Measurment of Laccase, Peroxidase and Xylanase Activity

Laccase Acitivity

The activity of the laccase was measured by monitoring the rate of oxidation of syringaldazine. One unit of activity (U) was defined as the change in absorbance at 530 nm of 0.001 per minute, per ml of enzyme solution, in a 100-mM phosphate buffer (2.20 ml) and 0.216 mM syringaldazine in methanol (0.30 ml). The test was performed at room temperature according to Sealey and Ragauskas

> *See Sealey, J. and Ragauskas, A., Residual lignin studies of laccase-delignified Kraft pulps. Enzyme and Microbial Technology, 1998.* ***23****: 422-426).*

Xylanase Activity

The activity was measured by the dinitrosalicylic acid (DNS method) described by Bailey using the DNS reagent described by Miller

> *See Bailey, M.J., Biely, P., and Poutanen, K., Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology, 1992.* ***23****: p. 257-270)*
> *Miller, G.L., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 1959.* ***31****(3): p. 426-428).*

In brief, the test DNS reagent was prepared as:

- 1% dinitrosalicylic acid
- 0.2% phenol
- 0.05% sodium sulfite
- 1% sodium hydroxide
- 20% sodium tartrate

The substrate solution was prepared by homogenizing 1.0% birchwood 4-O-methyl glucuronoxylan in 0.05 M Na-citrate buffer at pH 5. One gram (1.00 g) of xylan was homogenized in ca. 80 ml buffer at 60°C with a high shear blender and heated to its boiling point on a heated magnetic stirrer. The substrate was then cooled with continued stirring, covered and stirred slowly overnight. The solution was then made up to 100 ml with buffer and stored at 4°C until use. All enzyme and xylose dilutions were performed in 0.05 N sodium citrate buffer. The enzyme assay was performed as described below:

1. 1.80 ml of substrate solution was added to a 15 ml test tube using an automatic pipette. The sample was allowed to equilibrate in the water bath to 50.0 °C.
2. 200 ul of enzyme, diluted appropriately in citrate buffer was added and mixed.
3. The reaction was incubated for 300 s (5 min) at 50.0 °C.
4. After 300 s, 3.00 ml DNS was added. The reaction was then boiled for 15 min, followed by cooling in cold water.
5. The absorbance was read against a blank containing only the reagent and another blank containing only the enzyme at 540 nm and corrected accordingly.

Soybean Peroxidase Activity

Soybean peroxidase (SBP) activity was measured by monitoring the production of a red quinoneimine dye at 510 nm. In the presence of hydrogen peroxide, phenol and 4-aminoantipyrine (4-AAP), SBP catalyzes the formation of phenol free radicals which then react with 4-AAP to form the quinoneimine dye. (ε_{510} = 97,210 a.u. /M/cm)

> *see Kinsley C, Nicell JA (2000) "Treatment of aqueous phenol with soybean peroxidase in the presence of polethylene glycol." Bioresource Technology* ***73****:139-146).*

The standard reaction conditions were 10.00 mM phenol, 2.40 mM 4-AAP and 0.20 mM H_2O_2 at 25 °C, pH 7.0.

Note: For reactions involving hydrogen peroxide, the concentration of the stored stock solution (nominally 30%) must be determined. The assay procedure involves titration of an acidic solution of hydrogen peroxide with sodium dithionite in the presence of an indicator. Two (2.00) mL of peroxide solution is mixed with 100.00 mL distilled water, 10-15 mL of 25% sulfuric acid, 5.00 mL of 10% potassium iodide (KI) solution and 3 drops of saturated ammonium molybdate solution. This mixture is titrated with 0.10 N sodium thiosulfate ($Na_2S_2O_3$) until a pale yellow color appears. Then 10 mL of soluble starch solution is added and the thiosulfate titration continues until the blue color disappears. The equation for calculation is shown below:

$$\text{Grams/Liter} = \frac{0.10(\text{norm. thio.}) \times 34 (\text{equiv. } H2O2 - 50\%) \times}{2.00 \text{ mL (sample size)}} (\text{mL. thio used})$$