

Submission ID #: 71047

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Title: A Microfluidic Platform to Investigate Microbial Precipitation of Metal Oxides in Porous Media

Authors and Affiliations:

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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? **Yes, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 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: 34

Number of Shots: 59

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Jasquelin Peña:** We developed a microfluidic system to study the dynamic precipitation of metal oxide minerals by bacteria in a porous environment.
Videographer's note: Talent reworked this line slightly.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Eleanor Fadely:** This protocol enables in situ, real-time measurement of mineral precipitation, unlike conventional methods such as batch and column systems.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Jasquelin Peña:** This protocol allows researchers to study the timing and location of mineral precipitation in a porous medium.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Eleanor Fadely:** It is critical to prevent biofilm growth in the reactor inlet region, ensuring a constant Mn(II) boundary condition and restricting precipitation to the pore space. Videographer's note: Talent reworked this line slightly.
- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Jasquelin Peña:** Future studies can explore coupled microbe-mineral-fluid interactions under conditions relevant for environmental and engineering applications.
- 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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

~~What is the key takeaway you hope viewers remember after watching your video article?~~

- 1.6. **Eleanor Fadely**, **Enter author title**: (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How does the video format of your article help you to share your work with the broader scientific community?~~

- 1.7. **Jasquelin Pena**, **Enter author title**: (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

2. Bacterial Culture for Microfluidic Reactor Inoculation

Demonstrator: Eleanor Fadely

- 2.1. To begin, add 10 milliliters of LB (L-B) medium to a 50-milliliter conical tube [1]. Inoculate the medium using a single colony of *Pseudomonas putida* GB-1 (G-B-one) obtained from a plate that was streaked 20 hours earlier [2]. Incubate the LB preculture in a shaker incubator at 30 degrees Celsius and 180 revolutions per minute for 16 hours to reach the stationary phase [3].
 - 2.1.1. WIDE: Talent adding LB medium into a 50-milliliter conical tube.
 - 2.1.2. Talent picking a colony and inoculating the tube.
 - 2.1.3. Talent placing the conical tube into a shaker incubator.
- 2.2. After incubation, pellet the cells by centrifugation three times at 8,000 g for 1 minute [1] and resuspend the final pellet in 1 milliliter of salt solution without manganese [2].
 - 2.2.1. Talent placing the tube into a centrifuge.
 - 2.2.2. Talent resuspending the final pellet in SS0.
- 2.3. Then, dilute the washed cell suspension fivefold in salt solution without manganese in a cuvette [1] and measure the optical density at 600 nanometers using a cell density meter [2].
 - 2.3.1. Talent adding cell suspension to a cuvette with salt solution.
 - 2.3.2. Talent measuring optical density using a cuvette in a cell density meter.
- 2.4. Inoculate 20 milliliters of growth medium without manganese with the washed cell suspension to an optical density of 0.01 in a 50 milliliter Erlenmeyer flask [1]. Incubate at 30 degrees Celsius and 180 revolutions per minute for 6 hours to reach mid-exponential phase [2].
 - 2.4.1. Talent adding washed cell suspension into a 50 milliliter Erlenmeyer flask containing GM0.
 - 2.4.2. Talent placing the flask into a shaker incubator.

