Name: Date: Class/Hour:

BIOMASS CARBOHYDRATE COMPOSITION

INTRODUCTION

The chemical compositions of biomass feedstocks largely determine their usefulness for energy production by fermentation. Fermentation refers to a wide range of anaerobic metabolic pathways used by microorganisms. Bacteria and yeast produce various byproducts from fermentation pathways such as organic acids, alcohols, and gases (CO_2, H_2, H_2S, etc.). Biomass feedstocks can provide the necessary nutrients for fermentation and production of the desired byproducts, i.e. ethanol. Many fermenting microorganisms utilize simple sugars for energy. It is therefore useful to characterize types of biomass by their carbohydrate compositions.

Carbohydrates appear in plant cells for a variety of functions such as structural support in cell walls, energy storage in starch or glycogen, or cellular recognition with glycoproteins and glycolipids in cell membranes. Depending on the biomass, different types of carbohydrates will appear in different amounts.

Cellulose is the most common structural carbohydrate, constituting the cell walls of most plants. It is a glucan bonded by ß1,4 glycosidic linkages and can be tens of thousands of glucose units long. Extensive hydrogen bonding between chains makes cellulose extremely stable and water insoluble. Few organisms produce enzymes that can break it down.

Hemicelluloses are another common structural carbohydrate present in plant cell walls. Hemicelluloses can be xyloglucans, xylans, mannans, and glucomannans, and have shorter chain lengths than cellulose. Depending on the type of monomer and the length of the chain, hemicelluloses can be either water soluble or water insoluble.

Plants also use carbohydrates for energy transport and storage. Starch is a common storage glucan bonded by ß1,4 linkages and is water soluble.

Biomass streams with large water soluble carbohydrate contents are convenient in that they require little processing to extract and process their sugars. Most biomass, however, have the majority of their carbohydrates in the form of structural carbohydrates, mainly cellulose. These streams require more intensive processing to convert their carbohydrates to fermentable sugars. In this lab investigation, you will measure the carbohydrate contents of biomass types as percentages of total mass. Additionally, you will determine the ratio of water soluble to water insoluble carbohydrates.

PROCEDURE

Before you begin, answer the Pre-Investigation Questions on your Discussion sheet.

PART 1: ACID HYDROLYSIS

Materials Needed:

- Biomass Samples (Dried at 65°C overnight and milled with Wiley Mill through #40 sieve, 200mg per sample)
- 72% Sulfuric Acid, Reagent Grade (3mL per sample)
- Analytical Balance (± 0.0001g)
- Benchtop Autoclave
- 3.0mL Pipet
- 125mL Erlenmeyer Flasks (1 per sample)
- 400mL Beaker (1 per sample)
- 100mL Graduated Cylinder
- 500mL Volumetric Flasks
- Filter Flask
- Buchner Funnel
- Filter Paper (sized to fit Buchner funnel)
- Vacuum Nozzles Or Pumps
- Vacuum Hosing
- Aluminum Foil
- Wash Bottles
- Spatulas
- Parafilm

1. Weigh out 200mg of dried and milled biomass and record the mass to the nearest 0.0001g. Transfer into a 125mL Erlenmeyer flask.

2. Carefully pipet 3.0mL of 72% sulfuric acid into the flask. Observe the color change when the acid contacts the biomass. Tip and swirl the flask to completely saturate the biomass with acid.

3. Fill a 400mL beaker to about 200mL with hot tap water. Cover the sample flask with aluminum foil and place upright in the beaker. Allow it to rest for about 30 minutes.

4. Using a graduated cylinder, add 84mL deionized water to the flask and re-cover with aluminum foil.

5. Set the autoclave to 121°C for 15 minutes. The entire class should autoclave their sample flasks in one large batch.

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PROCEDURE (cont.)

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6. Note: The 15 minutes does not include ramp or cool down time. The entire process should take about an hour. Expect about 15 minutes for ramping and 20 minutes for cool down.

Do not open the autoclave until the pressure has returned to ambient levels; rapid depressurization of hot liquid samples will lead to boiling over!

7. Remove samples from the autoclave and allow them to cool enough to be handled with bare hands.

8. Obtain a filter flask, Buchner funnel with filter paper, and vacuum hose. Place the Buchner funnel in the filter flask, and connect the flask to a vacuum nozzle with the rubber tubing. Place the filter paper in the funnel, wet with deionized water, and apply light suction.

9. Swirl the contents of your sample flask to suspend the biomass material. Empty your sample flask into the Buchner funnel under suction. Rinse your flask with deionized water and pour into the funnel to ensure you completely transfer your sample. Apply suction until all the liquid has dripped into the filter flask.

10. Quantitatively transfer the filtrate from the filter flask to a 500mL volumetric flask, rinsing with deionized water to ensure complete transfer. Dilute the volumetric flask to the mark with deionized water, and mix well by capping and turning the flask over several times.

11. Label a clean dry test tube "Acid Hyd." and save about 10mL of the liquid. Parafilm and set aside for sugars analysis.

PROCEDURE

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Before you begin, answer the Pre-Investigation Questions on your Discussion sheet.

