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Protocol for Incubating Solid Plastics in Water for Microbiome Analysis

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We use this protocol and it's working

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Abstract

This protocol is meant to simulate microbiome development on plastic in the environment in a controlled lab setting for sampling purposes. It was developed from the plastic preproduction pellet incubations done by Ward et al. 2022 for microbiome sampling at various time points. The purpose of the setup is to continuously flow water into the jars containing the plastic material. The window screen on the jars allows for the water to overflow and escape the jar, ensuring the water is constantly refreshing.

Materials

- Solid plastic material
- Glass mason jars
- Fiberglass window screen (can be bought in rolls from the hardware store)
- Air line tubing
- Silicone tubing
- Air line splitter (with enough outflows for experimental procedure)
- Scissors
- Razor blade or Box Cutter
- Peristaltic pump or continuous flow system
- Drainage or water collection system
- Microcentrifuge tubes
- Freezer boxes
- Freezer (-80°C)

Protocol for the purpose of cultivating microbiomes on solid plastic particles

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Note

We developed this protocol for our own plastic experiments that focus on plastic preproduction pellets. The pellets come small enough to be classified as microplastics (<5mm) without altering them in any way. Cutting up post-consumer environmental plastics, consumer plastics, etc. to various sizes as needed for experimentation is acceptable and will work with the protocol. It is recommended to take the average metrics (mass, volume, density, etc.) of the plastic pieces prior to use.

This protocol is also identical across plastic types (the 7 recycling categories used to distinguish between commonly used plastics).

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Preparation

- 3 Determine how many mason jars will be needed for your set up.

[EX: We have 5 types of plastic preproduction pellets available: PET, HDPE, PVC, PP, PS and are running two different light conditions: ambient light and under a grow light. Therefore, we use 10 jars total]

- 3.1 Take the lids off all the jars, keep the lid screw bands and set aside the metal lids.



- 4 Cut a square of window screen off the roll. Cut 10 squares, larger than the metal lid of the jar, and stack them on top of each other. Using one of the metal lids as a template, cut the window screen into circles about 1 inch larger than the lid itself.



- 5 Decide what you're putting into the jars based on how much and how many times you plan to sample. [We put 100 pellets in each jar.

[EX: The PET jar has 100 PET pellets. We plan to sample 4 times taking 5 pellets in 4 replicates at each interval which totals 80 pellets total. We rounded up to 100 pellets in case we decide to run the experiment longer or if something were to go wrong and we needed the extra samples].

- 5.1 Label your jars accordingly. Put your plastic material into each jar.

- 6 Now, take your recently cut window screen circles. Lay them flat over the top of the jars and screw the screw band back onto the jar so that the window screen is pulled tight. Once the screw band is securely on the jar, you should have a little hangover of window screen- that is okay.



- 7 Then, using your razor blade or your box cutter, slice a small hole in the window screen that is only large enough to insert the airline tubing while not allowing any plastic material to escape. The window screen keeps all the material inside the jar while still allowing water to flow out of the jar. Once complete, set the jars aside.



- 8 Now, take all your tubing. The silicone tubing should be larger than the airline tubing. The silicone tubing is either for the peristaltic pump or to attach to a tap or spicket that can provide continuously running water for the duration of the experimental process. To adapt the silicone tubing to the airline tubing, you can use an adapter or a 1000 μL micropipette tip with the narrow end slightly cut to fit the airline tubing over it so that it seals.
- 9 Now, take your airline splitter and label each output accordingly. Cut a desired length of airline tubing and using the first piece as a template, cut as many pieces as desired of the same length. Hook each piece up to an output. Then connect the airline tube that's connected to the silicone tube to the inflow of the airline splitter.
- 9.1 Insert the airline tubing into the jar through the hole to ensure it fits.



- 10 At the end of the preparation process, you should have everything needed to set up the experiment: glass jars containing the plastic material with the window screen as lids & the tubing set up to flow water into each jar.

Experimental Protocol/Set Up

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Note

It is recommended to test your experimental set up in the lab before moving all materials to the experimental location. We found that the peristaltic pumps work better when placed higher than the jars and therefore used a shelf assembly for our experimental set up.



Remember the jars will be overflowing and therefore expelling water. If your experiment cannot be set up over a drain, you can drill a hole in a plastic tub (see image) and run a hose from the tub to a nearby drain.

- 12 Using all the previously assembled parts, set up the experiment. Ensure the air line tubing reaches the bottom of each jar.
- 13 Turn the water system on. If using a peristaltic pump, take note of the pump speed and ensure it remains the same through the duration of the experiment.



- 14 The jars should begin to fill with water and overflow. Your plastic material will sink or float based on density. When a microbial biofilm begins to develop, most if not all material will sink.



Sampling

- 15 Your sampling intervals should have been decided previously.

[EX: We take our samples at Week 1, 2, 4, and 8]
- 16 Turn the water system off. You will be disassembling one jar at a time to take samples.
 - 16.1 Pull the air line tube out of the jar. Pour half the water out of the jar. Move the jar away from the others to take your samples. Unscrew the screw band and remove the window screen.
- 17 Using tweezers, remove the necessary pellets for all your samples, including replicates. Place on paper towel or Kimwipe.
 - 17.1 Transfer proper amount into prelabeled microcentrifuge tubes. Tubes then get transferred to freezer box.
- 18 Close the jar, ensuring the window screen is back in place and tight again. Put the air line tubing back through the hole and into the jar.
- 19 Repeat this process for every type of plastic being sampled. Store samples in freezer (-80°C) until DNA extraction can be performed.

Protocol references

Ward, C. S., Diana, Z., Ke, K. M., Orihuela, B., Schultz, T. P., & Rittschof, D. (2022). Microbiome development of seawater-incubated pre-production plastic pellets reveals distinct and predictive community compositions. *Frontiers in Marine Science*, 8, 807327. <https://doi.org/10.3389/fmars.2021.807327>

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