3. Biomineralization Experiment

- 3.1. ~~Prepare for the experiment by pre heating the microscope incubator to 30 degrees Celsius~~ Before beginning the experiment, verify that the microscope incubator is maintained at 30 degrees Celsius [1] ~~and priming the flow control system and identify the flow control system [2].~~ NOTE: The VO is edited as per the videographer's note.
 - 3.1.1. ~~Talent setting the microscope incubator to 30 degrees Celsius.~~ Shot of a microscope incubator temperature display showing 30 degrees Celsius. Videographer's NOTE: Unslated. The microscope is already set to warm up to 30 degrees.
 - 3.1.2. Shot of the flow control system on the right side of the microscope setup. Videographer's NOTE: Talent and videographer pointed out the flow system on the right side of the screen.
- 3.2. Pour 10 milliliters of growth medium and salt solution with manganese into a 15-milliliter conical tube [1] and connect both tubes to the fluid reservoir rack [2].
 - 3.2.1. Talent pouring GM_{Mn} and SS_{Mn} into 15-milliliter conical tubes.
 - 3.2.2. Talent connecting both conical tubes to the fluid reservoir rack.
- 3.3. Degas the prepared microfluidic reactor in a vacuum desiccator for 20 minutes [1].
 - 3.3.1. Talent placing the reactor in a vacuum desiccator.
- 3.4. Draw 1 to 2 milliliters of salt solution with manganese into a 3-milliliter plastic syringe [1]. Attach a blunt-tip needle and 20 centimeters of 0.02-inch inner diameter Tygon tubing [2]. Press the syringe plunger until the tubing is fully saturated [3].
 - 3.4.1. Talent drawing SS_{Mn} into a 3-milliliter plastic syringe.
 - 3.4.2. Talent attaching a blunt-tip needle and 20 centimeters of 0.02-inch inner diameter Tygon tubing to the syringe.
 - 3.4.3. Talent pressing the syringe plunger to fully saturate the tubing.
- 3.5. After degassing, use angled tweezers to attach the tubing to the reactor outlet hole [1]. Manually saturate the reactor with the syringe until a droplet forms at the reactor inlet hole [2].
 - 3.5.1. Talent attaching the tubing to the reactor outlet hole with angled tweezers.
 - 3.5.2. ~~Talent pushing salt solution with the syringe until a droplet forms at the reactor inlet hole.~~ Shot of droplet formation at the reactor inlet hole. Videographer's NOTE: Talent would like the shot to start after the water begins to flow.

- 3.6. Next, attach 20 centimeters of 0.02-inch inner diameter Tygon tubing to the reactor inlet hole [1]. Continue dispensing salt solution with manganese into the reactor until all air bubbles are removed [2].
 - 3.6.1. Talent attaching 20 centimeters of 0.02-inch inner diameter Tygon tubing to the reactor inlet hole.
 - 3.6.2. Talent dispensing SS_{Mn} into the reactor until all air bubbles are removed.
- 3.7. To prepare the reactor inoculum, remove the mid-exponential culture from the shaker incubator 6 hours after inoculation [1], dilute fourfold in salt solution without manganese in a cuvette [2], and measure the optical density [3].
 - 3.7.1. Talent removing the mid-exponential culture from the shaker incubator.
 - 3.7.2. Talent diluting the inoculum in a cuvette.
 - 3.7.3. Talent measuring the optical density at 600 nanometers.
- 3.8. Then, dilute the mid-exponential culture in 2 milliliters of salt solution with manganese to an initial optical density of 0.005 in a 2-milliliter microcentrifuge tube [1]. Invert the tube several times to mix [2].
 - 3.8.1. Talent diluting the mid-exponential culture in 2 milliliters of SS_{Mn} in a 2-milliliter microcentrifuge tube.
 - 3.8.2. Talent inverting the microcentrifuge tube several times to mix.
- 3.9. Now, place the reactor inlet tubing in the microcentrifuge tube, ensuring that a droplet of fluid contacts the inoculum to prevent air introduction in the tubing [1]. Partially close the microcentrifuge tube and secure the lid with tape to prevent contamination [2].
 - 3.9.1. Talent placing the reactor inlet tubing in the microcentrifuge tube while maintaining contact between the droplet and the inoculum.
 - 3.9.2. Talent partially closing the microcentrifuge tube and securing the lid with tape.
- 3.10. Next, withdraw 4 to 5 milliliters of salt solution with manganese into a 5-milliliter glass gas-tight syringe to wet the barrel [1]. Dispense the solution until 0.5 milliliters remain in the syringe [2] and load the syringe into a syringe pump [3].
 - 3.10.1. Talent withdrawing SS_{Mn} into a 5-milliliter glass gas-tight syringe.
 - 3.10.2. Talent dispensing solution from the syringe until 0.5 milliliters remains.
 - 3.10.3. Talent loading the glass syringe into a syringe pump.
- 3.11. After disconnecting the plastic syringe from the needle, attach the needle to the glass syringe while avoiding trapping air in the needle hub [1]. To inoculate the reactor, withdraw the inoculum into the reactor for 20 minutes at a flow rate of 8.33 microliters per minute [2].

