APPENDIX 9

Assay procedure for estimation of carboxy methyl cellulase activity (Mandel et al., 1976; IUPAC, 1986)

Substrate: 2% Carboxymethyl cellulose (CMC) in 0.05 M citrate buffer of pH 4.8

Method:

1. 0.5 ml enzyme, diluted in citrate buffer, is taken in a 30 ml test tube. At least two dilutions must be made of each enzyme sample. One dilution should release slightly more and one slightly less than 0.5 mg of glucose in the reaction.

2. 0.5 ml substrate is added, mixed well and the mixture is incubated at 50°C for 30 mins.

3. 3 ml DNS reagent is added and the mixture boiled for 5 mins. in a vigorously boiling water bath. All samples, enzyme blanks, glucose standards and the spectro zero are boiled together.

4. The tubes are cooled by placing in a cold water bath. 20 ml water is added to each tube and mixed well by inversion.

5. The absorbance are measured against the spectrozero at 540 nm.

Spectro zero:

0.5 ml substrate solution is taken in a test tube and incubated at 50°C for 30 mins. 3 ml DNS reagent and 0.5 ml citrate buffer, in that order, are added and mixed well. The mixture is boiled vigorously for 5 mins. After cooling, 20 ml water is added and mixed completely by inversion. This is the spectro zero used to set the spectrophotometer at zero absorbance.

Enzyme blank:
0.5 ml substrate solution is taken in each test tube and incubated at 50°C for 30 mins. 3 ml DNS reagent and 0.5 ml of enzyme solution, in that order, are added and mixed well. The mixture is vigorously boiled for 5 mins. and cooled. 20 ml water is added and mixed completely. The absorbance is read at 540 nm against the spectro zero.

Standards:
Glucose stock solution is prepared with a concentration of 2 mg/ml. The following standards of different dilutions are prepared:

1) Undiluted = (1.0 mg/0.5 ml)
2) 1 ml glucose soln. + 0.5 ml buffer = 1 : 1.5 (0.67 mg/0.5 ml)
3) 1 ml glucose soln. + 1.0 ml buffer = 1 : 2 (0.5 mg/0.5 ml)
4) 1 ml glucose soln. + 3.0 ml buffer = 1 : 4 (0.25 mg/0.5 ml)

0.5 ml substrate solution is taken in each test tube and incubated at 50°C for 30 mins. 3 ml DNS reagent and 0.5 ml of each standard, in that order, are added into the respective tubes. The tubes are placed in vigorously boiling water bath for 5 mins. and cooled. 20 ml water is added to each tube and mixed well. The absorbance are measured against the spectro zero at 540 nm.

Unit calculation:

1. A linear glucose standard graph is plotted with absolute amounts of glucose (mg/0.5 ml) against the absorbance.

2. Using this graph, the absorbance value of the enzyme sample (after deducting the absorbance of the enzyme blank) is translated to glucose (mg glucose produced during the reaction).

3. The dilution used is translated into enzyme concentration as: concentration = 1/dilution
4. The concentration of enzyme which would have released exactly 0.5 mg of glucose is estimated.

5. CMCase is calculated as:

\[
\text{CMCase} = \frac{0.185}{\text{Enzyme conc. to release 0.5mg glucose}} \text{ units/ml}
\]

**Note:** In cases of low level activity, it is found that even undiluted enzyme releases less than the critical amount of glucose. In such cases, the calculation is done according to the relation:

\[
\text{CMCase} = \text{mg glucose released} \times 0.37 \text{ units/ml}
\]