EXPERIMENT 5

KINETICS ANALYSIS OF AMYLOGLUCOSIDASE HYDROLYSIS OF SOLUBLE STARCH

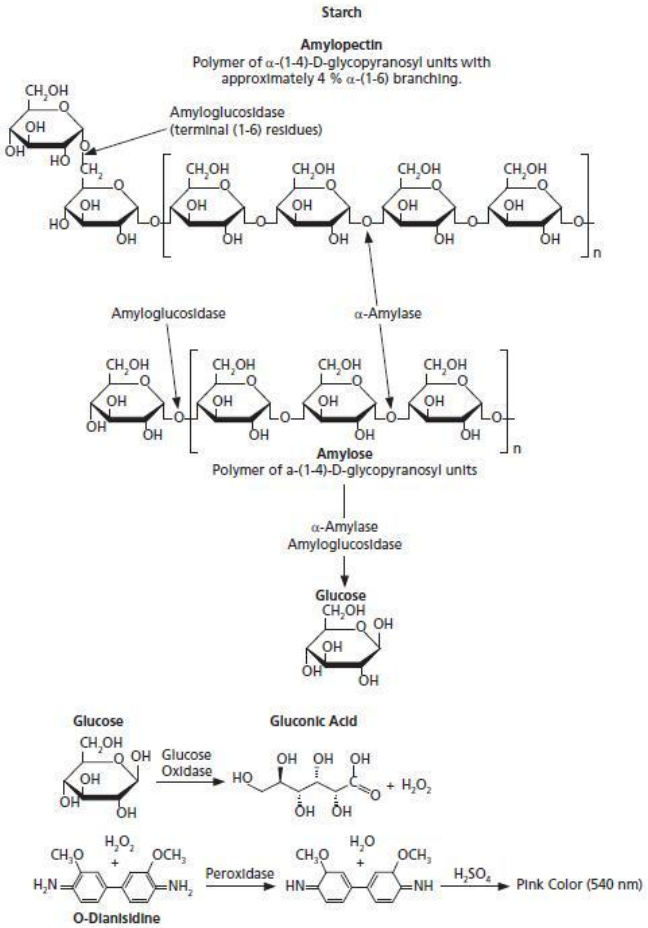

Figure 1: Starch hydrolysis with the final product is glucose (Source: http://www.sigmaaldrich.com/technical-documents/articles/analytix/enzymatic-food-analysis.html)

OBJECTIVES:

Student should be able to

1. To understand and explain the enzymatic hydrolysis of starch
2. To explain and calculate enzyme kinetics parameters.

MATERIAL

- Amyloglucosidase enzyme (Enzyme activity >3000 U/mL)
- Dinitrosalicylic acid solution (DNS)
- Sterile distilled water
- Universal bottles (10 each group)
- Glass pipettes
- Pipettor
- Starch (50 g/500 mL = 0.1 g/mL)

METHODOLOGY

1) Standard curve for reducing sugar analysis
   By now, each individual group should have their own standard curve. Use this standard curve for today's experiment.

2) Enzyme preparation
   Take 1 mL of enzyme solution and dilute it to 25 U/mL using distilled water. This will be the enzyme stock solution.

3) Starch solution and enzyme reaction
   i) Prepare 5 different or varying concentrations of starch solution.
      a) 1 mL stock solution + 1 mL distilled water
      b) 1ml stock solution + 3 mL distilled water
      c) 1 ml stock solution + 5 mL distilled water
      d) 1 ml stock solution + 7 mL distilled water
      e) 1 ml stock solution + 9 mL distilled water
   ii) Calculate the final concentration of each starch solution.
   iii) Take 1.0 mL of each concentration and mix with 1.0 mL of enzyme stock solution. Do the same procedures but this time add 1 mL of distilled water instead of enzyme. This will be your control.
   iv) Incubate the mixture in 55 C waterbath for 15 mins.
   v) Conduct reducing sugar analysis and estimate the concentration of glucose in each reaction.
   vi) Plot the Lineweaver-Burk curve and calculate the Michaelis-Menten constant and maximum reaction velocity.

4) Different concentration of enzyme
   i) Add 1.0 mL of starch solution (0.1 g/ mL) in a new bottle and add five different concentration of enzyme in different tubes.
      a) 1 ml of enzyme + 2ml distilled water
      b) 1 ml of enzyme + 4 mL distilled water
      c) 1 ml of enzyme + 6 ml distilled water
      d) 1ml of enzyme + 8 mL distilled water
      e) 1 ml of enzyme + 10 mL distilled water
   ii) Calculate the enzyme concentration of each enzyme solution.
   iii) Incubate in 55 C for 15 min and estimate the glucose produced.

iv) Plot an appropriate graph to estimate the glucose concentration for 10 U/mL enzyme activities.

QUESTIONS

1. Explain in details the starch hydrolysis pathway. Include all enzyme involve.