Name: Date: Class/Hour:

DISCUSSION

Beer's Law states that the absorbance of a colored sample varies linearly with substrate concentration at low concentrations. "Simple Sugar Concentration" refers to the concentration in the test tube after color development. Recall each test tube had a final volume of 8mL. Use this data to create a scatterplot of Simple Sugar Concentration versus Absorbance at 490nm in Microsoft Excel. Sketch below:

Create a trendline of this data and display the equation on the plot. This equation describes how the absorbance of a sample at 490 nm changes as the concentration of simple sugars changes. Rearrange this equation to obtain simple sugar concentration as a function of absorbance at 490 nm. Write your equation below:

Use your new equation to calculate the concentration of simple sugars present in each sample. Of the 8 mL in the test tubes, 0.5 mL of original solution were used to create the acid hydrolysis samples, while 1.0 mL of solution were used to create the hot water extraction samples. Recall that the hydrolyzate was diluted to 500 mL, and the hot water extracts were diluted to 25 mL. Use this information to fill in the tables on the Data and Tables sheet.

**BIOMASS CARBOHYDRATE COMPOSITION**

Name: Date: Class/Hour:

DISCUSSION (cont.)

Multiplying the simple sugar concentrations of the original samples by their respective volumes will yield the mass of simple sugars recovered by each method. This mass, however, is larger than the mass of cellulose and/or hemicellulose present in your original sample.

To see why, consider the disaccharide sucrose. Sucrose is easily hydrolyzed into its simple sugar monomers, glucose and fructose. Starting with 1 mole, 342.30g, of sucrose will yield 1 mole, 180.16g, each of glucose and fructose after hydrolysis. It doesn't take long to realize that you've gained 18.02 g in the process. The extra mass came from the addition of water (18.02g/mole) in the hydrolysis reaction:

$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

The same thing happens when hydrolyzing cellulose or hemicellulose into simple sugars. Cellulose and hemicellulose chains rarely have the same degree of polymerization, or number of simple sugar units. The question is, how do you account for the added mass of water to polymers of different lengths? The mass of 1 mole of cellulose with a degree of polymerization n is:

$$M(n) = \left[\left(180.16\frac{g}{mole}\right)n - \left(18.02\frac{g}{mole}\right)(n-1)\right] * 1\ mole$$

After complete acid hydrolysis, the mass of glucose present is:

$$m(n) = \left(180.16\frac{g}{mole}\right) * n\ mole$$

The mass ratio of cellulose prior to hydrolysis to glucose after hydrolysis is:

$$\frac{M(n)}{m(n)} = \frac{180.16n - 18.02(n-1)}{180.16n} = 1 - \frac{18.02}{180.16} - \frac{18.02}{180.16n} = 0.899978 - \frac{18.02}{180.16n}$$

As the degree of polymerization n becomes large, the mass ratio of cellulose prior to hydrolysis to glucose after hydrolysis approaches 0.899978:

$$\lim_{n\to\infty}\frac{M(n)}{m(n)} = \lim_{n\to\infty} 0.899978 - \frac{18.02}{180.16n} = 0.899978$$

Hemicellulose chains usually consist of 150 to 200 monomers, while cellulose can chains stretch to nearly 20,000 glucose units. The degree of polymerization in both hemicellulose and cellulose is sufficiently large to assume the mass ratio of the original polysaccharides to simples sugars after hydrolysis is roughly 0.9.

$$\frac{Mass\ of\ Cellulose}{Mass\ of\ Simple\ Sugars} \cong 0.9$$

## BIOMASS CARBOHYDRATE COMPOSITION

Name: Date: Class/Hour:

DISCUSSION (cont.)

You are now ready to calculate the percent composition of water soluble sugars and total sugars in your biomass sample. Calculate the mass of simple sugars recovered by each method, and multiply by 0.9 to obtain the mass of cellulose and hemicellulose. Water soluble carbohydrates are sometimes reported as the percentage of total sugars. Fill in the remaining portions of the tables on the Data and Tables sheet.

Show your work in the space below: