NEATH PORT TALBOT COLLEGE
COLEG CASTELL NEDD PORT TALBOT

School of Maths & Science
Science Practical

β – galactosidase induction

◆ Aim

To induce and measure the production of the enzyme - β galactosidase (lactase) by E.coli

◆ Introduction

The lac operon is the classic example of gene regulation, in which the production of β -galactosidase (lactase) is induced by the presence of lactose in the growth medium. In this practical task, ONPG, rather than lactose, is used as a substrate for the enzyme. After partial disruption of the cell membrane with methylbenzene, colourless ONPG is added. It moves into the cells where it is broken down to form the yellow-coloured product, ONP.

◆ Safety

Control Measures

- The wearing of safety glasses and a laboratory coat at all times will be sufficient to take account of most hazards and significant risks.
- Methylbenzene is harmful by inhalation and in contact with the skin, avoid inhalation and contact with skin.
- Avoid inhalation of fumes during the heating phase by ensuring good ventilation provision.
- Good microbiological practice must be observed when handling micro-organisms.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.

Hazards

Highly Flammable — Methylbenzene

Harmful — Methylbenzene
ONPG solution
E. Coli culture

◆ Procedure

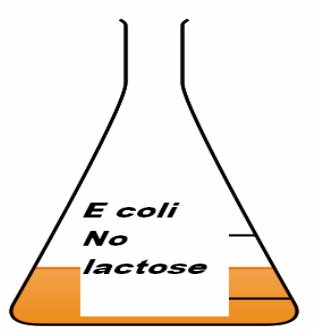
You will need cultures of *E.coli* from a strain that possesses the lacZ (β galactosidase) gene. These have been grown in broth 24-48 hours in advance. To induce the production of β -galactosidase, lactose must be added to the growth medium (0.1 g per cm^3 of broth). The ONPG solution should has been produced fresh (a day before at the earliest)

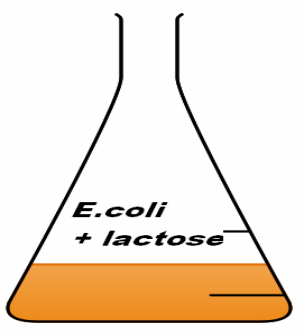
This activity takes about 60 minutes, including an incubation period of 10 minutes.

Quantitative method

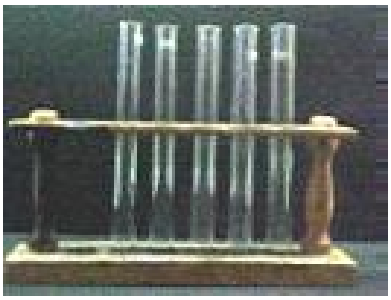
This is used to determine how much β -galactosidase is produced by a culture.

1 You are provided with two stock bacterial suspensions. E. coli in nutrient broth (control), E. coli in nutrient broth + lactose.





2 Label four test tubes Control 1, Control 2, Lactose 1 & Lactose 2.



3 Using a fresh sterile 1 cm^3 graduated pipette for each suspension, transfer 0.2 cm^3 of each microbial suspension to be tested into the appropriate test tube. Place used pipettes in the waste container.

4 Add one drop of methylbenzene to each tube, cap the tubes and shake well to mix. ***Methylbenzene kills the cells and partially disrupts the cell membrane, allowing the ONPG to diffuse into bacterial cells.***

5 Perform the next operation in a fume cupboard. Use a hair drier to evaporate the methylbenzene .Methylbenzene is lighter than water and will appear as a 'greasy' film on the surface. You must wait until all of this solvent has evaporated before proceeding to the next step.

6 Using a pipette add 3 cm3 of ONPG (ortho-nitrophenyl-β -D-galactoside) dissolved in Z-buffer to each test tube.

7 Transfer the test tubes to a water bath maintained at 35-37^{0}C. Record the time.

8 After 5 minutes transfer the test tube contents to a cuvette using a clean pipette.

9 Measure the absorbance of the samples at 460 nm using a blue filter in the colorimeter.

10 Record your results in a suitable table.

11 Return cuvette to water-bath. Ensure that you have marked the cuvettes appropriately on the non clear side. e.g. C1, C2, L1 & L2

12 Measure the absorbance of the four samples at every 5 minute intervals until there is no further colour change.

13 Plot a graph of the results (Absorbance against time).

14 Use the practical results and procedure to complete Assignment 3.