FINAL SCRIPT: APPROVED FOR FILMIN

Submission ID #: 68950
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21029798

Title: Aerobic Biodegradation Testing of Materials Using a Natural Marine Seawater Inoculum and Closed Loop Respirometer

Authors and Affiliations:
Micheline S. Labrie[1], Elizabeth Ells[1], Erica Roche[1], Jo Ann Ratto[2]

[1]School for Marine Science and Technology, University of Massachusetts, Dartmouth
[2]Francis College of Engineering, University of Massachusetts, Lowell

Corresponding Authors:
Micheline S. Labrie (mlabrie@umassd.edu)
Email Addresses for All Authors:
Micheline S. Labrie (mlabrie@umassd.edu)
Elizabeth Ells (eells@umassd.edu)
Erica Roche (eroche2@umassd.edu)
Jo Ann Ratto (JoAnn_RattoRoss@uml.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, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   Within the same building.

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length
Number of Steps: 23
Number of Shots: 51

Introduction

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

INTRODUCTION:

1.1. **Elizabeth Ells**: Quantifying the aerobic biodegradation rate and degree of natural materials in the marine environment using natural seawater inoculum for data comparison.
   1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What are the current experimental challenges?

1.2. **Elizabeth Ells:** Limited testing capacity and lack of infrastructure to meet growing demand for biodegradation testing, while ensuring uniform and transparent methods across laboratories.
   1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

What research gap are you addressing with your protocol?

1.3. **Elizabeth Ells:** This protocol addresses the gap in standardized, accessible methods for screening the biodegradability of materials in the marine environment for industry, academia, and regulatory bodies.
   1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2*

What advantage does your protocol offer compared to other techniques?

1.4. **Elizabeth Ells:** The protocol provides a faster, standardized way to directly measure this biodegradation with shorter test duration and simultaneous sample evaluation.
   1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.17*

How will your findings advance research in your field?

1.5. **Elizabeth Ells:** The protocol enables high-throughput, standardized testing for material and laboratory comparability for machine learning and predictive modeling of material performance.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Elizabeth Ells, Postdoctoral Researcher**: (authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Elizabeth Ells, Postdoctoral Researcher**: (authors will present their testimonial statements live)

2. Nutrient-Amended Seawater Reactor Preparation for Particulate and Dissolved Nutrient Analysis Under Controlled Incubation Conditions

Demonstrator: Elizabeth Ells

2.1. To begin collect 10 to 20 liters of seawater into a 20liter acidleached carboy using a large Niskin bottle **[1]**. Transport the filled carboy back to the laboratory **[2]**.

2.1.1. WIDE: Talent dropping a Niskin bottle into the sea.

2.1.2. Shot of the filled carboy in a laboratory setting.

2.2. Transfer 1 liter of whole seawater from the carboy into an acid-leached 1-liter amber HDPE bottle for particulate carbon and nitrogen analysis **[1]**. Filter it through a 0.2-micrometer low nitrogen cellulose acetate membrane **[2].** Then collect 60 milliliters of filtrate into an acid-leached polyethylene bottle for dissolved inorganic and organic nitrogen and phosphorus analysis **[3]**.

2.2.1. Talent pouring a subsample from carboy into amber HDPE bottle.

2.2.2. Shot of the water being poured through a 0.2 µm low nitrogen cellulose acetate membrane.

2.2.3. Talent collecting 60 mL of filtrate into acid-leached polyethylene bottle.

AUTHOR'S NOTE: Please move 2.6.1-2.6.2 after 2.2

2.3. Loosely cover the opening of the carboy to allow aerobic conditions **[1]**. Store in the dark at 30 degrees Celsius for up to 7 days **[2]**.

2.3.1. Talent covering the carboy opening with loose cover.

2.3.2. Talent placing the carboy inside a temperature-controlled dark incubator or storage area at 30 °C.

2.4. Next, place 10 to 15 grams of the experimental substrate in a 50-milliliter stainless steel milling jar with a 20-millimeter steel ball **[1]**. ~~Mill the substrate to a uniform particle size between 0.10 and 0.25 millimeter prior to the start of the test **[2].**~~ Submerge the milling jar in liquid nitrogen for fifteen minutes to embrittle it **[3]**.

