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Mini Winnies: scaled down and transparent Winogradsky columns for microscopy in microbiology education

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ABSTRACT Winogradsky columns were invented by Sergei Winogradsky in the 1880s and have commonly been used as a microbiology classroom learning tool in K-12 and collegiate education. However, they can be challenging to examine with microscopy. We scaled down Winogradsky columns into nuclear magnetic resonance (NMR) tubes and replaced the natural sediment with a transparent soil substitute toward the goal of observing the microbial growth under a bright-field microscope without column disassembly. Using this "Mini Winnie" approach, students can practice their microscopy skills while observing microbial growth inside the column after only days of incubation on the laboratory windowsill. Overall, we believe that the Mini Winnies provide a simple method for maximizing student engagement while giving them a greater understanding of how microorganisms interact in the environment.

KEYWORDS Winogradsky column, microscopy, environment, microbiology, student engagement

Winogradsky columns are model ecosystems that allow for the analysis of microorganisms in a simulated natural environment (1, 2). While the technique has been used for species enrichment and hypothesis testing in microbial ecology, it has also become a mainstream educational experience in many introductory biology courses (3–5). Traditionally, Winogradsky columns are grown in larger containers (50 mL and up) with soil from the natural environment and supplemented nutrients for microbial growth allowing students to observe community interactions at the macroscale. Observations of color change and the development of gas pockets (and sometimes smell) enrich the experience and can enhance student engagement. To further increase student interaction with large columns, Benoit demonstrated the substitution of the dark natural soil with a lighter-colored diatomaceous earth (DE) matrix, allowing for more vibrant colors and observations in color change to be made much sooner (6).

Microorganisms, as the name implies, experience life at the micro-scale; though, the traditional Winogradsky experience typically gives the observer only a macroscopic perspective of the overall ecology. Unfortunately, using larger columns does not allow students to observe community development at the microscopic scale because traditional bright-field microscopy cannot physically accommodate a large Winogradsky column—even if it could, the path length is too large, and the soil matrix is too opaque.

To give students a microscopic perspective, we grew Winogradsky columns in NMR tubes and replaced the soil matrix with cryolite, a transparent soil substitute (7). By growing the columns in NMR tubes, the path length is sufficiently short and by implementing cryolite as a soil substitute, the opacity barrier can also be overcome. Using this "Mini Winnie" approach, students can learn important microscopy skills and gain a better understanding of what is occurring in the column at the microscopic scale. Performing microscopy could allow students to identify some of the microorganisms

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The authors declare no conflict of interest.

Received 13 December 2023
Accepted 8 May 2024
Published 30 May 2024

found in the world around them, and gain knowledge into the complexities that are life at the microscopic scale.

PROCEDURE

The following values can make about 400 Mini Winnie columns. The procedure to make the Mini Winnies was based on earlier work by Benoit (6).

Materials

The following materials were used: 10-inch blunt tip needle (14–18 gauge needle), 1 mL syringe, three NMR tubes (or similarly sized column, e.g., sealed pasture pipet), 200 mL of cryolite (~590 g) or DE (~450 g), 200 mL of turbid water:sediment mixture (4:1 ratio), 0.16 g of finely shredded paper towels—1 mm by 20 mm strands (see Supplementary Material Appendix S1), 0.33 g each of calcium carbonate, sodium sulfate, and ammonium phosphate, 55 mM $MgSO_4$ in deionized water, bottle for mixing slurries, and bright-field or dissection microscope (see Supplementary Material Appendix S2).

Preparation

1. *Collect the sample*: Find a shallow area near a pond or stream, agitating the sediment and filling a wide mouth container with a sediment-water mixture. There should be approximately a 4:1 ratio of water to sediment in the container.
2. *Prepare inoculum slurry*: Gently agitate the sample to dislodge the microbes from the sediment and suspend them in the water. Let the large particles settle and then decant the water into a separate beaker. Measure and combine the desired matrix (cryolite or DE) in a 1:1 volume ratio of matrix to sediment water.
3. *Add nutrients*: Mix inoculum slurry with 0.33 g/200 mL sediment water of calcium carbonate, sodium sulfate, and ammonium phosphate, and combine them thoroughly.
4. *Prepare NMR tubes*: Soak a few fibers of shredded paper towel in water or 55 mM $MgSO_4$ and push them to the bottom of the NMR tube using a 10-inch blunt tip needle.
5. *Fill NMR tubes*: Using the blunt tip needle and a 1-mL syringe, pull up roughly 0.9 mL of well-mixed inoculum slurry. Carefully insert the needle into the NMR tube and slowly eject the mixture while backing the needle out to avoid trapping air bubbles in the column while it is being filled.
6. *Place NMR tubes vertically in the window and watch growth!* Perform microscopy on columns as desired (see Supplementary Material Appendix S2). Check water levels in NMR tubes and add DI water as needed to ensure proper hydration of the column.

