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Title: Mesocosm-Scale Constructed Wetland Design for Wastewater Treatment

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 19
Number of Shots: 43

Introduction

Videographer's NOTE:
Audio (interviews) – Channel 1 – On board shot gun mic
Channel 2 – Lav Mic on Authors
Clip0001 – Grey Card/White Card for interview space

Clip0062—Grey Card and White Card for lab ** Once the glow lights were turned on, the room had a very strong magenta tint. We might be able to correct it a bit.

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **~~Dani Degenhardt~~ Amy-lynne Balaberda:** Our research investigates the use of constructed wetland treatment systems as a passive, cost-effective approach to treat oil sands process-affected water, focusing on removing naphthenic acid fraction compounds through mesocosm-scale experiments and identifying key optimization factors **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll:3.1* Note: Clip0002 is wide shot; Clip0003 is medium shot

What are the most recent developments in your field of research?

1.2. **~~Dani Degenhardt~~ Amy-lynne Balaberda:** Recent advancements in CWTS for OSPW include genomics-based methods to enhance the efficacy of CWTS in degrading toxic NAFCs. Additionally, our research outcome will inform the optimization of plant selection and system design for improved contaminant removal **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Note: Clip0002 is wide shot; Clip0003 is medium shot

What research gap are you addressing with your protocol?

1.3. **Amy-lynne Balaberda:** Mesocosm studies bridge lab-to-field CWTS gaps by testing plant-microbe interactions and design factors under semi-realistic conditions to assess treatment efficacy and guide pilot-scale implementation **[1]**.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Note: Clip0004 is medium shot; Clip0005 is wide shot; Clip0006 – Headshot of Amy Balaberda

What advantage does your protocol offer compared to other techniques?

1.4. **Kaitlyn Trepanier:** Our protocol allows for replication and manipulation of variables while still incorporating ecological complexity, making mesocosms ideal for identifying water treatment mechanisms, testing system designs, and predicting scalability with reduced risk and cost **[1]**.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Note: Clip0007 is wide shot; Clip0008 is medium shot

How will your findings advance research in your field?

1.5. **Kaitlyn Trepanier:** Our findings will advance OSPW treatment research by demonstrating how mesocosm-scale CWTS can effectively reduce toxic compounds like NAFCs, while also identifying the specific biotic and abiotic mechanisms at play **[1]**.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll:4.4* Note: Clip0007 is wide shot; Clip0008 is medium shot; Clip0009 – Headshot of Kaitlyn Trepanier

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Setup and Maintenance of the Mesocosm

Demonstrators: Amy-lynne Balaberda and Kaitlyn Trepanier

2.1. To begin, place *Carex aquatilis* seeds into standard styroblock containers filled with peat as plug stock **[1]**. Once the seedlings have germinated, fertilize them three times a week using water-soluble plant food **[2-TXT]**.

2.1.1. WIDE: Talent placing seeds into styroblock containers filled with peat. Note: Use Clip0011; Clip0012 – 2.1.1a – Optional Added shot – CU of seeds

2.1.2. Talent fertilizing the seedlings with water-soluble plant food. **TXT: Let seedlings grow for at least 3 months**

2.2. Reinforce the greenhouse tables with plywood to support the weight of the mesocosms **[1]**. Distribute the mesocosms evenly across the greenhouse bay tables **[2]**. Position the plumbing to hang off the edge of the table **[3]**.

2.2.1. Shot of the reinforced plywood on the greenhouse tables.

2.2.2. Shot of evenly distributed mesocosms across greenhouse tables. Note: Clip0018 – 2.2.2a – Optional added shot – Medium shot of putting the Mesocosm on the table.

2.2.3. Talent positioning plumbing off the table edge. Note: Use Clip0017

2.3. Next, place a 57-liter open-top plastic industrial drum under the drainage plumbing **[1]**. Install a submersible powerhead circulation pump between the middle and bottom of the tank for continuous mixing **[2]**. Secure the power cord to the outside of the tank **[3]**.

2.3.1. Talent positioning the open-top plastic drum under the drainage plumbing. Note: Use Clip0016

2.3.2. Talent installing the submersible pump inside the tank.

2.3.3. Shot of the secured power cord running along the tank exterior.

2.4. Now, spread the substrate evenly in the mesocosm and tamp it down with moderate pressure to reach the desired height **[1]**. Fully saturate the substrate with reverse osmosis water **[2].** Measure the volume of water added, as it is equivalent to the volume of porewater in the substrate **[3]**.

