FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68499
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20892243

Title: Scalable Step-by-Step Approach of Sustainable Bioplastic Production from Food Waste

Authors and Affiliations:
Xueyao Zhang[1], Mingxi Wang[1], Yebo Li[2], Stephanie Lansing[3], Zhi-Wu Wang[1]

[1]Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University
[2]Quasar Energy Group
[3]Department of Environmental Science and Technology, University of Maryland

Corresponding Authors:
Zhi-Wu Wang wzw@vt.edu

Email Addresses for All Authors:

Xueyao Zhang	xueyao@vt.edu
Mingxi Wang	mingxiwang@vt.edu
Yebo Li	yli@quasareg.com
Stephanie Lansing	slansing@umd.edu
Zhi-Wu Wang	wzw@vt.edu

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? **Yes**

Videographer: Please record the computer screen for the shots labeled as SCREEN

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

Current Protocol Length

Number of Steps: 23

Number of Shots: 46 (5 SC)

Introduction

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

1.1. **Xueyao Zhang:** We developed a scalable process to convert food waste into biodegradable plastic using halophilic microbes, which addresses both plastic pollution and organic waste challenges.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges in your field of research?

1.2. **Mingxi Wang:** Key challenges include managing feedstock variability, scaling up without contamination, and achieving high-purity bioplastics while avoiding expensive or hazardous chemical processes.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What significant findings have you established in your field?

1.3. **Xueyao Zhang:** We achieved 93% recovery of biodegradable plastic from food waste using only water, offering a chemical-free solution for downstream processing.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.2*

How will your findings advance research in your field?

1.4. **Mingxi Wang:** Our work demonstrates a viable path from lab to pilot scale for converting food waste into high-quality bioplastics, paving the way for industrial implementation and broader circular economy practices.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

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

Protocol

2. Volatile Fatty Acid (VFA) Production Through Arrested Anaerobic Digestion (aAD)

Demonstrator: Mingxi Wang

2.1. To begin, add 10 kilograms of food waste into a 5-gallon bucket **[1]**. Pour 2.5 liters of water into the bucket containing the food waste **[2]**. Connect the blender to a power source **[3]**. Submerge the blade into the food waste and water mixture **[4]**, then press the **Start** button to begin blending **[5]**. Continue blending for at least 30 minutes until the mixture is fully homogenized **[6]**.

2.1.1. WIDE: Talent placing 10 kilograms pre-weighed food waste into a 5-gallon bucket. **NOTE**: The action was modified

2.1.2. Talent pouring 2.5 liters of water into the bucket.

2.1.3. Talent plugging the blender into a power source.

2.1.4. Talent lowering the blender blade into the mixture.

2.1.5. Talent pressing the **Start** button on the blender.

2.1.6. Close-up of the mixture being blended to a smooth consistency.

2.2. Prior to feeding, temporarily increase the digester stirring speed from 150 revolutions per minute to 200 revolutions per minute and maintain this speed for 20 minutes **[1]**.

2.2.1. Shot of the stirring speed setting being changed.

2.3. Then, open the discharge valve at the bottom of the pilot anaerobic acidogenic digester and release approximately 26.7 liters of digestate **[1]**. Collect the discharged digestate into labeled buckets **[2]** and store them at 4 degrees Celsius for volatile fatty acid analysis and subsequent solid-liquid separation **[4-TXT]**.

2.3.1. Talent opening the discharge valve and releasing digestate into buckets.

2.3.2. Talent positioning labeled buckets under the valve to collect the digestate.

2.3.3. Talent placing collected buckets in a refrigerator at 4 °C. **TXT: Organic loading rate (OLR): 2.5 g VS/L-day; VS of the food waste slurry: ~14.5%**

2.4. Now, add water to a separate bucket containing 4.1 kilograms of the wet food waste slurry until the total volume reaches 26.7 liters **[1]**. Using a peristaltic pump, feed the 26.7 liters of food waste slurry into the anaerobic acidogenic digester **[2]**.

2.4.1. Talent adding water to the slurry to bring total volume to 26.7 liters.

2.4.2. Talent operating a peristaltic pump to transfer the slurry into the digester.

2.5. Turn off the peristaltic pump and unplug it from the power source **[1]**. Reset the stirring speed of the pilot digester and continue stirring for 30 minutes **[2]**. Add sodium hydroxide pellets proportionally to the digestate volume to adjust the pH to 5.5 **[3]**.

2.5.1. Talent switching off the pump and unplugging it.

2.5.2. Talent resetting the stirring speed. **NOTE**: The action was modified

2.5.3. Talent adding measured sodium hydroxide pellets to the digester.

2.6. Check the pilot digester to ensure all valves are fully closed **[1]**.

2.6.1. Talent inspecting the digester.

3. Recovering VFA for Polyhydroxyalkanoate (PHA) Fermentation

3.1. Turn on the main power switch of the disc centrifuge **[1]** and wait until the word STANDSTILL *(stand still)* appears on the Human-Machine Interface system screen **[2]**. Check that the lubricant oil level is above the minimum threshold **[3]**.

