: 20 bp)

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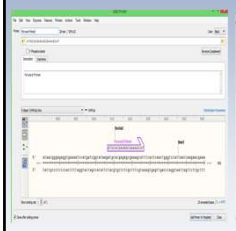
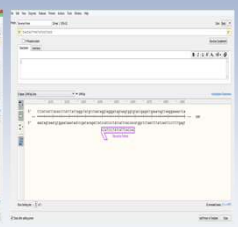
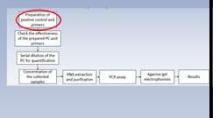
Work in Progress: RT-qPCR analysis







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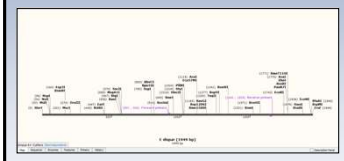
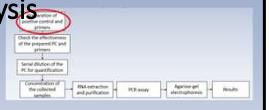
Work in Progress: RT-qPCR analysis





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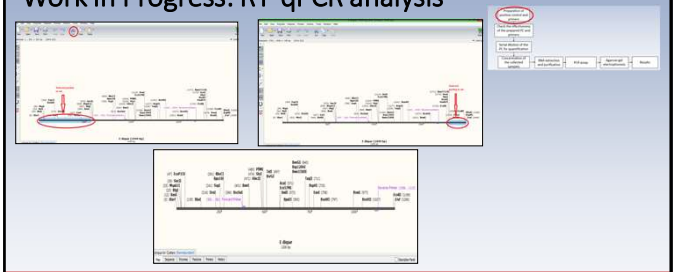
Work in Progress: RT-qPCR analysis



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Work in Progress: RT-qPCR analysis

- ❑Check the effectiveness of the PC and primers
  - Agarose-gel electrophoresis
  - PCR assay

Experiments	PC-1	PC-2	NC	Ladder
Agarose-gel electrophoresis	Prepared and ordered positive control	50% dilution of the PC-1	Nuclease-free water	1 kb
PCR assay				NA

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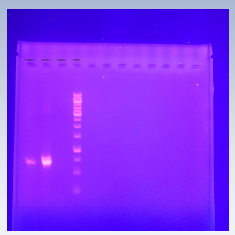
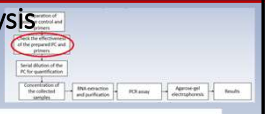
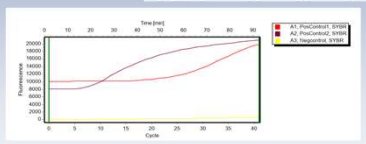
Work in Progress: RT-qPCR analysis







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Work in Progress: RT-qPCR analysis

Notation	Dilution (Times)	Addition of nuclease-free water (μL)	Addition of the plasmid
S	0 (Initial plasmid)	-	-
S_1	2	1	1 μL of S
S_2	4	1	1 μL of S_1
S_3	8	1	1 μL of S_2
S_4	16	1	1 μL of S_3
S_5	32	1	1 μL of S_4
S_6	64	1	1 μL of S_5

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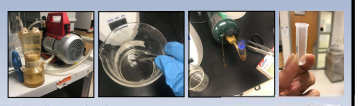
Work in Progress: RT-qPCR analysis

- ❑Concentration of 50 mL samples was achieved using filtration
- ❑The filter was dissolved into poly buffer saline (PBS) and vortexed
- ❑RNA extraction and purification was conducted following the protocols provided by the manufacturer GeneJET



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Future Work: Disinfection

Regions	Well types	Problems	Temperature (Deg C)	Cl_2 concentration (mg/L)	Contact times (hr)	References
Alaska	Water Well	Well rehabilitation	75-85	500	6	Butts (2017)
Venice, FL	Raw water extraction wellfield	Biofouling	-	6000	6-24	AWWA (2014)
Hollywood, FL	Water supply well	Membrane fouling	-	6000	6-24	AWWA (2014)

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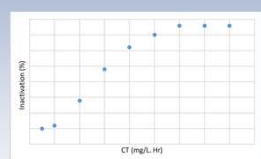
Future Work: Disinfection

Chlorine concentration (mg/L)	Contact Time (hr)					
	0	2	6	12	24	48
0						
500						
1000						
2000						
4000						
6000						



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Future Work: Plan

- FAU will continue to monitor the leachate water quality.
  - Periodic sampling and onsite water quality analysis
  - Laboratory experiment (COD, alkalinity, Ca hardness, ammonia)
  - Precipitation potentials (LSI and RI)
- FAU will conduct the qPCR and agarose-gel tests in details for the samples going to the deep injection well
  - Source detection of *Entamoeba dispar*
  - Quantify the number of the parasite in each of the sample
- FAU will conduct a lab based chlorination of the collected samples going to the deep injection well
  - Evaluate the effectiveness of chlorine disinfection as potential pretreatment for the well and its effect on the calcium carbonate precipitation.

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Major Accomplishments

- **A new detection method to identify *Entamoeba dispar* present in wastewater samples was developed**
- **Effect of chlorine disinfection dose in inactivating E. dispar will be evaluated**

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THANK YOU

Questions?