Long-term sacrificial bottle biomass breakdown experimental setup

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How to set-up a big batch of biomass breakdown experiments to track changes in carbon over time. This particular set-up is relevant ONLY to closed, anaerobic systems - there are other better ways to track respiration in oxic settings - see: respirometer.

This is a brute force bottle method, there are probably other more elegant updates to make as time goes on.

Goals

To track changes in carbon system and terminal electron acceptors/donors over time, while providing a food source (CDR-trial biomass), semi-relevant environmental constraints, and a natural environmental microbial community. Microbes are the drivers of base biogeochemical cycles, especially in anaerobic systems. This procedure aims to 1) quantify the durability of "stored" carbon; 2) track the metabolisms, nutrients, and electron acceptor/donor pairs that encourage or discourage growth; 3) qualify any predictable changes in microbial community dynamics or structures that might occur when natural communities are exposed to a large carbon source and external microbial groups.

-caveats: closed bottle microcosm experiments are idealized, and do not represent open system processes. Some microbes are just plain unhappy in bottles. Flow in the ocean is important to restore nutrient stocks. There is also the inability to completely represent environmental parameters such as high pressure at the bottom of the ocean. However, bottle experiments are useful to ask specific questions, such as rates of degradation, community metabolic capacity, while observing changes in a controlled environment.

Materials

X 500 mL media bottles (these hold ~600 mL full) + butyl rubber septa and aperture caps
35 uM mesh nylon sheeting + heat sealer
Optional: oxygen sensing optodes
Stocks of environmental water, microbes, and biomass
Big carboy or other water holder
N_2:CO_2 mix gas (we use 5%)
UHP Argon
Vacuum evacuating line

Hardware Prep

1) Clean bottles and septa
    a) Rinse and dishwash bottles, acid if needed
    b) Bleach boil new septa, rinse, acid wash all septa
2) Combust bottles - one of the things we want to collect is DOC, so good to fire bottles

3) *Optional, if using oxygen tracking optodes, set these into the bottles at this point*
4) Make nylon pouches
    a) Heat seal a number of 10x10 cm envelopes to hold biomass in each bottle
5) Add biomass to nylon envelope and heat seal closed
    a) "Control" experiments just have an empty nylon envelope
6) Mass biomass added to each envelope
    a) (easiest to mass the nylon envelope empty then full and take the difference - though a tiny bit of nylon is lost to the heat seal, keep track of that)
7) Add massed and sealed biomass envelope to its respective bottle, seal with septa and aperture cap
8) Degas bottles/septa/biomass by vacuum evacuating the interior and flushing with N_2 or Argon
    a) Repeat 2x and let sit with a N_2 or argon atmosphere overnight or several days

Amount of bottles that need to be prepared depends on the desired sampling scheme. We are doing seven rounds of sacrificial bottles, with three bottle replicates at odd sampling times and four bottle replicates at even sampling times. The extra bottle replicate on even sampling days allows S^{35} rads analysis of two bottle replicates while still having two bottle replicates for biomass massing purposes. See table 1 for breakdown.

OR, SB	T0	T1	T2	T3	T4	T5	T6	T7	Total
B2		1	4	3	4	3	4	3	22
B2 irrad		1	4	3	4	3	4	3	22
Sargassum		1	4	3	4	3	4	3	22
Control		1	4	3	4	3	4	3	22
Total water	1								**89**

Table 1: Example breakdown of incubation bottles prepped for LTB2 incubation. OR = Orca Basin experiment; SB = Santa Barbara experiment; B2 = heat treated bagasse; B2 irrad = gamma irradiated heat treated bagasse. 89 individual bottles are needed for each experiment setup.

Water and slurry community prep

The point of this step is to make sure there is a living relevant microbial community in each incubation bottle. General pelagic communities should be in live, unfiltered water, and this community is amended by adding a bit of live sediment slurry to increase the range of possible organisms/metabolisms in the final incubation system.

There needs to be~ 600 mL of water for each bottle prepared. For an 89 bottle incubation you need 53.4 L of water, total, but I would recommend making about 10 L extra

1) Add 90% total water volume as 0.2 um filtered environmental water
    a) If needed, this can be supplemented with artificial seawater (ASW) matching the nutrient and salinity needs of your system
2) Sparge incubation water mix with a known mix of N_2:CO_2 - we used 5% CO_2 to set the carbon system to a baseline and purge oxygen from the system
3) To the sparged anoxic base water, add in 10% total volume as live, unfiltered water through a 2.7 um filter
    a) These should be kept as anoxic as possible to start with, assuming you want to represent an anaerobic system
    b) These are filtered to remove any large particulates that might drive randomness between the bottles, do at discretion
4) Continue sparging the mixed incubation waters and prepare to load bottles
5) Take a DNA sample of the mixed slurry or of the original live water for comparative purposes

For the sediment slurry, take a sealed media bottle of sparged incubation water and add in a small amount of relevant sediment (similar to the surface seafloor the biomass might be exposed to if deposited). Take note of the mass of the sediment to know how much is added. Also take a solid sample of the sediments, a sample for DNA analysis, and a sample of the final slurry for DNA analysis.

Incubation setup

1) Set up an argon flow line to a large tub - argon will displace a lot of the air, giving a reasonably less oxygen-rich area to work in
2) Position the sparging jug of mixed incubation water over or nearby the argon-workspace
    a) If dispensing water out from the carboy, have over workspace…if using a peri-pump, then it doesn't matter
3) Have the sediment slurry on hand
4) Mass a degassed bottle (including cap and septa)
5) Transfer bottle to argon filled workspace
6) While working in the workspace:
    a) Pop the bottle cap and fill directly with UHP argon from the live line
    b) Inject 2 mL of mixed sediment slurry into the bottom of the bottle under argon
    c) Fill the bottle to the very tip top with sparged incubation water
    d) Recap the bottle
7) Wipe down any extra liquid on the sides of the bottle and re-mass
    a) The difference in empty and full weight gives you the mass of water added
8) For bottles planned for CH4 headspace sampling - leave ~20 mL of headspace or up to the entire neck of the bottle for future gas extraction
9) Note the time of water addition to make sure there is no systematic change in bottles properties over time as the water stocks get lower
10) Make sure to take an initial water sample as a baseline for system parameters
    a) In the initial bottle, take an extra bottle of water to use to calculate specific density in order to convert mass to mL added

11) Store all bottles at an appropriate temperature (i.e. 4C cold room)
12) Take optode measurements if applicable (these don't like temp. changes)