- 3.11.1. Talent attaching the needle to the glass syringe.
- 3.11.2. Talent programming the syringe pump and starting the inoculation.
- 3.12. Turn off the syringe pump following inoculation and allow the bacteria to settle and attach for 1 hour with no flow [1].
 - 3.12.1. Talent turning off the syringe pump.
- 3.13. Then, remove the glass syringe from the syringe pump while leaving it connected to the outlet tubing [1].
 - 3.13.1. Talent removing the syringe from the syringe pump while keeping it connected to the outlet tubing.
- 3.14. Next, move the reactor and syringe from the benchtop to the microscope incubator [1].
 - 3.14.1. Talent transferring the reactor and syringe to the microscope incubator.
- 3.15. On the microscope stage, remove the inlet tubing from the reactor [1] and push gently on the syringe plunger to create a droplet at the reactor inlet hole [2].
 - 3.15.1. Talent removing the inlet tubing from the reactor on the microscope stage.
 - 3.15.2. Talent gently pressing the syringe plunger to form a droplet at the reactor inlet hole.
- 3.16. Gently bend the polytetrafluoroethylene or PTFE (*P-T-F-E*) tubing from the flow control system at a right angle so that it interfaces perpendicular to the polydimethylsiloxane or PDMS (*P-D-M-S*) surface, minimizing lateral forces and air intrusion into the reactor inlet hole [1].
 - 3.16.1. Talent bending the PTFE tubing near its end to form a right angle for perpendicular alignment with the reactor inlet.
- 3.17. Dip the end of the PTFE tubing into a 1 percent polyethylene glycol diacrylate solution to wet the exterior of the hydrophobic tubing [1]. Ensure a droplet of growth medium with manganese is present at the end of the PTFE tubing [2] and attach the tubing to the reactor inlet hole [3]. NOTE: The sentence numbers are adjusted for the added shot.
 - 3.17.1. Talent dipping the end of the PTFE tubing into the polyethylene glycol diacrylate solution.
 - 3.17.2. Talent checking for a droplet of GMMn at the tubing tip ~~and attaching the PTFE tubing to the reactor inlet hole.~~ Videographer NOTE: I separated this into two shots. First Talent showing off droplet, second attaching to tubing. They're slated as such.
 - Added shot: Talent attaching the PTFE tubing to the reactor inlet hole.
- 3.18. After disconnecting the syringe, place the outlet tubing into a 5-milliliter microcentrifuge tube to collect the reactor effluent [1].

3.18.1. Talent placing the outlet tubing into a 5-milliliter microcentrifuge tube.

3.19. Set the flow rate to 1.33 microliters per minute in the flow control system software [1]. Program the system to supply growth medium with manganese for 15 hours to promote biofilm formation, followed by salt solution with manganese for 28.5 hours to induce manganese biomineralization [2].

3.19.1. SCREEN: 71047_3.19.1_T1.MP4

3.19.2. SCREEN: 71047_3.19.2_T1.mp4

3.20 Next, place a sample frame underneath the reactor for stability [1]. Rigidly mount the frame and reactor in the microscope stage insert to ensure full immobilization [2].

3.19.3. Talent placing a sample frame underneath the reactor.

3.19.4. Talent securing the frame and reactor firmly in the stage insert.

3.20. Affix the PTFE tubing to the stage insert with a piece of tape to prevent detachment from the reactor inlet hole [1]. Align the ultraviolet C (*ultraviolet-C*) arms to 3 millimeters from the pore space edge on the inlet side and 2 millimeters on the outlet side to minimize UV light scattering and biofilm damage [2].

3.20.1. Talent taping the PTFE tubing to the stage insert.

3.20.2. Talent adjusting the ultraviolet C arms to specified distances from the pore space edges.

3.21. Set up a program for reactor irradiation to treat the reactor inlet and outlet regions for 5 minutes at the experiment onset, then treat only the outlet region for 5 minutes 17.5 hours after the experiment onset to inactivate remaining biofilms [1].