2.4.1. Talent evenly spreading and tamping down the substrate in the mesocosm.

2.4.2. Talent pouring reverse osmosis water into the mesocosm and measuring the volume added. Note: Clip0023 – 2.4.2a – Optional added shot – CU of water

2.4.3. Talent measuring the volume of water added.

2.5. After selecting a retention time based on prior studies and study objectives**,** calculate the total water volume in the mesocosm using the formula shown **[1]**. Then calculate the flow rate **[2]**.

2.5.1. TEXT ON PLAIN BACKGROUND:

$$Water\ Volume\ per\ Mesocosm\ (L) = [Mesocosm\ width\ (m) \times\ mesocosm\ length\ (m) \times\ water\ level\ height\ (m)]\ \times\ 1000\frac{L}{m^3} + pore\ water\ volume\ (L)$$

2.5.2. TEXT ON PLAIN BACKGROUND:

$$Flow\ Rate\ \left(\frac{L}{d}\right) = \frac{Total\ water\ volume(L)}{Retention\ time\ (days)}$$

2.6. Position one pump between two adjacent mesocosms **[1].** Link all the pumps together using a male-male USB *(U-S-B)* cable, ensuring the last pump is connected to the controller **[2]**.

2.6.1. Talent positioning a pump between two mesocosms.

2.6.2. Talent connecting pumps using a male-male USB cable.

2.7. Now, place the in-valve tubing in the reservoir and secure it down to keep it in place **[1]**. Secure the out-valve tubing to the back top corner of the mesocosm, keeping it above the waterline **[2]**. Wrap the tubing in aluminum foil to help prevent algae growth **[3].** NOTE: VO is modified for the modified shot.

2.7.1. Talent ~~submerging and~~ securing the in-valve tubing in the reservoir. Note: Ensure that there is no water in the container at this point in the video

2.7.2. Shot of of the out-valve tubing secured above the waterline.

2.7.3. Talent wrapping tubing in aluminum foil.

2.8. Set up and calibrate the pumps, power bar, and controller according to the manufacturer's instructions **[1]**. Then adjust the pumps to the calculated flow rate **[2]**.

2.8.1. Talent sets up the pumps, power bar and controller.

2.8.2. Shot of the pumps being adjusted to the calculated flow rate.

2.9. Then, adjust the temperature and LED *(L-E-D)* grow-lights to optimal levels for plant growth while conditioning plant species to the mesocosm **[1] [2-TXT]**. Evenly plant six to twelve individual plant species to ensure equal biomass per unit area in the mesocosm **[3].** NOTE: Sentence numbers are only adjusted to accommodate the added shot**.**

2.9.1. Talent adjusting temperature and LED grow lights. Note: Clip0032 is a medium shot of the screen; Clip0033 is a close-up shot of the screen

Added shot: 2.9.1a (Clip0034) – To show Grow light settings for this facility. **TXT: Settings will differ across labs**

2.9.2. Talent planting individual plant species in the mesocosm.

2.10. Fill the reservoir with reverse osmosis water **[1]**. Gradually raise the reverse osmosis water level, maintaining each level for one to two days, and replace the PVC *(P-V-C)* pipe as needed to match the water level **[2]**. Turn on the pumps with the final desired flow rate **[3]**. Note: VO is added for the added shot, and sentence numbers are adjusted.

Added shot: 2.10.0 (Clip0036) – Filling the reservoir with reverse osmosis water.

2.10.1. Talent adjusting water levels gradually over one to two days. Note: Clip0038 – 2.10.1a – Optional shot – showing fill lines in container

2.10.2. Talent turning on pumps at the final desired flow rate.

2.11. Once the desired water level is reached, adjust the greenhouse light and temperature to experimental settings and allow the plants to acclimate for approximately 35 days **[1]**.

2.11.1. Shot of plants acclimating under experimental lighting and temperature settings.

2.12. To drain and flush the system, remove the PVC standpipe **[1]** and open the ball valve to completely drain the system **[2]**. Then flush the system with OSPW *(O-S-P-W)* and ensure complete drainage **[3-TXT]**.

2.12.1. Talent removing the PVC standpipe

2.12.2. Talent opening the ball valve to drain the system.

2.12.3. Talent flushing the system with OSPW. **TXT: OSPW: Oil Sands Process-Affected Water; Do not use flushing OSPW for experiment** Note: Use Clip0044 Take 2

2.13. Once flushed, close the ball valve **[1]** and add the PVC pipe to match the desired water level **[2]**. Carefully pour OSPW into each mesocosm to avoid disturbing the substrate or plants **[3-TXT]**, filling until the desired water level is reached **[4]**. NOTE: Only sentence numbers are adjusted to accommodate the added shot

2.13.1. Talent closing the ball valve ~~and inserting the PVC pipe~~.

Added shot: 2.13.1a (Clip0046) – To show inserting the PVC pipe.

