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APPENDICES

ศูนย์วิทยทรัพยากร
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APPENDIX A

Preparation of Buffer Solution

Mixing the two solutions as below according to the instruction indicated in Table B-1 to prepare 1,000 cm³ of a buffer solution of the desired pH [47].

Solution 1 : Dissolve 12.37 g of anhydrous boric acid, H₃BO₃, and 10.51 g of citric acid, H₃C₆H₅O₇·H₂O, in distilled water and dilute to 1,000 cm³ in volumetric flask, This makes a 0.20 mol dm⁻³ boric acid and 0.05 mol dm⁻³ citric acid solution.

Solution 2 : Dissolve 38.01 g of Na₃PO₄·12H₂O in distilled water and dilute to 1,000 cm³ in volumetric flask. This makes a 0.10 mol dm⁻³ tertiary sodium phosphate solution.

Table B-1 Preparation of Buffer Solutions

Desired pH	Solution 1, cm ³	Solution 2, cm ³
3.0	880	120
5.0	670	330
7.0	495	505
9.0	345	655
11.0	220	780

APPENDIX B

Preparation of Reagents for Enzymatic Hydrolysis

3,5-Dinitrosalicylate, DNS Reagent:

To prepare 100 cm³ of the DNS solution 1.6 g NaOH is dissolved in 20 cm³ distilled water, 1 g DNS is added and stir until completely dissolved. The solution may need to be heated to get complete dissolution. Then 30 g sodium potassium tetratae is dissolved in 5 cm³ distilled water. The two solutions are mixed, and they are made up to 100 cm³ with distilled water.

0.05 M Phosphate Buffer pH 7.0 :

106.2 g anhydrous Na₂HPO₄ and 53.1 g NaH₂PO₄ are dissolved in 1 L distilled water. The pH of the resulting solution then needs to be checked and adjusting to pH 7.0 by adding NaOH or HCl. This pH is very critical and must be exact. Unless the exact pH specifications are adhered to, enzymes will lose their ability to breakdown starch to glucose.

Glucose Standard:

To prepare a 1 g/l standard solution of glucose, 100 mg of glucose is dissolved in 100 cm³ distilled water.

Stock Enzyme Solution:

α -amylase from *Bacillus* sp.

These activities are usually measured in terms of the activity unit (U), which is defined as the amount which will catalyse the transformation of 1 mole of the substrate per minute under standard conditions.

$$\begin{aligned}
 20 \text{ g solid} &= 51 \text{ units/mg solid} \\
 &= 1,600 \text{ units/mg prot. (Lowry)} \\
 &= 1,020,000 \text{ units}
 \end{aligned}$$

4 g of α -amylase is dissolved in 20 cm³ of phosphate buffer (0.05M, pH 7.0) and stored at 0 °C in a tightly closed glass container. This makes a stock solution containing 10200 U/ml.

Benedict's Reagent:

4.325 g sodium citrate and 2.5 g sodium carbonate are dissolved in about 14 cm³ of distilled water. Then 0.4325 g copper sulfate is dissolved in approximately 11 cm³ of distilled water. The copper sulfate solution is added to a flask with constant stirring. The reagent does not deteriorate on long standing.

Iodine solution :

A 2% solution of potassium iodide and sufficient iodine is prepared to color the solution a deep yellow.

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VITA

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