

## TOTAL CARBOHYDRATES PROTOCOL

### 1. INTRODUCTION

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The total saccharides moiety in a sample can be estimated by the **anthron method** which is a simple colorimetric method with relative insensitivity to interferences from the other cellular components. The first step in total carbohydrates measurement is to hydrolyze the polysaccharides and to dehydrate the monomers (digestion with sulfuric acid addition and heat treatment). The 5-carbon (pentoses) and 6-carbon (hexoses) sugars are converted to furfural and hydroxymethylfurfural, respectively. When anthrone (an aromatic compound), it reacts with these digestion products to give colored compound. The amount of total carbohydrates in the sample is then estimated via reading the absorbance of the resulting solution against a glucose standard curve. Please refer to Section 22.3 at "*Methods for General and Molecular Bacteriology*" by Gerhardt *et al.* (1994) for further information and useful tips. We have this book at the Raskin Lab (Loc: 4217).

### 2. MATERIALS and CHEMICALS

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- COD vials : or any other type of vial durable to heating at 100oC.
- Heating-block : or a reliable water-bath that goes up to 100oC (Loc.: 4217)
- Clean Glassware
- 10 mL pipets : glass or acid-durable (disposable) pipettes
- Ice-buckets : to keep everything chilled all throughout the experiment
- Semi-micro cuvettes : Standard type plastic disposable cuvettes with 10 mm path length, sample capacity of 1.5-3 mL (FisherBrand 14-385-942): Using these cuvettes is optional for total carbohydrates measurements. If you prefer to measure at the HACH spec., you can directly use the COD vials and record the readings manually.
  
- Spectrophotometer
- Anthron reagent : Anthron (C<sub>14</sub>H<sub>10</sub>O), [9(10-H)-Anthracenone] Reagent, Spectrum, AN135 (Loc: 4217)
- Glucose : D-(+)-Glucose anhydrous, Sigma G-7528 (Loc: 4217)
- Reagent-grade H<sub>2</sub>SO<sub>4</sub>

### 3. PREPARING the SOLUTIONS

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- Prepare the **75% H<sub>2</sub>SO<sub>4</sub> solution** (preferably **1 day before** the experiment, or at least 4 hours before starting). **500 mL** is good for approx 70 measurements (6 standard dilutions and 17 samples in triplicates).
- Put 100 mL of ddl water in a 500 mL volumetric flask
- Add a magnetic stirring bar, **place the flask in an ice-bath**, and put this on a stirring plate
- Wearing goggles, preferably acid-durable thick gloves, lab coat, and working under the fume hood (COD station at 4130), measure 390 mL of 95-97% concentrated reagent-grade H<sub>2</sub>SO<sub>4</sub>.
- Turn on the stirrer and add the acid **slowly and carefully** to the volumetric flask
- Let it **cool down to room temp.** under the fume hood, add more ice to the ice-bath to speed up
- **Adjust the volume** to 500 mL with ddl water
  
- Prepare the **anthron solution freshly**, at the day of measurement:
- Weigh **0.5 g of anthron** and add to a small beaker containing some **absolute EtOH** (i.e., 5 mL)

- Use some more EtOH (total of 5 mL) to rinse the anthron particle on the weighing boat into the beaker (you can use a 1000  $\mu$ L pipetman at this point)
- Put a mini stirring bar and stir to dissolve
- Add the anthron, dissolved in 10 mL EtOH, to a 250 mL volumetric flask containing the 75%  $H_2SO_4$  solution, stir to mix well and fill up to 250 mL with the 75%  $H_2SO_4$  solution

#### 4. GETTING READY

- Take your samples out of -20oC freezer to thaw
- Place the freshly prepared anthron reagent and the 75%  $H_2SO_4$  solution on ice to chill
- Switch on the heating block and set the temperature to 100oC (actually for the heating-block at 4217, you need to set the temp. to 110-115oC to get a fairly stable 100oC reading. Check the temperature from the digital display and also with a thermometer)



- Prepare the glucose solution and dilutions for the standard curve (prepare freshly):
- Weigh 0.05 g of glucose and add to a 500 mL volumetric flask containing ddl water
- Stir well to dissolve and adjust the volume to 500 mL with ddl water: Final concentration of the stock is 100 mg glucose/L.
- Prepare dilutions\* in 15 mL Croning tubes, following the recipe at Table 4.1.