3.1.1. Talent switching on the main power of the disc centrifuge.

3.1.2. SCREEN: Show STANDSTILL status text appearing on the HMI screen. ***Videographer: Please record the computer screen for the shots labeled as SCREEN***

3.1.3. Talent inspecting the oil level indicator on the centrifuge.

3.2. Open the valve of the water utility line and adjust the pressure to 45 pounds per square inch **[1]** and then open the valve of the air utility line. Adjust the pressure to 90 pounds per square inch **[2]**.

3.2.1. Talent opening the water utility line valve and setting the pressure gauge to 45 psi.

3.2.2. Talent opening the air utility valve and setting the gauge to 90 psi.

3.3. Now, press the **Green** button on the HMI screen to start the production process of the disc centrifuge **[1]**. Wait for the centrifuge to complete its automated checks **[2]**.

3.3.1. SCREEN: Show the **Green** button being pressed on the HMI screen.

3.3.2. SCREEN: Display the system performing pre-checks with progress or status bars visible.

3.4. Monitor the system until it reaches the full set speed, indicated by the word STANDBY *(stand by)* on the HMI system screen **[1]**.

3.4.1. SCREEN: Show the word STANDBY appearing on the HMI system as the centrifuge stabilizes at full speed.

3.5. Ensure the inlet hose is fully submerged in the container holding discharged digestate **[1]** and connect the supernatant outlet pipe to an empty 5-gallon bucket for supernatant collection **[2]**. Then, attach the solid outlet pipe to a separate empty bucket to collect the residual solids **[3]**.

3.5.1. Talent placing the inlet hose into the digestate container.

3.5.2. Talent attaching the supernatant outlet to a labeled 5-gallon bucket.

3.5.3. Talent attaching the solid outlet to another labeled empty bucket.

3.6. Using a peristaltic pump, begin feeding the digestate to the inlet of the disc centrifuge **[1]**. Simultaneously, press the **PROD** *(production)* button on the HMI screen **[2]**.

3.6.1. Talent operating the peristaltic pump to deliver digestate to the centrifuge.

3.6.2. SCREEN: Show the **PROD** button being clicked on the HMI screen.

3.7. After 30 minutes of operation, press the **Discharge** button on the HMI system screen to release both solids and supernatant into their respective buckets **[1]**.

3.7.1. Show the **Discharge** button being pressed and the output flowing into labeled buckets.

4. Pilot-Scale PHA Fermentation and PHA Downstream Recovery

Demonstrators: Xueyao Zhang, Mingxi Wang

4.1. Turn on the water bath heating switch and set the temperature to 37 degrees Celsius **[1]**. Then, turn adjust the speed of the stirrer to 150 revolutions per minute **[2]**.

4.1.1. Talent flipping the switch on the water bath and adjusting the temperature to 37 degrees Celsius.

4.1.2. Talent setting the stirrer to 150 revolutions per minute.

4.2. Turn on the air pump of the pilot fermenter **[1]** to aerate 40-liters of food waste digestate supernatant, prepared using the optimal dilution factor with supplementation and inoculation, in the 50-liter glass fermenter **[2]**.

4.2.1. Talent switching on the air pump.

4.2.2. Shoot of the liquid level of 40 liters.

4.3. Collect a fermentation sample daily **[1]** and measure the optical density at 600 nanometers **[2]**.

4.3.1. Talent using a syringe to collect a sample and adding it into a 96-well plate.

4.3.2. Talent placing the sample in the 96-well plate in the spectrophotometer to measure OD.

4.4. For downstream recovery, feed the *Haloferax mediterranei* fermentation broth to the disc centrifuge to separate the salty supernatant and the cells **[1]**. Collect the harvested cells from the solid outlet for subsequent cell lysis **[2]**.

4.4.1. Talent pumping in fermentation broth into the inlet of the disc centrifuge.

4.4.2. Talent collecting the solids from the outlet into a labeled container for cell lysis.

4.5. Resuspend the harvested cells in water at a ratio of 100 milliliters per gram of wet cells **[1]** and mix the suspension at 150 revolutions per minute for 2 hours at room temperature to induce cell lysis by osmotic pressure shock **[2]**.

4.5.1. Talent adding measured water to harvested cells in.

4.5.2. Talent placing the beaker on a magnetic stirrer and setting it to 150 revolutions per minute.

4.6. Centrifuge the lysed cell suspension at 10,000 *g* for 30 minutes **[1]** and collect the PHA or PHA granules into a fresh container **[2]**.

4.6.1. Talent loading the lysed suspension in centrifuge tubes into a centrifuge.

4.6.2. Talent collecting the PHA or PHA granules into a separate container using a spatula. **NOTE**: The action was modified

4.7. Freeze-dry the PHA granules at minus 50 degrees Celsius for approximately 48 hours until a constant weight is achieved to obtain crude PHA powder **[1]**.

