A Study of Carbohydrates

- Characterize different sugars and identify an unknown sugar.
- The sugars tested will be sucrose, lactose, xylose, fructose, glucose, honey and an unknown solution of sugar.
- First test is fermentation where the sugar is converted to ethanol and CO$_2$. The fermentation process allows yeast to utilize certain sugars by enzymatic reactions as food. The CO$_2$ will bubble forming foam. Can yeast produce enzymes that breakdown sugars beside glucose?
- Benedict's test reacts with all reducing sugars to produce a red precipitate of copper oxide. The reagent is a copper solution prepared under mildly basic condition.

$$RCHO + 2Cu^{+2} + 4OH^- \rightarrow RCOOH + Cu_2O + 2H_2O$$

Ketose tautomerises to aldose
- Glucose is an example of a reducing sugar.
- Glucose occurs in nature both in aldehyde form and hemiacetal form.

\[
\begin{align*}
\text{D-}(+) \text{ Glucose (hemiacetal form)} & \quad \text{D-}(+) \text{ Glucose (aldehyde form)} \\
\end{align*}
\]

- The essential structural features of a hemiacetal are an \(-\text{OH}\) and an \(-\text{OR}\) group attached to the same carbon atom.

Reducing vs. non-reducing sugar
The Barford test distinguishes reducing monosaccharides from reducing disaccharides. The reagent is a copper solution prepared in a mildly acidic solution.

\[
RCHO + 2\text{Cu}^{+2} + 4\text{OH}^- \rightarrow \text{RCOOH} + \text{Cu}_2\text{O} + 4\text{H}^+ 
\]

- The Barford reagent reacts with reducing monosaccharides faster than the reducing disaccharides.

- Seliwanoff test depends on the relative rates of dehydration of carbohydrates.
- Seliwanoff reagent is a solution of resorcinol dissolved in concentrated HCl.
- A ketohexose reacts rapidly to form 5-hydroxymethyl furfural than an aldohexose.

Once the reaction to 5-hydroxymethyl furfural is completed, the product reacts resorcinol to give a dark red condensation product.
- Glucose exists in equilibrium as an open chained structure and as hemiacetal (alpha and beta glucose.)
• Starch is a glucose polymer connected through an alpha (1-4) glycosidic linkages.

• Cellulose is also a glucose polymer connected through a beta (1-4) glycosidic linkages.

• Add amylase to both starch and cellulose solution to test if amylase cleaves either or both polysaccharides.

• I$_2$ solution (Lugol’s reagent) reacts with starch to form a dark blue starch/I$_2$ complex. Does cellulose react with the I$_2$ solution also?

• Use the benedicts test to determine if the starch and cellulose was broken down to alpha or beta glucose.
Part E procedure modification.

Procedure modification:
1. To another test tube add 1 mL of 0.5 % cellulose solution and add an equal volume of amylase solution. Incubate both starch/amylase and cellulose amylase into the incubator for 10 to 15 minutes.
2. Place 1 mL of 0.5 % starch solution and 0.5% cellulose solution to two separate test tubes. Add 10 drops of iodine solution to each test tube and not the color.
3. Once incubation of 5 % starch solution and 0.5% cellulose solution is completed, transfer half of each test tube into a two separate test tubes. To the original test tubes, add 10 drops of iodine solution and note your observations.
4. To the second set of test tubes, add 20 drops of Benedict’s solution. Heat the test tubes in boiling water for 2 minutes. Record your observations.

HYDROLYSIS OF STARCH BY AMYLASE

Reaction of starch with iodine solution.

Reaction of cellulose with iodine solution.

Reaction of starch/amylase solution with iodine solution

Reaction of cellulose/amylase solution with iodine solution

Reaction of starch/amylase solution with Benedict’s reagent.

Reaction of cellulose/amylase with Benedict’s reagent.

Post lab Questions:
1. Find the structures for the following carbohydrates (sugars) in a reference work or textbook and decide whether they are reducing or non-reducing carbohydrates (sugars): sorbose, mannose, ribose, maltose, raffinose and cellulose.

2. From your results, can you determine whether amylase cleaved the starch and cellulose? Explain.

3. Was there any residual starch in your hydrolyzed sample? Explain.