**Table 4.1** Dilutions from the glucose stock solution (100 mg/L) for the standard curve

V ddl water	V of stock glucose solution	Final conc.
10 mL	0 mL	0 mg/L
8 mL	2 mL	20 mg/L
6 mL	4 mL	40 mg/L
4 mL	6 mL	60 mg/L
2 mL	8 mL	80 mg/L
0 mL	10 mL	100 mg/L

**\*POINT OPEN FOR DISCUSSION:** I've been preparing standard curves at the above range for my measurements. I do not need to dilute the BLANK and SUPERNATANT samples from the EPS extraction experiments, but I usually need to dilute my DOWEX-extracts (1:2 to 1:5 dilutions). Depending on the general character of the sample and the expected total carbs values for the supernatant and the DOWEX-extract, a higher-range calibration curve can be prepared to avoid sample dilution.

- Dilute your samples with ddl water if necessary\*.

## 5. PROCEDURE

- **Wear gloves, goggles, lab coat, and work very carefully**
- Be careful while preparing and dispensing the 75% H<sub>2</sub>SO<sub>4</sub> solution and the anthron solution: Work on a bench covered with paper towels. You can easily recognize an acid spill on a paper towel since strong acid burns the paper, turning the color to black. But during the experiment, **DO NOT use paper towels to wipe** the plastic pipettes (especially the tips) or the flasks containing the solutions (even if there are drops or spills on/around the containers). Acid containing reagents hydrolyze the cellulose, present in paper towels, to sugars, resulting in a high background and interfering significantly with the measurements. Though, clean the work area thoroughly after finishing the experiment.

- Work in triplicates and on ice
- Vortex your **samples/dilutions (or standards)** well to mix and transfer **1 mL** to a COD tube. You can use a 1000 uL pipetman at this step
- After transferring all of your samples/dilutions/standards to the COD tubes, place the tube rack on ice to chill



- **Add 2 mL** of already chilled **75% H<sub>2</sub>SO<sub>4</sub>** solution to the tubes. You can use a 10 mL disposable plastic pipette to dispense 2 mL of the acid solution to the samples.
- **Cap** and **vortex** briefly to mix (decrease the speed of the vortex to approx. 3-4)



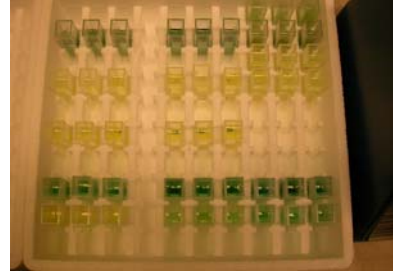
- **Add 4 mL** of already chilled **anthron solution** to the tubes
- **Cap** and **vortex** to mix



- Place the COD tubes on the heating-block and **boil at 100°C for 15 min**
- Remove the tubes from the heating-block carefully and cool down to room temp.

- While waiting, turn on the UV-VIS spec to warm up and stabilize (Loc.:4215)
- Before using the UV-VIS spec, please get the general training from the Lab. Manager.

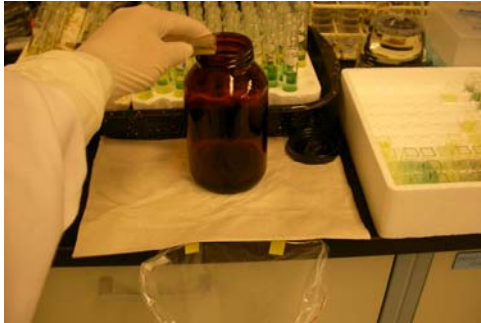
- Transfer sufficient amount of acid-heat digested samples to semi-micro disposable cuvettes
- Working at the **Quantitative** mode and **@578 nm**, auto-zero the spec. with 2 semi-micro cuvettes filled with ddI water. Then record the absorbance values for the standards (standards mode) and the samples (unknown mode).



- Prepare the calibration curve from the abs. readings of the standards and calculate the total carbohydrates of the samples in mg glucose/L from this curve.

## 6. CLEAN-UP and WASTE DISPOSAL

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- When done with the spec. readings, collect all the samples (both in the COD vials and in the semi-micro cuvettes) and the extra anthron solution in a acid-durable container; label
  - Collect the emptied semi-micro disposable cuvettes in a plastic bag; label
  - Collect all the waste generated to a designated area to be picked-up.
- Fill out a CWM-TRK-01 form (Request for Pickup of Chemical Waste) for the liquid and solid wastes to be picked up and disposed.
  - Rinse all the glassware and then clean with soapy water, rinse well again
  - Clean the work space well

## 7. REFERENCES

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**Gerhardt, P.; Murray, R.G.E.; Wood, W.A.; Krieg, N.R. (1994)** “*Methods for General and Molecular Bacteriology*”, ASM, Washington DC, ISBN 1-55581-048-9, p 518.

**Frolund, B.; Palmgren, R.; Keiding, K.; Nielsen, P.H. (1996).** “Extraction of extracellular polymers from activated sludge using a cation exchange resin”, *Water Res* **30**(8), pp. 1749-1758.