3.21.1. SCREEN: 71047_3.21.1_T1.MP4: 00:00-00:28.

4. Optical Time-lapse Microscopy

4.1. Manually adjust the Köhler alignment following the manufacturer's instructions to ensure even illumination [1].

4.1.1. Talent adjusting the microscope controls to achieve Köhler alignment.

4.2. To prevent quilting artifacts, select **Acquire** and open the **Shading Correction Panel**, then capture a shading image and set it as the current shading image [1].

4.2.1. SCREEN: 71047_4.2.1_Zoom.mp4: 00:00 – 00:45

4.3. Set the center coordinate of the stitched image by selecting **ND Acquisition** (*N-D-Acquisition*) and choosing **XY** [1].

4.3.1. SCREEN: 71047_4.3.1_Zoom.mp4

4.4. Set up the stitched image scan area as 5 by 4 fields of view. Apply a 1 percent overlap by selecting **ND Acquisition** and choosing **Large Image [1]**.

4.4.1. SCREEN: 71047_4.4.1_Zoom.mp4

4.5. Next, set up acquisition of color brightfield images with the 4x objective by selecting **ND Acquisition** and choosing **Lambda [1]**. Set the imaging frequency with intervals of 1 hour for 15 hours and 0.5 hours for 28.5 hours by selecting **ND Acquisition** and choosing **Time**, then run the time-lapse program **[2]**.

4.5.1. SCREEN: 71047_4.5.1_Zoom.mp4

4.5.2. SCREEN: 71047_4.5.2_Zoom_T2.mp4.

Results

5. Results

5.1. Brightfield images demonstrated that *Pseudomonas putida* GB-1 biofilms were uniformly distributed throughout the microfluidic pore space at 18.5 hours before the onset of manganese oxide precipitation [1].

5.1.1. LAB MEDIA: Figure 5A.

5.2. Subsequently, manganese oxides preferentially accumulated on the inlet side of the pore space closest to the influent manganese source [1].

5.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the left side of the image where the darker brown mineral signal is concentrated*

5.3. The difference between the reference image and subsequent image timepoints revealed the contribution of manganese oxide precipitates to the overall image intensity [1].

5.3.1. LAB MEDIA: Figure 5C.

5.4. Quantification of the observed mineral accumulation gradient was enabled at the reactor scale [1].

5.4.1. LAB MEDIA: Figure 5D. *Video editor: Highlight the line plot showing a higher signal near the left side that decreases across the distance axis*

5.5. At the biofilm scale, the image subtraction approach captured the gradual precipitation of manganese oxides on biofilm surfaces [1] and accumulation of minerals at the edges of biofilms in contact with pore fluid [2].

5.5.1. LAB MEDIA: Figure 6A. *Video editor: Show the time series and highlight the growing dark mineral coating on the biofilm surfaces from 19 hours to 43 hours*

5.5.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the outer edges of the biofilm shapes where the colored signal becomes strongest along the borders touching the surrounding fluid*

5.6. The resulting estimated rate of manganese oxide precipitation was non-monotonic, increasing steeply between 19.5 hours and 21 hours [1], peaking at 0.29 microgram per hour [2], and decelerating gradually until 43.5 hours [3].

5.6.1. LAB MEDIA: Figure 7. *Video editor: Highlight the steep rise in the curve between about 19.5 hours and 21 hours*

5.6.2. LAB MEDIA: Figure 7. *Video editor: Mark the highest point of the curve near 0.29 microgram per hour*

5.6.3. LAB MEDIA: Figure 7. *Video editor: Highlight the gradual downward trend in the curve from after the peak to 43.5 hours*

Pronunciation guides:

