Potential Rate Measurements for Uptake of Bicarbonate and Acetate by Microbes in Crustal Fluids

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Abstract

These were developed to measure potential microbial activity for uptake of acetate and bicarbonate by microbes in venting hydrothermal fluids. The protocol was designed for Axial Seamount diffusely venting fluids, but can be applied to other fluid systems (e.g. cold, oxic fluids, seawater, etc). Concentrations of label will depend on environment of interest.

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Before start

This protocol assumes you have already collected the sample (seawater, vent fluid, etc) of interest.

Protocol

Step 1.
Prepare sterile, anoxic stocks of bicarbonate and labeled acetate. For these experiments, we prepared a 600mM bicarbonate solution that was 10:1 unlabeled:labeled. We added 175 uL of this mixed bicarbonate solution to acid-washed, sterile balch tubes in an anaerobic chamber, then sealed with rubber stoppers and aluminum crimps. Final targeted concentration after sample addition was 5 mM DIC. For acetate, we prepared a 10mM acetate solution that was 10:1 unlabeled:labeled. We added 53 uL of this mixed acetate solution to acid-washed, sterile balch tubes in an anaerobic chamber, then sealed with rubber stoppers and aluminum crimps. Final targeted concentration after sampled addition was 25 uM acetate.

Step 2.
At sea, use acid-washed tubing and a peristaltic pump (Masterflex) with sterile needles to distribute 20 mL of fluid into each pre-prepared balch tube. Fill the 3 sterile controls for each isotope (6 total) first using a disposable syringe filter, then discard and fill the rest of the tubes. Do all the DIC first, then switch tubing and do the acetate. Use an exit needle. Can use same exit needle in same time series.

Step 3.
Add 2 bar hydrogen by setting regulator to 2 bar and sticking the needle in for 2 seconds to see dip in regulator then back up to 2 bar, giving an overlying atmosphere of 50% N2/50% H2. Incubate at appropriate temperature. For RT/20, place in main lab and put a thermometer nearby if possible.

Record the temperature. We incubated at room temperature, 30, 55, and 80 degree C.

Step 4.
When it is time to sacrifice a time point, first sample the head space for methane for the DIC samples. Need to allow it to cool to room temp before sampling head space. Can run under cool water to do this. For 80ºC, this can take up to 30 minutes, so plan accordingly. First shake tube for 1 minute vigorously, then use a 10 mL syringe with stopcock to equilibrate headspace. Close stopcock and remove syringe, and record volume of gas in syringe. Inject into GC and record concentration on sample sheet.

Step 5.
Use a 30 cc sterile syringe with a needle to remove all of the sample from the balch tube. Need to use an exit needle. Get rid of any air bubbles in the syringe.

Step 6.
Attach an acid-washed 25 mm syringe filter holders with a pre-combusted GF/F filter onto the syringe and then gently filter. Do this slowly and watch for leaks. Note if any leaks occurred on the sample sheet.

Step 7.
Rinse filters with 30 mL of 0.5M NaCl solution (fresh syringe). Follow this with an air-filled 30 cc syringe (same syringe) to get rid of any residual liquid.

Step 8.
Take the tape off of the tube and place onto a 30 mm petri dish and place the filter in there.

Step 9.
Dry filter overnight at 55 °C. Wrap in electrical tape and store dry and at room temperature.10. Back on land, place in dessicator until analyses of enrichment of biomass in stable isotope facility.

Warnings

Hydrogen is used to over-pressurize for some of these experiments. Use caution.