Protocol for Growing *Synechococcus* strain CB0101 with Variable Iron Concentrations

Make media

1. Prepare 1 liter of SN media (without iron) using this SN Medium recipe: https://www-cyanosite.bio.purdue.edu/media/table/SN.html
2. Aliquot SN media for the five experimental conditions: 0 mM, 0.00023 mM, 0.0023 mM, 0.023 mM, 0.23 mM Ferric Ammonium Citrate.
3. Dissolve 6 grams of Ferric Ammonium Citrate in 100 mL of dH20. This is the 1000x stock for the 0.23 mM Ferric Ammonium Citrate SN media. (For example, if making 100mL of 0.23mM FAC SN media, add 100uL of this stock to 100mL of SN media.)
4. Serial dilute the Ferric Ammonium Citrate solution at 1:10 dilutions to get the 1000x stock for the 0.023 mM, 0.0023mM, 0.00023mM FAC SN media.
5. Add the 1000x stocks to the appropriate SN media aliquots. (For example, if making 100mL of 0.23mM FAC SN media, add 100uL of 1000x stock to 100mL of SN media.)

Culture cyanobacteria

6. Rinse cyanobacteria to remove media that contained iron
    a. Centrifuge,
    b. Pour off supernatant,
    c. Add water
    d. Resuspend by tapping bottom,
    e. Centrifuge again,
    f. Pour off supernatant,
    g. Add fresh media at same volume
7. Start with a culture of cyanobacteria with OD at 0.2
8. Label five sterile 50 ml conical tubes with the experimental conditions: 0 mM, 0.00023 mM, 0.0023 mM, 0.023 mM, 0.23 mM Ferric Ammonium Citrate.
9. For each experimental condition, add 20 ml of the appropriate Ferric Ammonium Citrate SN media to the corresponding conical tubes.
10. Add 2 ml of cyanobacteria culture to conical tubes
11. Cover conical tubes
12. Store tubes into the under ambient light (with day and night cycles) and at room temperature.

Measure Secchi depth

13. Set up a timer. Measure about every 12 hours for 10 days. Can be more or less frequent.
14. Record the time at which the measurement was taken
15. Record the temperature when you take the measurement
16. Record light intensity with light meter if available
17. Mix the sample by inverting the tube gently before taking measurements.
18. Measure and record the first sample for cyanobacteria growth using secchi sticks. Lower secchi stick into culture. Record the depth at which you can no longer see the target on the secchi stick (https://www.youtube.com/watch?v=du1PA0pzdnQ&feature=youtu.be)
19. Sterilize secchi stick with ethanol between taking measurements for each iron level