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Title: Bacterial cellulose spheres that encapsulate solid materials

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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. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option. We would appreciate this as a back-up option if one of the students cannot travel to Missoula for filming.

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

Current Protocol Length

Number of Steps: 06
Number of Shots: 16

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Laurel Bitterman:** Our protocol is significant as it expands on current methods of producing a biodegradable, naturally occurring biomaterial. Our method emphasizes production of reproducible bacterial cellulose spheres.

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

1.2. **Adolfo Martinez:** The main advantage of this technique is the ease of access to the necessary materials: sugar, water, tea, vinegar, bacterial culture starter, a baffled flask, and an orbital shaker.

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

OPTIONAL:

1.3. **Catherine Mulholland:** Someone performing this technique for the first time may not be able to correctly identify the spheres and remove irregular bacterial cellulose masses. We have demonstrated these steps in the video, but do not be discouraged if it does not work the first time.

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

1.4. **Catherine Mulholland:** Visual demonstration of this method is helpful for researchers to visualize what sizes, amounts, and shapes of bacterial cellulose masses are preferred and not preferred.

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

Introduction of Demonstrator on Camera

1.5. **Tyler Somerville:** Demonstrating the procedure will be myself, Tyler Somerville and Adolfo Martinez, Catherine Mulholland, and Laurel Bitterman. We are all undergraduate research assistants in the Aqueous Technologies Lab at Montana Tech.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Production of Bacterial Cellulose (BC) Spheres

2.1. Begin by boiling 350 milliliters of deionized water using a tea kettle **[1]**, then transfer the hot water to a 500-milliliter beaker. Use heat-protection gloves and make sure that the glassware can withstand the boiling water temperatures **[2].** Completely dissolve 42.5 grams of granulated sucrose into the hot water using a stir rod **[3].** *Videographer: This step is important!*

2.1.1. Shot of water in a container, kept for boiling.

2.1.2. Talent transferring hot water to the beaker.

2.1.3. Talent dissolving sucrose in hot water.

2.2. Steep a bag with 2.54 grams of black tea in the flask containing sucrose solution for 1 hour **[1].** Remove the tea bag with a stir rod without breaking it open **[2]** and dispose of it in the trash **[3].**

2.2.1. Talent keeping black tea bag in the flask.

2.2.2. Talent removing the tea bag.

2.2.3. Talent disposing the tea bag.

2.3. Add 100 milliliters of distilled white vinegar to the beaker **[1]** and thoroughly stir the mixture **[2].** Transfer 80 milliliters of the prepared acidic tea mixture to a 250-milliliter baffled flask **[3]** and allow the tea mixture to cool to 20 to 25 degrees Celsius **[4].** *Videographer: This step is important!*

2.3.1. Talent adding white vinegar to the beaker.

2.3.2. Talent stirring the mixture.

2.3.3. Talent adding tea mixture to baffled flask.

2.3.4. Shot of baffled flask containing the tea mixture, sitting on a lab bench.

2.4. Add 20 milliliters of microbial starter culture liquid to the baffled flask when the liquid is at room temperature **[1]** and cover the flask with parafilm **[2].** *Videographer: This step is important!*

2.4.1. Talent adding culture liquid to the flask.

2.4.2. Talent covering the flask with parafilm.

2.5. Place the baffled flask on an orbital shake table **[1]** and allow it to shake at 125 rotations per minute for 3 days at 20 to 25 degrees Celsius to produce BC spheres **[2].** *Videographer: This step is important!*

2.5.1. Talent placing the flask on shake table.

2.5.2. Shot of the flask, rotating on shake table.

2.6. Remove unwanted BC masses of non-spherical shapes with tweezers, to prevent further irregular BC masses from forming **[1].** Once the BC spheres have formed, gently pour them from the flask for further use **[2].** *Videographer: This step is difficult and important!*

2.6.1. Talent removing unwanted BC masses from the flask.

2.6.2. Talent pouring BC spheres from the flask.

Results

3. Results: Encapsulation Ability and Thermal Analysis with Microscopic Observation of BC Spheres

3.1. In this protocol, the BC spheres showed a high growth rate for the first 48 hours of culture **[1]** and the size remained constant after reaching a maximum **[2].** The BC spheres started to form tendrils around the eighth day of culture **[3].**

3.1.1. LAB MEDIA: Figure 2 A *Video Editor: Emphasize on first half of figure.*

3.1.2. LAB MEDIA: Figure 2 A *Video Editor: Emphasize on second half of figure.*

3.1.3. LAB MEDIA: Figure 2 D, E.

3.2. The size distribution of spheres after encapsulation of solid contaminants like biochar, polymer beads, and mine waste was tested **[1].** It was observed that the addition of solids to the BC spheres does not have a consistent effect on sphere size or frequency **[2].**

3.2.1. LAB MEDIA: Figure 3.

3.2.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize on highest bar of each figure.*

3.3. The orbital shaking speed, ambient temperature, and formation of irregular particles seem to be the main factors that affect shape, size, and frequency of spherical particles **[1].**

3.3.1. LAB MEDIA: Figure 4.

3.4. It was noticed that too high of a room temperature or improper removal of irregular masses changed the shape of an intact BC sphere **[1]** to stellate particles **[2]** or stringy clumps **[3]**.

3.4.1. LAB MEDIA: Figure 4 B.

3.4.2. LAB MEDIA: Figure 4 A.

3.4.3. LAB MEDIA: Figure 4 C.

3.5. To determine the fraction of encapsulated solids in the BC spheres, a thermal gravimetric analysis was done **[1].** Thermal and microscopic evaluation together confirmed the effective encapsulation of solid particles within BC spheres **[2-TXT].**

3.5.1. LAB MEDIA: Figure 5 A.

3.5.2. LAB MEDIA: Figure 5 C, D, E. *Video Editor: Emphasize on red arrows and label C "***biochar***", D "***plastic microbeads***", and E "***mine waste***".*

3.6. The differential TGA profile of plain BC appeared in nearly identical magnitudes with the BC with polystyrene beads **[1].** However, an additional peak corresponding to thermal decomposition of polystyrene beads was observed **[2].**

3.6.1. LAB MEDIA: Figure 6.

3.6.2. LAB MEDIA: Figure 6 A. *Video Editor: Emphasize on red peak.*

Conclusion

4. Conclusion Interview Statements

4.1. **Tyler Somerville:** It is most important to add the microbial starter culture when the tea has cooled down to room temperature. Additionally, one must ensure the culture has active and healthy organisms.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1 for microbial starter culture.*

4.2. **Laurel Bitterman:** This method can be used for contaminant removal in environmental remediation. The spheres can be utilized for controlled release of a substance of interest.

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

4.3. **Adolfo Martinez:** This technique enables us to encapsulate a variety of materials within bacterial cellulose spheres. This may be useful for biodegradable controlled-release or remediation platforms.

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