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

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


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 CaCO₃ 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.
Technical Advisory Group Meeting
December 14, 2020

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

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

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)
Agenda

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

Another Busy Year

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

Geri Mayer
Technical Advisory Group Meeting
December 14, 2020

http://labees.civil.fau.edu/leachate

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-
Wishing you
A HAPPY
Holiday Season
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 odors
- 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

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
**MECHANISM**

\[ \text{hOBPIIa} + \text{Fluorophore (1-AMA)} \rightarrow \text{Complex} \]

\[ \lambda_{\text{excitation}} = 380 \text{ nm} \]
\[ \lambda_{\text{emission}} = 485 \text{ nm} \]

**HUMAN ODORANT BINDING PROTEIN**

- Member of lipocalin superfamily
- 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

**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

**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

**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

**TASK 1: DEVELOP BIOSENSOR COMPLEX**

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

Biosensor complex
**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
  - H₂S, 25 ppm balanced with N₂
  - NH₃, 25 ppm balanced with N₂
  - CH₄, 25 ppm balanced with N₂
  - CH₄S, 50 ppm balanced with air
  - Mixture 1 (NH₃, 25 ppm; CH₄, 25 ppm) balanced with air
  - Mixture 2 (H₂S, 25 ppm; CO, 50 ppm and CH₄S, 50 ppm) 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

**Sample collection**
- 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**

**RESULTS: BINDING CURVE**

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

Titrination curve of 1-AMA at different concentrations to NHBPIa

**RESULTS: MODEL COMPOUND (H₂S)**

Verification experiment with 0.5 slpm H₂S

- 100 mL solution
- 25 mL solution (verification)
RESULTS: MODEL COMPOUND (H$_2$S)

- 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$_3$SH)

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

RESULTS: MODEL COMPOUNDS (PURE GASES)

- H$_2$S
- NH$_3$
- CH$_3$SH

Slopes:
- 0.9 slpm: -0.76
- 0.7 slpm: -0.52
- 0.5 slpm: -0.62

RESULTS: MODEL COMPOUNDS (MASS vs INTENSITY)

- H$_2$S
- NH$_3$
- CH$_3$SH

Mass:
- H$_2$S: 35-45 µg
- NH$_3$: 12-18 µg
- CH$_3$SH: 83-95 µg

Slopes:
- 0.9 slpm: 0.76
- 0.7 slpm: 0.52
- 0.5 slpm: 0.62
RESULTS:

MODEL COMPOUND (Mixture 1: NH₃+CH₄)

- CH₄ (0.5 slpm)
- CH₄ + NH₃ (0.5 slpm)

<table>
<thead>
<tr>
<th>Slopes</th>
<th>Mixture</th>
<th>NH₃</th>
<th>CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.38</td>
<td>-0.53</td>
<td>-0.59</td>
</tr>
</tbody>
</table>

RESULTS:

MODEL COMPOUND (Mixture 2: H₂S+CH₄+CO)

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

<table>
<thead>
<tr>
<th>Slopes</th>
<th>Mixture</th>
<th>H₂S</th>
<th>CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.36</td>
<td>-0.52</td>
<td>-0.59</td>
</tr>
<tr>
<td></td>
<td>-0.46</td>
<td>-0.55</td>
<td>-0.59</td>
</tr>
<tr>
<td></td>
<td>-0.44</td>
<td>-0.76</td>
<td>-0.55</td>
</tr>
</tbody>
</table>

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:
  - 33-45 μg of hydrogen sulfide (H₂S)
  - 12-18 μg of ammonia (NH₃)
  - 83-95 μg of methyl mercaptan (CH₃SH)
  - 15 μg of methane (CH₄)

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

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

By
Bishaw Shaha, PhD, EI

Under Supervision of
Daniel E. Meeroff, Ph.D (PI)

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

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

- 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
- UV unit retrofit
- 1 ft removable section (1” dia)

Methods

pH adjustment setup

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

Cyclone Separator

Methods

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

Bacteriological Analysis

Saturation Index (LSI)

Equations:

\[ \text{LSI} = \text{pH} - \text{pH}_{\text{s}} \]

- \( \text{pH}_{\text{s}} = k_2 - k_1 + p(\text{Ca}^{2+}) + p(\text{HCO}_3^-) + p(\text{H}_2\text{CO}_3) \)

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

Leachate Characteristics

Parameters | Literature reviewed | Range
---|---|---
pH | 2.0 – 11.3 | 6.3 – 7.5
Conductivity (µS/cm) | 3.2 – 95,000 | 3,600 – 38,300
Total Solids (mg/L) | 551 – 135,000 | 3,000 – 34,800
Calcium (mg/L as CaCO_3) | 10 – 7,200 | 800 – 4,100
Magnesium (mg/L as CaCO_3) | 50 – 15,000 | 100 – 4,000
Alkalinity (mg/L as CaCO_3) | 810 – 11,000 | 900 – 4,000

Major Observation

Leachate water quality parameters are consistent with literature review.
Results and Data Analysis

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)

Saturation Index

Location

Results and Data Analysis

Solid separation (Cyclone Separator)

<table>
<thead>
<tr>
<th>DivTime</th>
<th>Turbidity NTU</th>
<th>TSS mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single pass</td>
<td>Batch</td>
<td>Single pass</td>
</tr>
<tr>
<td>0</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>2</td>
<td>10.9</td>
<td>av</td>
</tr>
<tr>
<td>5</td>
<td>12.1</td>
<td>12.3</td>
</tr>
<tr>
<td>10</td>
<td>12.2</td>
<td>16.2</td>
</tr>
</tbody>
</table>

