Lab 3: Polymerization of lignin like compounds

Objective

To understand the process of lignification within the cell wall and how to interpret molecular weight distribution curves.

Background

Trees use enzymes (protein catalysts) to convert monomers into polymers. During lignification, phenolic monomers undergo dehydrogenative polymerization after they are transported into the cell wall and are polymerized by enzyme catalysts deposited in the framework. From this sequence of events the lignin ends up surrounding the cellulose components, causing the cell wall to increase in rigidity. The cell wall is typically described as reinforced concrete, with the cellulose component serving as rebar and the hemicellulose and lignin as the cement. Today we will provide an example of the enzymatic polymerization of phenolics based on similar materials as found in the cell wall.

Q: Do you think there is an upper limit to the size of the polymers during this polymerization? Explain.

Laboratory format

The lab will take place over two lab sessions. The first week the lab groups will polymerize the compounds. During the following week the class will measure the molecular weight distribution using gel permeation chromatography.

Data Analysis

For the first week, there is no hard data to collect. However, as a good scientist you must observe the three reaction vessels and make notes and take pictures.

Lab 1, Part 1: Phenolic polymerization

There are four essential components to this polymerization process: solvent (methanol), monomer (phenolic monomer: tyrosine or phenol), enzyme (horseradish peroxidase) in phosphate buffer, and hydrogen peroxide (~30%, or glucose oxidase/glucose). The monomer is activated by the enzyme to polymerize into the polymer, while the hydrogen peroxide activates the enzyme. The solvent allows diffusion of the monomers and the phosphate maintains enzyme activity. You will add hydrogen peroxide that will activate the enzyme catalysts to start the polymerization process. The system works best if the hydrogen peroxide is added slowly, but we will determine the impact of hydrogen peroxide addition with three different experiments - Tube 1) we will add H_2O_2 all at the same time, Tube 2) we will produce H_2O_2 using a second enzyme catalyst, glucose oxidase, that breaks down glucose and releases H_2O_2, and Tube 3) we will slowly add the H_2O_2 over 60mins.

Each group (except Groups 4 & 5 will work together) to do the reaction with three different test tubes as noted below. Divide responsibility of each test tube amongst the members in your groups.

Section A

Group 1:	Tube 1	_____	Group 3:	Tube 1	_____
	Tube 2	_____		Tube 2	_____
	Tube 3	_____		Tube 3	_____
Group 2:	Tube 1	_____	Groups 4/5	Tube 1	_____
	Tube 2	_____		Tube 2	_____
	Tube 3	_____		Tube 3	_____

Section B

Group 6:	Tube 1	_____	Group 8:	Tube 1	_____
	Tube 2	_____		Tube 2	_____
	Tube 3	_____		Tube 3	_____
Group 7:	Tube 1	_____	Group 9:	Tube 1	_____
	Tube 2	_____		Tube 2	_____
	Tube 3	_____		Tube 3	_____

Combining the reagents:

Each group will have 3 test tubes capped with special rubber stoppers, called "septa" in the fume hood. These test tubes contain 5 ml of methanol and 500 mg of monomers. You will add your enzyme catalyst into each test tube via a syringe through the septa. The enzyme has been suspended in phosphate buffer solution at a concentration of 1 mg/ml, so by adding a known volume you can determine the mass by the concentration as marked on the solution. The glucose oxidase enzyme is suspended in buffer at 2mg/ml, and the glucose solution is 125 mg/ml.

NOTE—MONOMERS ARE TOXIC: FOR THESE REASONS YOU WILL NOT HAVE TO WEIGH OUT THE MONOMERS AS THEY HAVE ALREADY BEEN ADDED TO THE SOLUTION. WORKING IN THE FUME HOOD AND USING SEPTA CAPPED TUBES PREVENTS EXPOSURE TO MONOMERS. ****BE CAUTIOUS WHEN ADDING REAGENTS TO THE TUBES.**

Test tube 1: You will first add 5ml of enzyme- phosphate buffer solution into the test tube by piercing the septa with your needle. After mixing the solution, add 0.25 ml of H_2O_2 into tube 1. After you add the H_2O_2, observe any immediate changes to your solution. During the 60 minutes observe any additional changes to your solution by noting color change and viscosity change (slowly rotate the test tube at an angle). There is nothing else to do with this vial for the experiment besides observe changes.

Test tube 2: You will first add 5ml of enzyme- phosphate buffer solution into the test tube by piercing the septa with your needle. You will add the glucose oxidase in a buffer (2 ml of solution) and then glucose solution (2ml) using the syringe. Observe immediate changes in the solution. As polymerization proceeds note changes in appearance of the solution such as color and viscosity. Before you leave, add another 2ml of glucose solution to your reaction (you should end up with approximately 16ml solution). These samples should continue to polymerize after class.

Test tube 3: You will first add 5ml of enzyme buffer solution into the test tube by piercing the septa with your needle. For the third experiment you will slowly add 0.25 ml H_20_2 over 60 minutes. Make periodic additions (0.04 ml) every ten minutes so it takes a full ~60 minutes to add the same volume of H_2O_2 as Tube 1. As polymerization proceeds note changes in appearance of the solution such as color and viscosity.

At the end of this lab session, each set of groups will have 3 tubes containing oligomers/polymers, which will be tested prior to the following lab session. Each group must sign up to help with recovering the polymer during the following week prior to the lab session. Polymers will be isolated, washed of excess reactants, and dried in a vacuum oven prior to analysis.

Lab 3, Part 2: Testing

The samples will be dissolved in a solvent (tetrahydrofuran, THF) and analyzed using a gel permeation chromatography (GPC), collecting the molecular weight distribution of the samples. The samples will be analyzed prior to your lab, but you will be walked through the methods of analyzing the samples on the GPC. We will email you the class data.

Laboratory report

Follow the guidelines for general lab reports included in the laboratory handout. For your results section, insert a photo of your samples, as well as a table containing the molecular weight characteristics of the class samples including an estimated average DP_n. Describe what the image shows in your results section and annotate (place arrows or boxes, etc. to call the reader's attention to details in the image) as required. Some topics to be discussed—what was the impact on the different methods to add H_2O_2 to your reaction solution? Did this impact both the number average molecular weight (M_n) and weight average molecular weight (M_w) equally? Which sample ended up having the largest/smallest MW? Did this meet your expectations? Is the molecular weight size reported similar to the molecular weight sizes of industrial polymers?