PART 2: HOT WATER EXTRACTION

Materials Needed:

- Biomass Samples (dried at 65°C overnight and milled with Wiley Mill) Through #40 Sieve (150mg per sample)
- Crushed Ice
- Analytical Balance (± 0.0001g)
- Hot Plates
- Centrifuge
- Vortex Mixer
- 15mm x 125mm Test Tubes (2 per sample)
- 13mm x 100mm Test Tubes (4 per sample)
- 5.0mL Pipet
- Thermometer
- 400 Ml Beaker
- 25 Ml Volumetric Flasks (2 per sample)
- Thin Neck Funnel
- Whatman #1 Filter Paper
- Stir Rod
- Spatulas
- Ice Bucket
- Markers
- Rubber Bands

1. Prepare a hot water bath by filling a 400mL beaker with about 200mL hot tap water and heating on a hot plate to a vigorous boil.

2. Weigh 75mg of dried and milled plant material into two separate 15mm diameter test tubes labeled 1 and 2. Record the mass of plant material in each to the nearest 0.0001g.

3. Pipet 5.0mL deionized water into both test tubes.

4. When the hot water bath is boiling, place the tubes into the bath for 5 minutes.

5. Centrifuge the sample test tubes at 2500rpm for 3 minutes. Be sure to counterbalance samples in the centrifuge before spinning.

PROCEDURE (cont.)

6. Carefully decant the supernatant from each test tube into separate 25mL volumetric flasks labeled 1 and 2.

7. Pipet another 5.0mL deionized water into the sample tubes and briefly vortex or agitate to re-suspend the pellet.

8. Following the same procedure as before, water bath the samples for 5 minutes, centrifuge, and decant the supernatents into the appropriate volumetric flask.

9. Dilute your extracts in each volumetric flask to 25.0mL with deionized water. Obtain two pieces of large Whatman #1 filter paper and fold to fit within a thin-neck funnel. Gravity filter the contents of your volumetric flasks into clean and dry test tubes labeled 1 and 2. You will only need a few mL. Cover these tubes with Parafilm and set aside for sugars analysis.

PART 3: COLORIMETRIC SUGARS ANALYSIS

Materials Needed:

- Acid Hydrolysis Sample (~ 1mL per group)
- Ethanol Extraction Samples (~ 2.5mL per sample per group)
- 18.3M Concentrated Sulfuric Acid (45mL per group)
- 2.1% (w/w) Phenol (1mL USP certified ≥89% liquefied phenol in 49mL water, 18mL per group)
- 1.0 mg/mL Glucose In Water (~ 0.5mL per group)
- Fume Hood
- UV/Vis Spectrophotometer
- Vortex Mixer
- 18mm X 150mm Test Tubes (12 per group)
- Test Tube Racks
- Cuvettes
- 1 – 10mL Adjustable Pipet And Tips
- 200 – 1000µL Adjustable Pipet And Tips
- 50 – 200µL Adjustable Pipet And Tips
- 500mL Erlenmeyer flasks
- 1000mL Beakers
- Safety Gloves
- Kim Wipes

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PROCEDURE (cont.)

1. Obtain and label nine 18×150mm test tubes as follows: 0, 50, 200, 400, 700, HW 1, HW 2, AH 1, AH 2.

2. Prepare solutions for your standard curve by pipetting the 0.25mg/mL glucose solution and deionized water as listed in Table 1 on the Discussion sheet. Use good pipetting technique and change tips whenever switching between solutions.

3. Prepare your acid hydrolysis sample for sugars analysis in duplicate. Pipet 300µL of hydrolysate and 700µL deionized water into the two test tubes labeled AH 1 and AH 2.

4. Prepare each water extraction sample for sugars analysis individually. Pipet 1000µL extract 1 into the test tube labeled HW 1 and 1000µL extract 2 into HW 2.

5. Wearing gloves and goggles, bring your test tubes in a rack to the fume hood. Dispense 2.0mL of 2.1 % phenol into each test tube. Next, dispense 5.0mL concentrated sulfuric acid into each test tube. Briefly vortex each tube, still within the fume hood, and allow the tubes to rest for 5 minutes.

6. While waiting, obtain a 500mL Erlenmeyer flask, a 1000mL beaker, and two cuvettes. Turn on your UV/Vis Spectrophotometer to allow it to warm up.

7. Set the UV/Vis Spectrophotometer to read absorbance at 490nm. Fill one clean cuvette with deionized water. Wipe the outside clean with a Kim wipe, and insert it into the sample tray, closing the tray afterwards. Set the reference of the instrument to display 0.000 absorbance with deionized water.

8. Retrieve your test tubes from the fume hood. Be sure you are wearing gloves and goggles. Carefully pour the contents of the 0 test tube into the other cuvette over the large beaker so as to catch any spills. Wipe the outside of the cuvette clean with a Kim wipe.

9. Place the cuvette into the sample tray of the spectrophotometer, close the cover, and record the absorbance at 490 nm in Table 2 on the Discussion sheet. Pour the 0 standard solution into the large Erlenmeyer waste flask.

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PROCEDURE (cont.)

10. Transfer a small amount of 50 standard solution into the empty cuvette and slowly invert while turning to coat the cuvette with the new solution. Empty the cuvette into the waste flask. Fill the cuvette with the remaining amount of 50 standard solution, wipe the outside clean, and record the absorbance at 490nm in Table 2.

11. Repeat this procedure for the remaining standards and samples, recording absorbance at 490nm for the samples in Table 3 on the Discussion sheet.

    Note: It may be necessary to re-blank your spectrophotometer if absorbance readings begin to wander. Be sure to check periodically that the water cuvette still reads 0.000 absorbance at 490nm.

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