% Removal @ 10 min

- 33% 22% 75% 60%
- 33% 20% 50% 4%

*Note = non-removal

- 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)

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

Components

- Major Components: (10 samples)
  - Calcite (CaCO₃)
  - Halite (NaCl)
- Conforms with historical precipitate composition analysis

Results and Data Analysis

pH Adjustment and Precipitation

- Initial pH enhances precipitation

\[ \text{LSI} = \text{pH} - \text{pH}_s \]
\[ \text{SUM} = \text{pH}_{CO_2} + \text{pH}_{HCO_3^=} + \text{pH}_{OH^-} \]

- 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)
Microbial Activity and Precipitation

### Results and Data Analysis

**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

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

### Alternate Submerged/Dry Condition and Precipitation

#### Alternate submerged and dry condition enhances surface adhesion of precipitates

#### Impacts on Deep Injection Well

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Control</th>
<th>Sterilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial-1</td>
<td>13.42</td>
<td>10.86</td>
</tr>
<tr>
<td>Trial-2</td>
<td>18.42</td>
<td>13.30</td>
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<tr>
<th>Sample ID</th>
<th>Control</th>
<th>Sterilized</th>
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</thead>
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<tr>
<td>Trial-1</td>
<td>8.72</td>
<td>11.43</td>
</tr>
<tr>
<td>Trial-2</td>
<td>9.66</td>
<td>12.50</td>
</tr>
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**Microbiological Composition**
- No algae
- Highest microbial community at surface zone
- Methanogenic archaea
- Sulfate-reducing
- Entamoeba dispar (Biofilm-producing protist)

**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 produces 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

2. Investigate the triggers/factors of precipitation
   - Impacts of pH adjustment, disinfection, and flow regime were studied
     - 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

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 an 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
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

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

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

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Does anyone have any questions?

Thanks
Current works

- **Sampling**
- **Water quality parameters test**
  - pH, TDS, Ca hardness, alakalinity, COD, ammonia, turbidity
- **Saturation indices**
  - LSI, RI
- **Microscopic analysis for Entamoeba dispar**
- **RT-qPCR analysis**
- **Chlorination of the samples**

Completed works

Works in progress

Future works

Current Work: Sampling

Current Work: Water Quality Parameters
Current Work: Water Quality Parameters

- DIW sample collected on 12/06/2019 and 08/24/2020
- DIW sample collected on 03/05/2020

### 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)

### Current Work: Saturation Indices

#### Sample TDS (mg/L)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water Temperature (°C)</th>
<th>Ca hardness (mg/L)</th>
<th>Alkalinity (mg/L)</th>
<th>pHs</th>
<th>LSI</th>
<th>RI</th>
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<tbody>
<tr>
<td>NEFCO In</td>
<td>830.00</td>
<td>33.80</td>
<td>450.00</td>
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**Avg.**

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**Observed values**

**Estimated values**

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

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
Current Work: Microbiological Detection Methods

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Cost</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>Microscopic analysis</td>
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<tr>
<td>ELISA</td>
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<tr>
<td>RT-qPCR analysis/Agarose-gel electrophoresis</td>
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Current Work: Microscopic Analyses

- Entamoeba histolytica (control sample)

Current Work: Microscopic Analyses

- NEFCO influent
  - Sampling on 11/12/19, 11/14/19, 11/20/19

Current Work: Microscopic Analyses

- NEFCO effluent
  - Sampling on 11/12/19, 11/14/19, 11/20/19

Current Work: Microscopic Analyses

- Class III/Dyer
  - Sampling on 11/12/19

Current Work: Microscopic Analyses

- Class I
  - Sampling on 11/12/19
Current Work: Microscopic Analyses

- 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

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
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)

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’
    - (GC = 50%; Tm = 60°C; Length: 20 bp)
  - Reverse primer: 5’-CAC CAC TTA TCC CTA CC-3’
    - (GC = 50%; Tm = 52°C; Length: 20 bp)
Work in Progress: RT-qPCR analysis

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

### Experiments
- **PC-1**
  - Preparations and ordered positive control
  - 50% dilution of the PC-1
  - Nuclease-free water
  - 1 kb

### Notation Dilution (Times)
<table>
<thead>
<tr>
<th>Addition of nuclease-free water (µL)</th>
<th>Addition of the plasmid</th>
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</thead>
<tbody>
<tr>
<td>S0 (Initial plasmid)</td>
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<tr>
<td>S1</td>
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<tr>
<td>S2</td>
<td>1 µL of S1</td>
</tr>
<tr>
<td>S3</td>
<td>1 µL of S2</td>
</tr>
<tr>
<td>S4</td>
<td>1 µL of S3</td>
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<tr>
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<td>1 µL of S4</td>
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<tr>
<td>S6</td>
<td>1 µL of S5</td>
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**Future Work: Disinfection**

<table>
<thead>
<tr>
<th>Regions</th>
<th>Well types</th>
<th>Problems</th>
<th>Temperature (deg C)</th>
<th>C22 concentration (mg/L)</th>
<th>Contact times (hr)</th>
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<tbody>
<tr>
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Future Work: Disinfection

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<th>Chlorine Concentration (mg/L)</th>
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<td>+</td>
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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.

Future Work: Plan

- 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

Major Accomplishments

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Thank you

Questions?