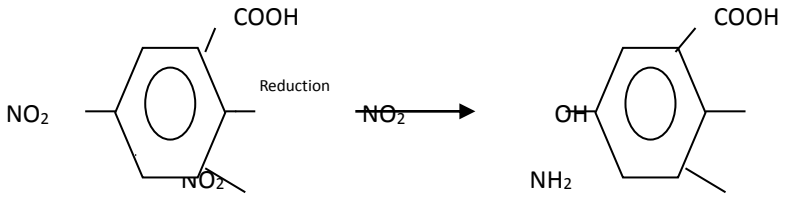
Objective:
Estimation of reducing sugars (Glucose) and non-reducing sugars (Sucrose) by DNS[1] method, in a sample of culture broth.

Principle:
Sugars are assayed by using their reducing properties. One such compound is 3,5-dinitrosalicylic acid, which in alkaline solution, gets reduced to 3-amino, 5-nitro salicylic acid. The degree of color intensity formed due to the presence of reduced compound in the reaction mixture can be directly correlated to the amount of reducing sugar present in that.



3, 5-dinitro salicylic acid 3-amino, 5-nitro salicylic acid

Procedure:
1. Preparation of DNS reagent

   Solution (i) To 300ml of 4.5% NaOH, 800ml of 10% di-nitrosalicylic acid (DNS) and 225g Rochelle salt (Sodium-potassium tartrate) are added.

   Solution (ii) To 10g of crystalline phenol, 22ml of 10% NaOH is added and the volume is made up to 100 by distilled water.

   Solution (iii) To 69ml of solution (ii), 6.9g sodiumbisulphite is added.

   For making the DNS reagent, solution-(iii) is added to solution-(i) and mixed thoroughly till all the salts dissolve. The DNS reagent so obtained is filtered through glass wool and stored in dark brown bottle to avoid light, which may degrade the reagent.

2. Preparation of standard curve (Glucose/ Sucrose)

   The range of sugar estimation by the DNS method is 0.1-2.0 mg/ml sugar in sample. Therefore the standard curves should be made only within this range.

   (i) To make a sugar standard solution (SSS) containing sugar concentration of 2mg/ml, dissolve 200mg sugar in distilled water (DW) and make up the volume to 100ml in a measuring flask.
   (ii) In 5X30ml capacity test tubes, pour suitable volumes of SSS and add required volume of distilled water so as to obtain 1ml of diluted SSS in the range 0.2mg to 2.0 mg in the test tubes. In the sixth test tube (blank), take only 1 ml distilled water (follow tables given in observations).

   (iii) In case of sucrose, add 0.5 ml 2N HCl to each tube and boil for 10 minutes (this will ensure hydrolysis of sucrose to glucose & fructose). After cooling the tubes, add 0.5 ml NaOH to neutralize the reaction mixture. In case of glucose, instead of adding HCl or NaOH, it will be sufficient to add 1ml water to each tube.
   (iv) Add 3ml DNS reagent to all the tubes, and keep the tubes in boiling water bath for exactly 5 minutes. Thereafter, cool all the tubes under a running tap. Add 20 ml distilled water to all the tubes and mix them thoroughly.

(v) Read OD at 540nm wavelength on a spectrophotometer using sixth tube as a blank.

3. Estimation of reducing sugars in unknown samples:

(i) The unknown sample (from fermentation broth) should be centrifuged (1000rpm, 10 minutes). The sugar must be estimated in cell free supernatant.
(ii) Suitably dilute the supernatant of unknown sample so that its sugar concentration falls in the estimation range of the method.
(iii) Perform steps 2-(iii) to (v) above.
(iv) Use standard curve to compute sugar concentration in unknown sample.

Observations:

(a) Standard Curve for Glucose

TEST TUBE #	SSS Added (ml)	DW Added (ml)	Sugar conc. in test tube (mg/ml)	DW added (ml)	DNS reagent added (ml)	DW added (ml)	OD 540nm
1	0.1	0.9	0.2	1	3	20	
2	0.3	0.7	0.6	1	3	20	
3	0.5	0.5	1.0	1	3	20	
4	0.7	0.3	1.4	1	3	20	
5	0.9	0.1	1.8	1	3	20	
6	0	1.0	0	1	3	20	

(b) Standard Curve for Sucrose

TEST TUBE #	SSS Added (ml)	DW Added (ml)	Sugar conc. in test tube (mg/ml)	2N HCl added (ml)	2N NaOH added (ml)	DNS reagent added (ml)	DW added (ml)	OD 540nm
1	0.1	0.9	0.2	0.5	0.5	3	20	
2	0.3	0.7	0.6	0.5	0.5	3	20	
3	0.5	0.5	1.0	0.5	0.5	3	20	
4	0.7	0.3	1.4	0.5	0.5	3	20	
5	0.9	0.1	1.8	0.5	0.5	3	20	
6	0	1.0	0	0.5	0.5	3	20	

Results:
Make a plot of glucose and sucrose standard curves by fitting a regression line and report the correlation coefficient. Attach the plot with this handout. Give estimated sugar concentration value in unknown sample.

Reference: Miller, G.L. (1959), "Use of di-nitro-salicylic acid reagent for the determination of reducing sugars", Anal Chem, 31, 426-428