2.4.1. Talent placing substrate fragments into the milling jar.

~~2.4.2. Talent running the mill to produce uniform particle size.~~

NOTE: Authors filmed this as 2.5.3 and moved VO accordingly

2.4.3. Talent lowering the milling jar into a Dewar of liquid nitrogen.

2.5. Secure the milling jar in the ball-mill attachment **[1].** Then set the instrument to 30 hertz and two minutes thirty seconds and press **Start.** Perform milling 4 times with 30 seconds of cooling in liquid nitrogen between each cycle **[2]**. Mill the substrate to a uniform particle size between 0.10 and 0.25 millimeter prior to the start of the test **[3-TXT]**.

2.5.1. Talent mounting jar on ball mill.

2.5.2. Talent setting the frequency and time on the instrument then pressing Start.

2.5.3. ~~Shot of the mill jar being placed in liquid nitrogen.~~ Talent running the mill to produce uniform particle size.
TXT: If substrate is a polymer with a low glass transition temperature, perform additional milling cycles

2.6. Now, fill a 1, 2 or 4-liter volumetric flask halfway with seawater **[1]**. If the seawater has high particulate organic matter, sieve it through a 20-micrometer mesh to remove large heterogeneous particles **[2]**.

2.6.1. Talent pouring seawater into flask to halfway mark.

2.6.2. Talent pouring seawater through 20 µm mesh sieve to remove large particulates.
AUTHOR'S NOTE: 2.6.1-2.6.2 were filmed together

2.7. Using a precision balance with 0.001-gram sensitivity **[1],** weigh out 0.5 grams per liter of ammonium chloride and 0.1 grams per liter of monobasic potassium phosphate **[2]**.

2.7.1. Talent taring the balance.

2.7.2. Talent weighing NH_4Cl and KH_2PO_4 quantities.

2.8. Add the inorganic nutrients to the flask **[1]**, bring the volume to mark using seawater **[2]**, then drop in a stir bar and stir on a stir plate until all salts dissolve **[3]**.

2.8.1. Talent adding NH_4Cl and KH_2PO_4 to seawater in flask.

2.8.2. Talent filling to final volume mark.

2.8.3. Talent placing the flask with stir bar on a stir plate.

2.9. On an analytical balance, weigh out 20 milligrams of the experimental or control substrate **[1].** Record the full readout weight, and set the vial aside **[2-TXT].**

2.9.1. Talent weighing out ~20 mg of substrate

2.9.2. Talent recording the full readout weight and placing vial aside.
TXT: Repeat for replicate vials

2.10. To set up the reactor vessels, use an acid-leached 75 milliliter volumetric pipet with a motorized pipet controller and aspirate the seawater **[1]** to dispense 75 milliliters of the nutrient-supplemented seawater into each reactor vessel **[2]**.

2.10.1. Shot of the 75 mL volumetric pipet being attached with a motorize controller and aspirating 75 mL of seawater.

2.10.2. Talent dispensing 75 mL nutrient-supplemented seawater into a reactor vessel.

2.11. Remove approximately three milliliters of seawater from each vessel **[1].** Then place it on a clean surface next to the vessel to rinse the milled substrate from the tared vial **[2]**.

2.11.1. Talent pipetting out ~3 mL from each reactor.

2.11.2. Talent placing the pipettel onto a clean surface beside the vessel.
AUTHOR'S NOTE: Please combine 2.11.1-2.11.2

2.12. Now transfer the milled substrate into the corresponding reactor vessel from the tared vial **[1]**.

2.12.1. Talent pouring or tipping the substrate from vial into the reactor vessel.

2.13. Place an O-ring on the reactor vessel rim **[1]** then place a two-port lid over the O-ring **[2]** and secure with a screw cap **[3]**.
AUTHOR'S NOTE: Please combine 2.13.1-2.13.3

2.13.1. Talent fitting the O-ring onto vessel rim.

2.13.2. Talent placing the two-port lid over the O-ring.

2.13.3. Shot of the lid being secured with a screw cap.

2.14. Connect each reactor vessel to its matching gas or condensation lines **[1].** Firmly insert the gas lines into the appropriate inlet and outlet ports on the lid **[2]**.

2.14.1. Talent matching the vessels to its matching gas/condensation lines.

2.14.2. Shot of the gas lines being inserted into the appropriate inlet and outlet ports on the lid.

2.15. Turn on the shaker platform to 0.05 *g* in continuous mode **[1]**. Visually check that all vessels are secure, then close the incubator door **[2]**.