Safety issues

When prepping the columns avoid inhaling the cryolite and DE powder as it could be harmful to your lungs. We advise that the instructors fill the NMR tube for the students as even a blunt tip needle could pose hazards.

NMR tubes can break easily. To best reduce the risk of breaking the column, the column should be held toward the top between your thumb and 1–2 fingers. The biggest risk of breaking the column occurs when placing the needle too forcefully into the column (e.g., when trying to put the wet paper towel shreds at the bottom).

CONCLUSION

Using scaled-down Winogradsky columns (Mini Winnies) in a classroom setting can help students gain an understanding of how microorganisms interact with each other in their natural environment at the microscopic scale. Here, multiple matrices were tested in the Mini Winnies, each with their own benefits and drawbacks (Supplementary Material Appendix S3). The natural soil has the benefit of giving the organisms

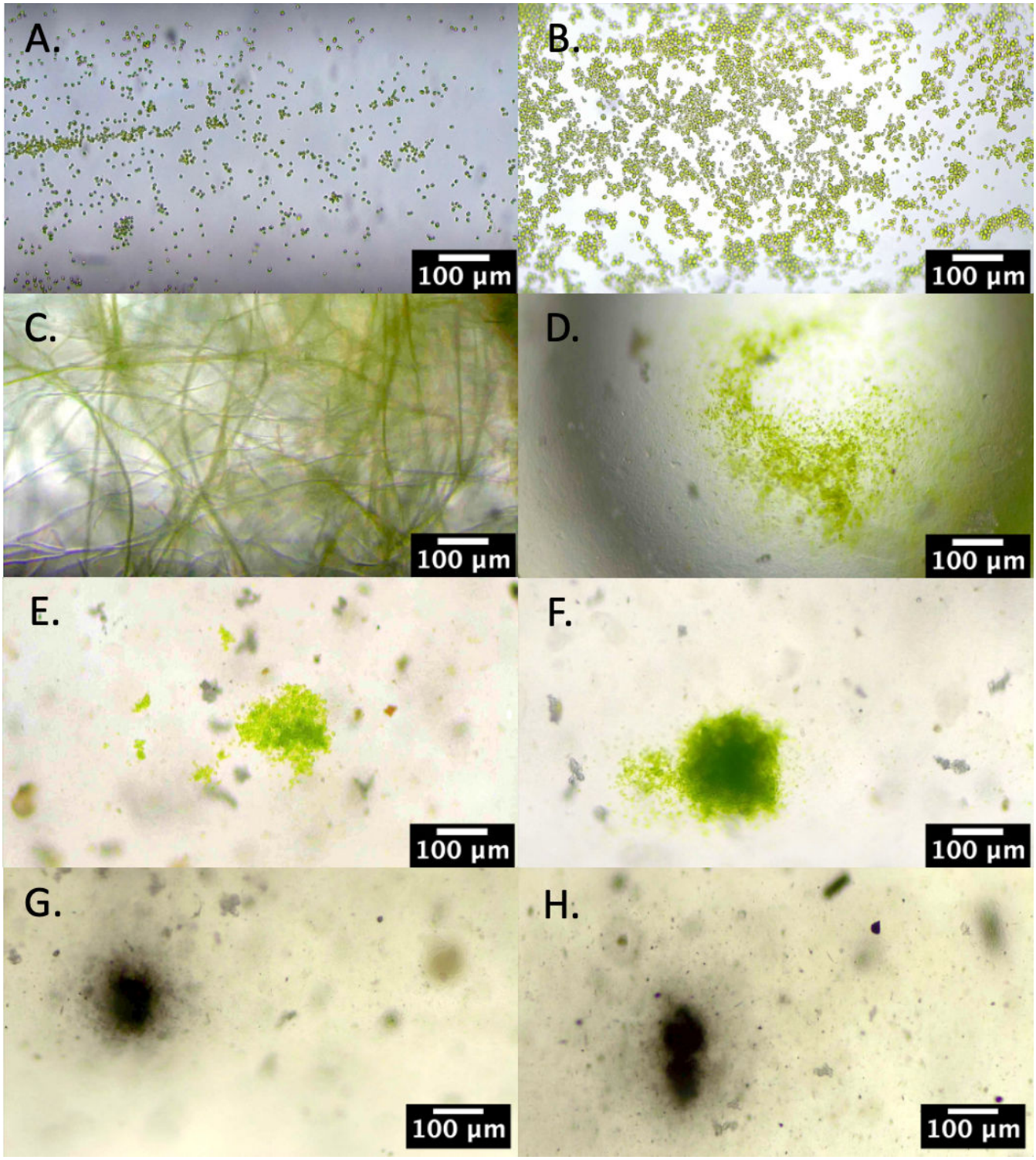

FIG 1 Microscopy images of cryolite Mini Winnie columns. (A, B) Images of the microorganisms in the headspace water. Image (C) was taken at the cryolite-water interface. Images (D–F) were taken in the bulk cryolite [~2–3 cm below image (C)]. (G, H) Images of the light-blocked columns spiked with $MgSO_4$ (bottom ~2 cm) All images, here, were taken after ~1 month of growth on a Leica DM500 bright-field microscope with an ICC50W Leica camera and the Leica Acquire application.

a replica of the natural environment; however, its dark color precludes meaningful microscopy and makes it hard to see the color changes in large columns (Supplementary Material Appendix S3). Additionally, the large grain size of the soil makes it difficult

to load into the Mini Winnie column. Diatomaceous earth, while useful for observing early color changes in large-scale columns (Supplementary Material Appendix S3), is also an ineffective matrix substitute for observing microscopic growth inside the Mini Winnie (Supplementary Material Appendixes S4 and S5). DE's ability to show early color development makes it work well for larger columns but poorly for microscopy on the Mini Winnies due to its opacity.

