

### Phenol Sulfuric Acid Assay to Quantify Carbohydrates (EPSs)

#### Sources:

- o Dubois *et al.*, 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.*; Vol. 28, pgs 350-356.
- o Neilsen, S., 2009. Phenol-Sulfuric Acid Method for Total Carbohydrates. *Food Analysis Laboratory Manual*. Pgs 47-58

#### Materials:

- Phenol, 5% by weight (add 95mL DI water to 5g reagent grade phenol, crystals)
- Sulfuric acid, reagent grade 95.5% (2.5 L, white storage, >98% purity grade Fischer Scientific S25894, CAS No. 7664-93-9)
- Glucose 1mg/mL stock solution
- 5 mL, 100 $\mu$ L, 1mL pipette
- Gloves
- Scale
- Spectrophotometer and semi-micro (1.5-3mL) glass cuvettes or UV-cuvettes (NOT polystyrene)
- Hood

#### Methods:

##### Prepare glucose standard curve:

- o Standards: glucose 100mg/L stock solution (DI water and EDTA)
- o Prepare the following standard solutions (glucose dissolved in DI water and EDTA)

|  |     |     |     |     |     |     |     |     |
|--|-----|-----|-----|-----|-----|-----|-----|-----|
| $\mu$ g glucose/200 $\mu$ L                      | 0   | 2   | 2   | 6   | 8   | 10  | 12  | 14  |
| $\mu$ L glucose stock solution                   | 0   | 20  | 40  | 60  | 80  | 100 | 120 | 140 |
| $\mu$ L deionized, distilled water or 100mM EDTA | 200 | 180 | 160 | 140 | 120 | 100 | 80  | 60  |

- o Perform steps 2-7 below
- o Determine the equation of the line for the standard curve

##### Sample analysis

1. Take 200 $\mu$ L of sugar or EPS solution (containing 20-140 $\mu$ g of sugar) in a 1.5 mL tube
2. Add 200 $\mu$ L 5% phenol
3. Rapidly add 1mL of concentrated sulfuric acid
4. Invert 3 times or briefly vortex to mix
5. Let stand for at least 60 minutes before transferring to cuvette
6. Take absorbance readings at 490nm, 1000nm (subtract noise)
7. Compare to glucose standard curve, 490 for hexoses, 480 for pentoses and uronic acids
8. Include a check standard
9. Calculate the concentration of  $\mu$ g glucose in terms of g per dried soil sample

#### NOTES:

- Larger reactions will result in precipitate particulates formed from EDTA/sulfuric acid mixture and will mess up absorbance readings. If precipitate does form, subtracting noise at 1000nm is critical.
- Properly and dispose of phenol and sulfuric acid waste.