2.15.1. Talent operating shaker control to 0.05 × g.

2.15.2. Talent checking vessels and closing door.

2.16. On the instrument software, navigate to **Experiment** and click **Setup [1].** Enter the start and end channel numbers from the drop down menus corresponding to occupied channels **[2]**.
Video Editor: Please slow down or freeze frame where necessary

2.16.1. SCREEN: 68950_screenshot_1-(1).mp4 00:00-00:02.

2.16.2. SCREEN: 68950_screenshot_1-(1).mp4 00:03-00:16.

2.17. Set the **Refresh Threshold** to **0.50**, **Refresh Interval** to **N.A.** *(N-A)*, and **Refresh Window**

to **Auto [1].** Then check the box for **Purge Sensors** under **Misc** *(Miscellaneous)* **Setup [2].** Ensure that the **Auto Volume Measurement**, **O_2** *(Oxygen)* **Consumption Positive**, and **Enable Open Flow Mode** are not selected **[3].**

2.17.1. SCREEN: 68950_screenshot_2-(1).mp4 00:00-00:09.

2.17.2. SCREEN: 68950_screenshot_2-(1).mp4 00:09-00:12.

2.17.3. SCREEN: 68950_screenshot_2-(1).mp4 00:13-00:17 .

2.18. Set the **Sample Interval** to **8.00** ~~and **Experiment Duration** to **N.A.**~~ **[1]** then set the gas and time units as required **[2]**.

2.18.1. SCREEN: 68950_screenshot_3-(1).mp4 00:00-00:05.

2.18.2. SCREEN: 68950_screenshot_3-(1).mp4 00:06-00:14 .

2.19. If using a primary temperature probe for incubation temperature correction, ensure **Manually Enter Chamber Temps** is not selected **[1].** Check the box for **Venting Mode**, and ensure **Drain Mode** is not selected **[2]**. ~~Ensure **Anaerobic Mode** is not selected **[3].**~~

2.19.1. SCREEN: 68950_screenshot_4-(1).mp4 00:00-00:01 .

2.19.2. SCREEN: 68950_screenshot_4-(1).mp4 00:02-00:05 .

~~2.19.3. SCREEN: 68950_screenshot_4-(1).mp4 00:04-00:05 .~~

2.20. Now, select **Chamber Setup** and enter the descriptive channel labels [**1**]. Then ensure the O-ring is clean, seated, and free of particles or fibers **[2]**.

2.20.1. SCREEN: 68950_screenshot_5-(1).mp4 00:00-00:08 .

2.20.2. Talent inspecting and seating the O-ring on the vessel before closure.

AUTHOR'S NOTE: 2.20.2 and 2.21.1 were filmed together

2.21. Tighten the screw cap **[1]**, inspect the tubing connections at inlet and outlet **.** Trim worn or deformed tubing ends with a sharp blade to produce clean cuts **[2]**.

2.21.1. Talent tightening screw cap.

2.21.2. Talent inspecting tubing connections.

2.21.3. Talent trimming tubing ends with blade.

AUTHOR'S NOTE: 2.21.2-2.21.3 were filmed together

2.22. From the menu, select **Tools** then click on **Service Menu** and confirm that the primary temperature probe measures 30 degrees Celsius with 2-degree variations and is stable **[1]**. Click **Run** to begin the experiment **[2]**.

2.22.1. SCREEN: 68950_screenshot_6-(1).mp4 00:00-00:10.

2.22.2. SCREEN: 68950_screenshot_6-(1).mp4 00:11-00:16 .

2.23. Save the experiment file under a descriptive name including the date and record the filename on the data sheet **[1]**.

2.23.1. SCREEN: 68950_screenshot_7-(1).mp4 00:00-00:20

Results

3. Results

3.1. Cumulative carbon dioxide production over 84 days confirmed expected performance **[1]**. Over 84 days, cellulose showed rapid carbon dioxide production after a short lag and reached about 700 micromoles **[2],** PHA *(P-H-A)* showed a slower initial rate over 3 days **[3-TXT]**, and the seawater control remained low at 150 micromoles **[4]**.