2.13.2. Talent carefully pouring OSPW into mesocosms to reach the desired level. **TXT: If using multiple batches of water, ensure chemical properties are consistent**

Added shot – 2.13.3 (Clip0048) – To show the desired level of water.

2.14. Now fill the reservoir tank with OSPW, leaving approximately five centimeters of space from the top **[1]**. Manage evaporation by refilling the reservoir tank with OSPW water as needed, maintaining the water level approximately five centimeters below the top **[2]**.

2.14.1. Talent filling the reservoir tank, leaving space at the top. Note: Use Clip0050

2.14.2. Talent refilling the reservoir tank with reverse osmosis water while monitoring the water level.

3. Plant Health and Water Quality Measurements

3.1. During every retention time cycle, measure plant health and growth metrics **[1]**. Assess plant health based on visible signs of stress such as chlorosis and insect damage **[2]**. Measure plant growth metrics, including mortality, height, and percentage cover **[3]**.

3.1.1. Talent looking at a plant.

3.1.2. Shot of leaves showing signs of chlorosis or insect damage.

3.1.3. Talent measuring plant height and percentage cover using a ruler or caliper.

3.2. Before adding substrates or OSPW to each mesocosm, conduct baseline characterization by measuring the on-screen parameters **[1].** During the first retention cycle, collect substrate and water samples from random locations in each mesocosm to establish a baseline for general chemistry **[2]**.

3.2.1. TEXT ON PLAIN BACKGROUND:
Parameters for measurement:
pH
Electrical Conductivity
Oxidation-Reduction Potential
Major Anions/Cations
Nutrients
Naphthenic Acid Fraction Compounds (NAFCs)
Other Relevant Contaminants

3.2.2. Talent collecting substrate samples from different locations in a mesocosm.

3.3. Measure substrate oxidation-reduction potential using an appropriate ORP *(O-R-P)* probe during every retention time cycle **[1]**. At the end of the experiment, collect substrate samples from each mesocosm and measure the same parameters recorded during baseline characterization **[2].**

3.3.1. Talent inserting an ORP probe into the substrate to take readings.

3.3.2. Talent collecting final substrate samples from mesocosms for laboratory analysis.

3.4. After adding OSPW, collect initial OSPW samples from each mesocosm at the end of the first retention cycle, allowing sediment to settle and filling the pore water space **[1]**. Then collect the samples from the front of each mesocosm **[2]**.

3.4.1. Shot of sediment settled in the mesocosm.

3.4.2. Talent using a container to collect OSPW samples from the front of the mesocosm.

3.5. During each retention time cycle, measure dissolved oxygen, ORP, pH, electrical conductivity, and temperature using the referenced instrument **[1]**. At the end of the experiment, collect final water samples to analyze general chemistry **[2]**.

3.5.1. Talent using a multi-parameter probe to record water quality metrics in a mesocosm.

3.5.2. Talent filling a sample bottle with final water samples from the mesocosm for analysis.

Results

4. Results

4.1. The height of *Carex aquatilis* increased steadily, reaching approximately 150 centimeters by day 40 before plateauing **[1].**

4.1.1. LAB MEDIA: Figure 5. *Video editor: Highlight the upward trend in plant height*

4.2. Dissolved oxygen levels were consistently higher in unplanted mesocosms compared to those with *C. aquatilis*, with levels above 5 parts per million in both conditions **[1].**

4.2.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the curve of Unplanted then highlight the curve of Carex aquatilis*

4.3. Soil redox potential in unplanted mesocosms remained between 50 millivolts and 100 millivolts, whereas in planted mesocosms, it occasionally approached 0 millivolts **[1].**

4.3.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the curve of Unplanted then highlight the curve of Carex aquatilis*

4.4. Total naphthenic acid fraction compounds in planted mesocosms showed a 76% reduction from 72.1 milligrams per liter to 17.1 milligrams per liter over 82 days, whereas unplanted mesocosms only showed an 8.5% reduction **[1].**

4.4.1. LAB MEDIA: Figure 7. *Video editor: Sequentially highlight the points of of Carex aquatilis then sequentially highlight the points of Unplanted*