**KOH-Trap Microbial Respiration Method**

Materials

* Mason jars with lids – one per sample
* Scintillation vials – 2 per sample (acid washed)
* Qorpak 60 mL vials with lids – one per sample (acid washed)
* EC reader
* CO2 cartridge
* Syringe
* Needles
* KOH
* Volumetric flask with stopper (acid washed)
* Glass bottle large enough to hold all your KOH solution with lid (acid washed)
* 15 mL pipettor that screws onto glass bottle (micropipettor with tips if unavailable)
* Glass bottle large enough to hold all your CO2 free water
* 5 mL pipettor that screws onto glass bottle (micropipettor with tips if unavailable)
* Plastic DI water bottle
* 2 beakers for water waste
* 1 beaker for KOH waste
* Hot plate
* Large Erlenmeyer flask for boiling water
* Boiling stones
* Oven mitt gloves
* Scupula
* 2 glass weigh dishes (acid washed)
* Funnel (acid washed)
* Paper towels
* Tape
* Sharpie
* 4mm sieve
* Scale
* Electric tea kettle

Methods

Preparation

1. Sieve your field moist soil to 4 mm, this is a compromise between unsieved soil which is not homogeneous and soil sieved to 2 mm which is highly disturbed. Make sure to wash and dry sieve between samples.
2. Wash all your mason jars, scintillation vials, and Qorpak vials. Acid wash the scintillation and Qorpak vials.
   1. Calculate the number of sample jars you will need by adding: the number of soil samples you are testing + one blank per 12 samples + 4-5 jars with septum lids for a standard curve.
3. Weigh 15.00 g ± 0.03 of field moist soil into Qorpak vials. Keep in cooler when not weighing and store all soil in walk in cooler when finished.
4. Keep soil in cooler for 2 weeks ± 1 day from sample date before starting respiration measurements. This should help the microbes re-acclimate after the sampling and sieving disturbance.

Mason Jar Set Up

1. Make enough CO2 free water for all Mason jars and for the KOH solution the day before. Store in a clean, air tight glass bottle.
   1. Boil DI water for 10 minutes at a rolling boil. Store in air-tight container. Boil water in two batches to help speed up boiling. Also, start heating some of the water in an electric tea kettle, then add the water to the Erlenmayer flask to boil for 10 minutes. Use boiling stones to help with boiling.
2. Make your 0.09M KOH solution the day of setup using the CO2 free water. Dissolve the required amount of KOH pellets with some water in volumetric flask first, and then fill to the line. Mix thoroughly by inverting a few times. Once complete, transfer immediately into dry glass bottle with lid.
   1. For 1 L of KOH use 5.05 g of KOH
   2. For 2 L of KOH use 10.10 g of KOH
3. Put KOH solution immediately into incubation room to let the solution come to temperature.
4. Transfer all necessary equipment into incubation room to allow the temperatures to become the same (you can also do this the night before if no one needs the equipment).
5. Wait at least 1.5 hours before sampling.
6. Bring your soil from the walk-in cooler up to the incubation room in a portable cooler.
7. Take out one tray (12 mason jars) at a time. Take a Qorpak vial of soil out of the cooler, uncap, place inside labeled mason jar, add 5 mL of CO2 free water to the bottom of the mason jar, fill a scintillation vial with 15 mL of KOH, and place scintillation vial inside mason jar. Immediately cap and place back on the tray.
8. Complete this process for every sample. Do at least one blank per 12 samples as well as the standard curve jars. For a blank or a standard curve jar do the same process, but place an empty scintillation vial inside the mason jar.
9. Periodically measure the EC of the KOH coming directly out of the glass bottle, about once per tray. To do this dispense 15 mL of KOH into clean scintillation vial, take EC probe out of it’s holding jar, rinse with DI water, dry off with paper towel being careful not to touch the probe, and place inside scintillation vial. Stir up the KOH solution slightly and wait for EC meter to read “ready”. Try to take every EC reading at about the same temperature: 30**°**C ± 3°C. Although the EC reader corrects for temperature differences, this helps to improve accuracy. To warm up the KOH hold the scintillation vial in your hand. These base EC measurements help you know that the EC is starting out about the same for every sample.
10. Once all the jars are full wash and acid wash all the used glassware. Let acid washed dishes soak for at least 2 hours before removing, rinsing, and drying so they are ready for your next round of measurements.

Reading EC

1. Make CO2 free water the day before and place DI water in incubation room to come to temperature.
2. Make KOH in the morning and put directly into incubation room along with all other necessary supplies.
3. At least 1.5 hours after putting everything into incubation room begin readings.
4. Take down one tray at a time. Open a jar, take an EC reading of the KOH inside the jar. Pour out the old CO2 free water and the old KOH (*after* taking the reading). Replace with a new 5 mL of CO2 free water and new KOH in a clean scintillation vial. Recap the mason jar and put back.
5. Do this for all the jars. Periodically take base EC measurements as before.
6. Wash and acid wash all glassware when finished.

Standard Curve

1. To create the standard curve, add known volumes of CO2 to 5-6 of your mason jars without soil (the ones with septum lids). First remove the amount of air you plan to add to each jar from that jar. For example, if you plan to add 5 mL of CO2 to standard curve jar #3, first insert the needle and remove 5 mL of air by pulling up on the stopper until it is at the 5 mL mark. Remove the needle and expel the air. Do this to all your standard curve jars.
2. Fill the piping of your CO2 canister with 100% CO2. Release all of this air by inserting a needle and syringe with the plunger removed into the end of the piping. This will purge your piping.
3. Refill the piping with more CO2 and this time purge your syringe by putting the stopper back on and inserting the needle into the piping only until the syringe is full of air, but before the plunger has popped off. The syringe will fill automatically as the air in the piping is pressurized.
4. Expel all the air in the syringe.
5. Again, fill the syringe with air, leaving the plug in, but this time eject air only until the 10 mL mark. Now your syringe has 10 mL of a known concentration of CO2. Inject known quantities of this air into each mason jar, covering what you believe the range of respiration will be. For example: 8 mL, 5 mL, 3 mL, 2 mL, and 1 mL.
6. Next time you take an EC measurement, also read the EC of your standard curve jars. The EC should have changed linearly with the amount of CO2 added. Use this curve to calculate how much CO2 was emitted by each jar of soil.

Take respiration measurements at appropriate intervals for as long as you deem necessary. Do not let the EC of your KOH drop below 7, this then runs the risk of becoming an anoxic environment and could change the chemistry and ecology of your jar.

Calculations

2KOH + CO2 🡪 K2CO3 + H2O

EC meter settings: calibration 0.9295

S-1 2.0 temp correction coefficient

S-3 25