3.1.1. LAB MEDIA: Figure 2A.

3.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the cellulose line*

3.1.3. LAB MEDIA: Figure 2A. *Video editor: Highlight the PHA line*
TXT: PHA: Polyhydroxyalkanoates

3.1.4. LAB MEDIA: Figure 2A. *Video editor: Highlight the yellow seawater line*

3.2. Individual negative control replicates showed low variability, with all curves remaining close together **[1]**. Cellulose positive control replicates showed consistently low variability, confirming stable performance across all four replicates **[2]**.

3.2.1. LAB MEDIA: Figure 3A.

3.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the overlapping lines labeled R1 through R4*

3.3. In the negative result dataset, replicate 4 exhibited an anomalous spike in carbon dioxide production rate around day 12, likely due to labile organic matter **[1]**, but its cumulative production still plateaued near 100 micromoles **[2]**.

3.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the sharp peak in the R4 line occurring around day 12 in 4 B*

3.3.2. LAB MEDIA: Figure 4. *Video editor: Highlight the R4 curve flattening and plateauing near the 100 mark on the Y-axis in 4 A*

3.4. Replicates 1 and 6 had lower cumulative carbon dioxide production compared to others, suggesting potential technical issues **[1]**.

3.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the R1 and R6 lines*

3.5. The standard error of average cumulative carbon dioxide production in the negative control was 2.5 percent, indicating low variability among replicates **[1]**. The standard error for the cellulose positive control was 0.83 percent, further confirming high

reproducibility of the biodegradation assay **[2]**.

3.5.1. LAB MEDIA: Table 1. *Video editor: Highlight the "CO_2 Evolution" cell for the "Blank" (Seawater) row showing 153.44 ± 3.79*

3.5.2. LAB MEDIA: Table 1. *Video editor: Highlight the "CO_2 Evolution" cell for the "Positive Control" (Cellulose) row showing 710.93 ± 5.89*

Pronunciation Guide:

1. **Polyhydroxyalkanoates**
   Pronunciation link: https://www.howtopronounce.com/polyhydroxyalkanoates How To Pronounce
   IPA (American): /ˌpɑːliˌhaɪˌdrɒkˌsiˈæl.kə.noʊ.əts/
   Phonetic Spelling: pah-lee-HY-drox-ee-AL-kuh-noh-uts
2. **Respirometer**
   Pronunciation link: https://dictionary.cambridge.org/pronunciation/english/respirometer Cambridge Dictionary
   Also: Merriam-Webster: "res·pi·rom·e·ter" Merriam-Webster
   IPA (American): /ˌrɛspəˈrɑːmətər/
   Phonetic Spelling: res-puh-RAH-muh-ter
3. **Inoculum**
   Pronunciation link: https://www.merriam-webster.com/dictionary/inoculum Merriam-Webster (though "inoculum" page)
   IPA: /ɪˈnɒkjələm/
   Phonetic Spelling: in-OCK-yuh-luhm
4. **Cellulose**
   Pronunciation link: https://www.merriam-webster.com/dictionary/cellulose Merriam-Webster
   IPA: /ˈsɛljʊˌloʊs/
   Phonetic Spelling: SEL-yoo-lose
5. **Amber** (in "amber HDPE")
   Pronunciation link: https://www.merriam-webster.com/dictionary/amber Merriam-Webster
   IPA: /ˈæmbər/
   Phonetic Spelling: AM-ber
6. **HDPE**
   This is an acronym (High-Density Polyethylene). Typically spelled out: **H-D-P-E**
   (No special IPA needed beyond saying the letters.)
7. **Variability**
   IPA: /ˌvɛriəˈbɪlɪti/
   Phonetic Spelling: VAIR-ee-uh-BIL-ih-tee
8. **Cumulative**
   IPA: /ˈkjuːmjəˌleɪtɪv/
   Phonetic Spelling: KYOO-myoo-LAY-tiv
9. **Replicate** (as noun/verb in experimental context)
   IPA: /ˈrɛplɪkət/ (noun), /ˈrɛplɪkeɪt/ (verb)
   Phonetic Spelling: REP-li-kut (noun) / REP-li-kate (verb)
10. **Incubation**
    IPA: /ˌɪnkjuˈbeɪʃən/
    Phonetic Spelling: in-kyoo-BAY-shuhn

11. **Substrate**
    IPA: /ˈsʌbstreɪt/
    Phonetic Spelling: SUB-strate
12. **Temperature**
    IPA: /ˈtɛmprətʃər/
    Phonetic Spelling: TEM-pruh-chur