

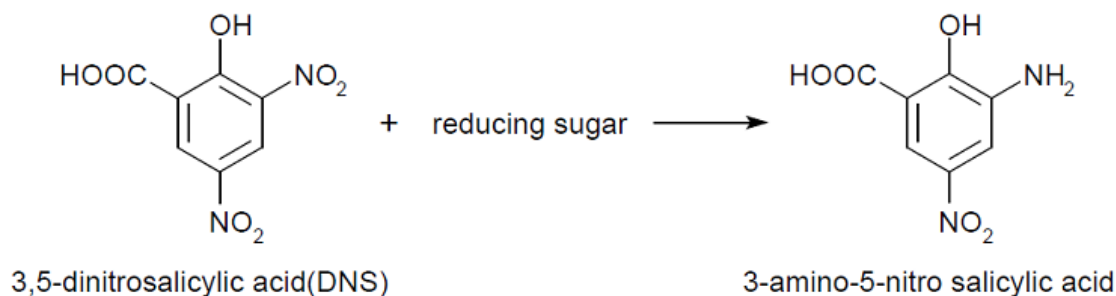
EXPERIMENT 4
CELLULOSE DEGRADATION AND GLUCOSE ANALYSIS


Figure 1: Schematic reaction of Dinitrosalicylic acid assay (Source: <http://2011.igem.org/Team:Kyoto/Digestion>)

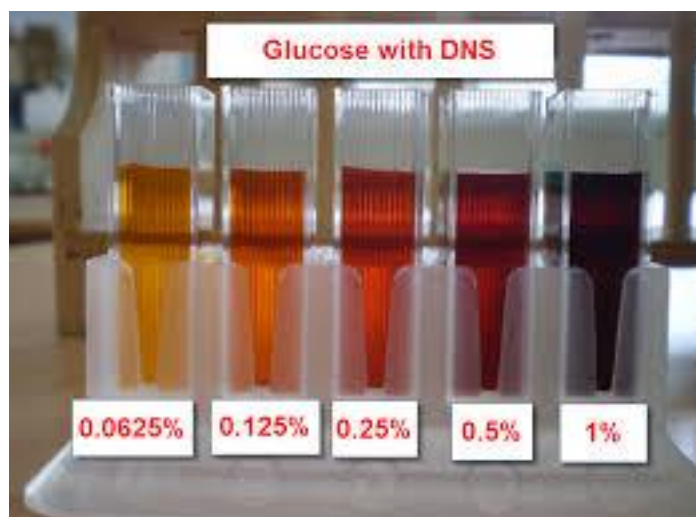


Figure 2: Color changes after DNS reaction based on glucose concentration (Source: <http://lawrencekok.blogspot.my/2011/06/ib-biology-enzyme-kinetics-sucrose.html>)

OBJECTIVES:

Student should be able to

1. To understand and explain the methods of cellulose degradation and analysis of glucose.
2. To conduct and explain the glucose analysis-using dinitrosalicylic assay.

MATERIAL

- Microbial solution (overnight culture)
- Dinitrosalicylic acid solution (DNS)
- Sterile distilled water
- Universal bottles (10 each group)
- Glass pipettes
- Pipettor
- D-glucose solution (1 mg/mL)

METHODOLOGY

1) Standard curve for glucose analysis

- i) Make four dilutions of the D-glucose solution (e.i. 0.1, 0.3, 0.5, 0.7 mg/ml) using distilled water. These will be your glucose standard. Show the dilution calculation in your report.
- ii) Take 1.0 mL from each dilution in a new bottles and then 1.0 mL of DNS reagent.
- iii) Boiled all tubes for exactly 5 minutes in 90 C water bath and cooled before adding 3 mL of distilled water.
- iv) OD540 is used to measure the glucose content in all tubes in the UV/Vis spectrophotometer.

2) Cellulose degradation

You are given one 5 different cellulose degradation trials, which were conducted the day before the experiment.

- i) Put in 2-mL of centrifuge tube and centrifuge for 5 min at the highest speed to separate the cells from the solution.
- ii) Pipette the solution part and analyze the reducing sugar content.

REMINDER: In the meantime you should also remember to have appropriate controls and blanks for the analysis.

QUESTIONS

1. Give one example of other methods for analyzing glucose and explain briefly the principle of the method.
2. Give examples of other material that contains cellulose and find out some information on the research of degradation of this material.