Dissolved Organic Carbon Protocol

•Ellen Esch, May 2019, adapted from Bartlett RJ, Ross DS (1988) Colorimetric determination of oxidizable carbon in acid soil solutions. Soil Sci. Soc. Am. J 52:1191-1192. And JRBP protocols.

•Note: this assay is inverse, the pink Mn(III) reacts with organic C and becomes clear. So the standard curve has a negative slope. Therefore the "blank" is the most pink, and so you must set the zero to be a standard, not a blank, or it will subtract a high value from all samples, leaving negative values. Also, samples may need to be diluted or else all Mn(III) will be consumed and samples will be completely clear, indistinguishable from the highest standard. For example, media with 20 g/L glucose (0.111 M) would need to be diluted by about 200 to be measured by this method.

Make solutions:

H_2SO_4 concentrated

No need to do anything

0.5M H_2SO_4 concentrated

- Since there are 2 free H ions in H_2SO_4 for every mol H_2SO_4, a 1M solution = 2N solution.
- Add 5mL 10N H_2SO_4 into a 50ml volumetric with nanopure.
    - 10N=5M; 5M*(v2)=(0.5M)*(50ml)

0.1M Sodium pyrophosphate; $Na_4P_2O_7$

- Dissolve 0.4461g $Na_4P_2O_7 \bullet 10H_2O$ into 10ml nanopure
    - (0.1 mol / L) * (10 ml) * (446.068 g / mol) * (1 L / 1000 ml)

0.1M Potassium manganate; K_2MnO_4

- Dissolve 0.1971g into 10mL nanopure
    - (0.1 mol /1L) * (197.134 g K_2MnO_4 / mol) * (10 ml) * (1 L / 1000 ml)
    - This is rather tricky as it kind of dissolves metal. Store the mixed reagent and rest of the chemical under N gas. Work rather quickly.

0.1M Manganese(II) sulfate; MnSO4

- Dissolve 0.1690g $MnSO_4 \bullet H_20$ into 10ml nanopure
    - (169.01g/mol)*(0.1mol/L)*(10ml)*(1L/1000ml)

Mn(III) Reagent: (make fresh weekly) → will make enough for ~10 plates

5.54 mL H_20
3.0 mL of 0.1M $Na_4P_2O_7$
0.46 mL of 0.5M H_2SO_4
0.2 mL of 0.1M K_2MnO_4

Mix, then carefully add:

0.8 mL 0.1M MnSO4

Mix thoroughly to avoid precipitation - this is an oxidation-reduction reaction, producing Mn(III) from Mn(II) and Mn(IV).

Make standard curve:

1. Standards: 0-700 µM Glucose (700 M glucose is equivalent to 50.4 mg C per L (ppm). Sometimes, this high standard is just out of the linear range).
    1. Make 1000uM stock solution
        i. Dissolve 0.09005 glucose (C6H12O6) into 500ml nanopure
            1. (180.1g/mol)*(1000umol/L)*(1mol/1x106umol)*(500ml)*(1L/1000ml)
        ii. Dilute according to the following table
            1. The mgC/L (ppm) is calculated like this: (700uM glucose)*(72gC/1mol glucose) *(1mol/1x106umol)*(1000mg/1g)=50.4mgC

Desired uM glucose	mL 700uM stock solution	mL nanopure	mgC/L (ppm)
0	NONE!	5 (all nano!)	0
100	1	10	7.2
200	1	5	14.4
300	1	3.33	21.6
400	1	2.5	28.8
500	1	2.0	36.0
600	1	1.67	43.2
700	1	1.43	50.4

Run analysis:

NOTE: run samples in triplicate (or quadruplicate!!), and add the standard curve to one plate. Add the samples to the wells first, and then use the multichannel pipette to add the reagent. Make sure to label plates, and create a diagram for your sample lay-out.

Add the following to each well:

1. 80 µL sample or standard
2. 40 µL Mn(III) reagent
3. 40 µL H_2SO_4 (concentrated)

Tap corner of plate to mix well, cover with foil and incubate for 8 hours or overnight (might need 18 hrs). Read plate at 490 nm.