4.7.1. Talent placing PHA granules in the freeze dryer.

4.8. Next, add ethanol to the crude PHA powder at a ratio of 10 milliliters per gram to eliminate residual impurities **[1]**. Centrifuge the mixture at 10,000 g for 30 minutes to collect purified PHA granules **[2-TXT]**.

4.8.1. Talent adding ethanol to the crude powder in a conical tube using a pipette.

4.8.2. Talent placing the sample tube in a centrifuge. **TXT: Dispose of the supernatant**

4.9. Finally, freeze-dry the purified PHA pellets at minus 50 degrees Celsius **[1]** for approximately 48 hours to obtain final purified PHA powder **[2]**. **NOTE**: VO modified to accommodate the extra shot

4.9.1. Talent placing the sample in the freeze dryer.

Added shot: Talent presenting the purified PHA powder and showcasing a final product sample.

Results

5. Results

5.1. After bench-scale PHA fermentation, *Haloferax mediterranei* showed the highest optical density at 600 nanometers and intracellular PHA content when cultured in food waste digestate supernatant diluted two times **[1]**, while no growth or PHA production occurred in the undiluted medium, confirming the presence of inhibitory compounds **[2]**.

5.1.1. LAB MEDIA: Figure 7. *Video editor: Highlight the line labeled "2-time dilution" in A and bar "2" in B*.

5.1.2. LAB MEDIA: Figure 7. *Video editor: Highlight the line labeled "1-time dilution" in A and bar labeled "1" in B*.

5.2. During pilot-scale fermentation, optical density at 600 nanometers peaked at 192 hours **[1]**, and intracellular PHA content peaked at 120 hours **[2]**.

5.2.1. LAB MEDIA: Figure 8A*. Video editor: Highlight the peak of the curve around 192 hours*.

5.2.2. LAB MEDIA: Figure 8B. *Video editor: Highlight the bar at "120" hours, which reaches the tallest height.*

5.3. The PHA powder recovered through the 2 hour water treatment exhibited a purity of around 84% with the colour indicating the presence of impurities, such as carotenoids **[1, 2]**.

5.3.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the reddish-pink colored powder at the center of the image*. *Show 5.3.1 and 5.3.2 side by side*

5.3.2. LAB MEDIA: Table 5. *Video editor: Highlight the box showing 84*.

5.4. Ethanol purification after water-based extraction improved PHA purity to around 96%, resulting in a white powder free of pigment impurities **[1, 2]**.

5.4.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the white powder in the center of the image. Show 5.4.1 and 5.4.2 side by side*

5.4.2. LAB MEDIA: Table 5. *Video editor: Highlight the box showing 96*.

Pronunciation guide:

---

1. Anaerobic

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/anaerobic
- **IPA**: /ˌæn.əˈroʊ.bɪk/
- **Phonetic Spelling**: an-uh-ROH-bikmerriam-webster.com+4merriam-webster.com+4merriam-webster.com+4

---

2. Acidogenic

- **Pronunciation link**: https://www.merriam-webster.com/medical/acidogenic
- **IPA**: /ˌæs.ɪ.dəˈdʒɛn.ɪk/
- **Phonetic Spelling**: as-ih-doh-JEN-ikmerriam-webster.com+1merriam-webster.com+1

---

3. Peristaltic

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/peristaltic
- **IPA**: /ˌpɛr.əˈstɔːl.tɪk/
- **Phonetic Spelling**: per-uh-STAWL-tikmerriam-webster.com+1merriam-webster.com+1

---

4. Digester

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/digester
- **IPA**: /daɪˈdʒɛs.tər/
- **Phonetic Spelling**: dy-JESS-termerriam-webster.commerriam-webster.com

---

5. Optical

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/optical
- **IPA**: /ˈɑːp.tɪ.kəl/
- **Phonetic Spelling**: OP-tih-kuhlmerriam-webster.com+1merriam-webster.com+1

---

6. Polyhydroxyalkanoate

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌpɒl.i.haɪˌdrɒk.siˌæl.kəˈnoʊ.eɪt/
- **Phonetic Spelling**: pol-ee-hy-DROK-see-al-kuh-NOH-atemerriam-webster.com

---

7. Haloferax mediterranei

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌheɪ.loʊˈfɛr.æks ˌmɛd.ɪ.təˈreɪ.ni.aɪ/
- **Phonetic Spelling**: HAY-loh-FER-aks MED-ih-tuh-RAY-nee-eye

---

8. Peristalsis

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/peristalsis
- **IPA**: /ˌpɛr.əˈstɔːl.sɪs/
- **Phonetic Spelling**: per-uh-STAWL-sismerriam-webster.com

---

9. Densitometer

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/densitometer
- **IPA**: /ˌdɛn.sɪˈtɒm.ɪ.tər/
- **Phonetic Spelling**: den-sih-TOM-ih-termerriam-webster.com

---

10. Acetogen

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/acetogen
- **IPA**: /əˈsiː.tə.dʒən/
- **Phonetic Spelling**: uh-SEE-tuh-jenmerriam-webster.com