Growing Winogradsky columns inside NMR tubes with a cryolite matrix allows students to perform bright-field microscopy on the columns and see the growth of a variety of phenotypes at the microscopic scale (Fig. 1). The relatively short path length of the NMR tube combined with the low refractive index of cryolite makes this configuration optimal for simultaneously educating students on bright-field microscopy and phenotypic variance in the natural environment (7). While very useful for microscopy, cryolite does not work as well as DE in the larger columns because the rate and variation in color development are limited visually (Supplementary Material Appendixes S3 and S4). The Mini Winnies also allow you to select different organisms by spiking different nutrients or varying the light exposure. We 3D printed a column rack that restricts light from the bottom half of the Mini Winnie while sitting in the window (Supplementary Material Appendix S6 and the associated GitHub link in the caption). In this case, the growth of green clusters (associated with oxygenic photosynthesis) in the light-restricted region was reduced (Supplementary Material Appendix S7). By spiking the bottom of the light-restricted column with $MgSO_4$, the growth of dark clusters was observed in the bottom 1–2 cm of the column (see Supplementary Material Appendixes S7, S8, and Appendix Video S9).

To expand on the mainstream educational experience, we adapted the Winogradsky column to an NMR tube and a transparent soil substitute toward the goal of enhancing the student's experience. With Mini Winnies students can observe the growth of microorganisms inside a Winogradsky column at the microscopic scale—an observation that typically requires column disassembly or major perturbance.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under grant no. 1944204 and the Colorado School of Mines through the MURF program.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Material (jmbe00212-23-s0001.docx). Appendixes S1 (Paper towel shreds), S2 (Mini Winnies on different microscopes), S3 (Large scale Winogradsky columns), S4 (Images of Mini Winnies with diatomaceous earth or cryolite), S5 (Bright-field microscopy images of Mini Winnies with diatomaceous earth or cryolite), S6 (Column rack for Mini Winnies), S7 (Impact of light exposure and sulfate on Mini Winnies), and S8 (Dark clustered growth inside Mini Winnies).

Appendix Video S9 (jmbe00212-23-s0002.mov). Bright-field microscopy video that scans the column.

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