1. Pseudomonas
Pronunciation link: <https://www.merriam-webster.com/dictionary/Pseudomonas>
IPA: /ˌsuːdəˈmoʊnəs/
Phonetic Spelling: soo-duh-moh-nuhs
2. Inoculate
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪˈnɔːkjəˌleɪt/
Phonetic Spelling: ih-naa-kyuh-layt
3. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /ˌsentrəˈfjʊˈɡeɪʃən/
Phonetic Spelling: sen-truh-fyoo-gay-shuhn
4. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /ˌriːsəˈspend/
Phonetic Spelling: ree-suh-spend
5. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /ˈnænəˌmiːtər/
Phonetic Spelling: nan-uh-mee-ter
6. Erlenmeyer
Pronunciation link: <https://www.merriam-webster.com/dictionary/Erlenmeyer%20flask>
IPA: /ˈɜːlənməˈɪər/
Phonetic Spelling: er-luhn-my-er
7. Biomineralization
Pronunciation link: <https://www.merriam-webster.com/dictionary/biomineralization>
IPA: /ˌbaɪoʊˌmɪnərələˈzeɪʃən/
Phonetic Spelling: bye-oh-min-er-uhl-uh-zay-shuhn
8. Microfluidic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidics>
IPA: /ˌmaɪkroʊfluˈɪdɪk/
Phonetic Spelling: my-kroh-floo-id-ik
9. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>

IPA: /'dɛsɪ kɛtər/

Phonetic Spelling: dess·ih·kay·ter

10. Tygon

Pronunciation link: <https://www.howtopronounce.com/tygon>

IPA: /'tɑɡɑ:n/

Phonetic Spelling: tie·gon

11. Inoculum

Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculum>

IPA: /ɪ'nɑ:kjələm/

Phonetic Spelling: ih·naa·kyuh·luhm

12. Microcentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>

IPA: /ˌmaɪkroʊ'sentrəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

13. Polytetrafluoroethylene

Pronunciation link: <https://www.merriam-webster.com/dictionary/polytetrafluoroethylene>

IPA: /ˌpɑːliˌtetrəˌflʊroʊˈeθəˌliːn/

Phonetic Spelling: pol·ee·tet·ruh·floor·oh·eth·uh·leen

14. Polydimethylsiloxane

Pronunciation link: <https://www.howtopronounce.com/polydimethylsiloxane>

IPA: /ˌpɑːliˌdaɪˌmɛθəl'sɪləˌkˌseɪn/

Phonetic Spelling: pol·ee·dye·meth·uhl·sil·ock·sayn

15. Polyethylene

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>

IPA: /ˌpɑːliˈeθəliːn/

Phonetic Spelling: pol·ee·eth·uh·leen

16. Diacrylate

Pronunciation link: <https://www.howtopronounce.com/diacrylate>

IPA: /daɪˈækɹəˌleɪt/

Phonetic Spelling: dye·ak·ruh·layt

17. Effluent

Pronunciation link: <https://www.merriam-webster.com/dictionary/effluent>

IPA: /'ɛfluənt/

Phonetic Spelling: ef·loo·uhnt

18. Irradiation

Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiation>

IPA: /ɪˌreɪdiˈeɪʃən/

Phonetic Spelling: ih·ray·dee·ay·shuhn

19. Köhler

Pronunciation link: <https://www.howtopronounce.com/kohler>

IPA: /'kɜ:lər/

Phonetic Spelling: kur·ler

20. Precipitation

Pronunciation link: <https://www.merriam-webster.com/dictionary/precipitation>

IPA: /prɪˌsɪpəˈteɪʃən/

Phonetic Spelling: prih·sip·uh·tay·shuhn

21. Quantification

Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>

IPA: /ˌkwɑːntəfəˈkeɪʃən/

Phonetic Spelling: kwon·tuh·fuh·kay·shuhn

22. Non-monotonic

Pronunciation link: <https://www.howtopronounce.com/non-monotonic>

IPA: /ˌnɑːnɪˈmɑːnəˈtɒnɪk/

Phonetic Spelling: non·mon·uh·ton·ik

23. Biofilm

Pronunciation link: <https://www.merriam-webster.com/dictionary/biofilm>

IPA: /ˈbaɪoʊˌfɪlm/

Phonetic Spelling: bye·oh·film

24. Manganese

Pronunciation link: <https://www.merriam-webster.com/dictionary/manganese>

IPA: /ˈmæŋɡəniːz/

Phonetic Spelling: mang·guh·neez

25. Optical Density

Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>

IPA: /ˈɒptɪkəl ˈdensəti/

Phonetic Spelling: op·tih·kuhl den·suh·tee

26. Putida

Pronunciation link: <https://www.merriam-webster.com/medical/putida>

IPA: /ˈpuːtɪdə/

Phonetic Spelling: pyoo·tih·duh