Day 2 - Enzyme Inhibition

Note: Test tube racks will be sitting outside of the 95°C bath. This temperature needs to be strictly controlled, so your TA will place the racks into the bath EVERY HALF HOUR for the 10 minutes necessary to incubate the color change. Keep this in mind while you are performing your experiment.

Effect of Copper Sulfate on Low Sucrose (Table 6)

- Prepare a set of solutions of various concentrations using the mixtures indicated in Table #6 of your prelab.
- Start the timer and add 1 mL of the working enzyme solution in succession to each of the test tubes at the same fixed interval (i.e. every 5 seconds another test tube gets the enzyme added).
- Allow the reaction mixture to incubate for precisely 5 min. Here the initial rate is being measured, therefore the length of the reaction must be controlled as accurately as possible.
- At the end of the incubation period add 1 mL of DNS reagent. Add the DNS to each of the test tubes in the same order and at the same fixed interval as before. Again, this is to ensure that each test tube is incubated for the same length of time.
- Shake each test tube to stop the reaction. The addition of the alkaline DNS reagent should effectively stop the sucrose hydrolysis reaction.
- Incubate the test tubes in a 95°C water bath for 10 min.
- Place the test tubes in a beaker of cold water to cool.
- Dilute your samples 1:1.
- Measure the absorbance at 540 nm. The reducing sugar concentration will be determined using the DNS colorimetric method by reading absorbance at 540 nm.

Effect of Copper Sulfate on High Sucrose (Table 7)

- Prepare a set of solutions of various concentrations using the mixtures indicated in Table #7 of your prelab.
- Start the timer and add 1 mL of the working enzyme solution in succession to each of the test tubes at the same fixed interval (i.e. every 5 seconds another test tube gets the enzyme added).
- Allow the reaction mixture to incubate for precisely 5 min. Here the initial rate is being measured, therefore the length of the reaction must be controlled as accurately as possible.
- At the end of the incubation period add 1 mL of DNS reagent. Add the DNS to each of the test tubes in the same order and at the same fixed interval as before. Again, this is to ensure that each test tube is incubated for the same length of time.
- Shake each test tube to stop the reaction. The addition of the alkaline DNS reagent should effectively stop the sucrose hydrolysis reaction.
- Incubate the test tubes in a 95°C water bath for 10 min.
- Place the test tubes in a beaker of cold water to cool.
• Dilute your samples 1:1.
• Measure the absorbance at 540 nm. The reducing sugar concentration will be determined using the DNS colorimetric method by reading absorbance at 540 nm.

Effect of Aniline on Low Sucrose (Table 8)

• Prepare a set of solutions of various concentrations using the mixtures indicated in Table #7 of your prelab.
• Start the timer and add 1 mL of the working enzyme solution in succession to each of the test tubes at the same fixed interval (i.e. every 5 seconds another test tube gets the enzyme added).
• Allow the reaction mixture to incubate for precisely 5 min. Here the initial rate is being measured, therefore the length of the reaction must be controlled as accurately as possible.
• At the end of the incubation period add 1 mL of DNS reagent. Add the DNS to each of the test tubes in the same order and at the same fixed interval as before. Again, this is to ensure that each test tube is incubated for the same length of time.
• Shake each test tube to stop the reaction. The addition of the alkaline DNS reagent should effectively stop the sucrose hydrolysis reaction.
• Incubate the test tubes in a 95°C water bath for 10 min.
• Place the test tubes in a beaker of cold water to cool.
• Dilute your samples 1:1.
• Measure the absorbance at 540 nm. The reducing sugar concentration will be determined using the DNS colorimetric method by reading absorbance at 540 nm.

Effect of Aniline on High Sucrose (Table 9)

• Prepare a set of solutions of various concentrations using the mixtures indicated in Table #9 of your prelab.
• Start the timer and add 1 mL of the working enzyme solution in succession to each of the test tubes at the same fixed interval (i.e. every 5 seconds another test tube gets the enzyme added).
• Allow the reaction mixture to incubate for precisely 5 min. Here the initial rate is being measured, therefore the length of the reaction must be controlled as accurately as possible.
• At the end of the incubation period add 1 mL of DNS reagent. Add the DNS to each of the test tubes in the same order and at the same fixed interval as before. Again, this is to ensure that each test tube is incubated for the same length of time.
• Shake each test tube to stop the reaction. The addition of the alkaline DNS reagent should effectively stop the sucrose hydrolysis reaction.
• Incubate the test tubes in a 95°C water bath for 10 min.
• Place the test tubes in a beaker of cold water to cool.
• Dilute your samples 1:1.
• Measure the absorbance at 540 nm. The reducing sugar concentration will be determined using the DNS colorimetric method by reading absorbance at 540 nm.

Determination of Inhibitor Type (Table 10)

• Prepare 20 mL of the 2.5 mM CuSO₄ solution by diluting 0.5 mL of stock CuSO₄ in 19.5 mL of dH₂O.
• Prepare a set of solutions of various concentrations using the mixtures indicated in Table #10 of your prelab.
• Start the timer and add 1 mL of the working enzyme solution in succession to each of the test tubes at the same fixed interval (i.e. every 5 seconds another test tube gets the enzyme added).
• Allow the reaction mixture to incubate for precisely 5 min. Here the initial rate is being measured, therefore the length of the reaction must be controlled as accurately as possible.
• At the end of the incubation period add 1 mL of DNS reagent. Add the DNS to each of the test tubes in the same order and at the same fixed interval as before. Again, this is to ensure that each test tube is incubated for the same length of time.
• Shake each test tube to stop the reaction. The addition of the alkaline DNS reagent should effectively stop the sucrose hydrolysis reaction.
• Incubate the test tubes in a 95°C water bath for 10 min.
• Place the test tubes in a beaker of cold water to cool.
• Dilute your samples 1:1.
• Measure the absorbance at 540 nm. The reducing sugar concentration will be determined using the DNS colorimetric method by reading absorbance at 540 nm.