**Carbohydrate extraction protocol Degerhamn 2015-03-12.**

For the measurement of intracellular carbohydrate concentrations, based on DuBois (1956).

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Chemicals;

1 M H2SO4

5% Phenol (wt/v)

72% H2SO4

Materials;

Freeze dried algae biomass of known quantity.

Autoclave safe tubes

Centrifuge able glass vials

Clean glass tubes

Water bath

Spectrophotometer

**When working with phenol and strong acids, work in a fume hood or bench. Inform other people in the lab about what chemicals you are using. Wear acid resistant gloves, lab coat and safety goggles**. **Phenol is poisonous, corrosive, and flammable. Read up on safety instructions before proceeding from this point.**

1. Dissolve freeze dried biomass in 5ml 1 M H2SO4.
2. Incubate samples in autoclave 30 min at 120 °C.
3. Let the samples cool down in room temperature.
4. Transfer the samples to centrifugeable glass tubes.
5. Centrifuge the samples for 10 min at 4000 rpm.
6. Prepare a standard curve 0.2 – 1.0 mg/ml using D(+) glucose.
7. Transfer 0.1 ml of the supernatant and the standard curve + blank to clean glass tubes.
8. Add 1 ml Phenol to all samples and standards
9. Add 3ml 72% H2SO4 to all samples and standards.
10. Vortex gently
11. Seal the glass tubes with aluminium foil and parafilm paper
12. Incubate samples in water bath 30 min in 90 °C inside of a fume hood.
13. When samples are at room temperature, vortex gently and measure absorbance in spectrophotometer at 490 nm.
14. For quantification, compare your samples with the standard curve.

References

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

Miranda, J.R., Passarinho, L., Gouveia, L. 2011. Pre-treatment optimization of *Scenedesmus obliquus* microalgae for bioethanol production. Bioresource Technology 104 342–348.