Experiment (02) Title: Assessment of Cell Disruption by Ultrasonication

Principle: The rate of protein released by mechanical cell disruptions is usually sound to the proportional to the amount of releasable protein.

Materials required:

i) A Sonicator
ii) 2.0 mL centrifuge tubes
iii) Overnight grown *E. coli* cell suspension
iv) Spectrophotometer

Procedure:

i) *E. coli* DH5α cells were inoculated (3%) in 100 ml LB (Luria-Bertani) media and kept for overnight incubation at 37°C at 120 rpm
ii) This homogenous suspension of overnight grown cells of *E. coli* DH5α was made in sterile water
iii) After this an aliquot of 1.0 mL of *E. coli* DH5α cell suspension was then taken in 10 separate 2.0 mL centrifuge tubes
iv) The cell suspension in the centrifuge tubes (Tube No. 1-10) were each subjected to ultrasonication for various time periods (as shown in the table below).
v) For each time period, ultrasonication was accomplished with 30% amplitude setting and 10 seconds ON and 20 seconds OFF cycle.
vi) After ultrasonication, for the respective time periods, a 20 µL aliquot of the sample was aspirated from each tube and added to 480 µL of water. To each sample, 0.5 mL of Bradford reagent was added and the tubes were incubated in the dark for 10 minutes.
vii) Subsequently, the absorbance for each sample was recorded at 595 nm

Ultrasonication Duration	Sample Volume (µL)	Diluent Volume (µL)	Fold Dilution	Absorbance A_{595}
5.0 minutes	20	480		
3.0 minutes	20	480		
1.0 minute	20	480		

Using a standard Bradford assay (using 2.0 µg - 18 µg BSA), a prior calibration plot was generated in order to obtain a calibration equation for estimation of the amount of protein released from each sample. The calibration equation is: **$y = 0.0316x$**, where y is absorbance measured at 595 nm and x is the amount of protein in microgram (µg). A prior experiment indicated that the maximum amount of protein released from 1.0 mL of overnight grown cells of *E. coli* DH5α is 28575 microgram (µg). Based on the data obtained (as shown in the table), graphically calculate and report the cell disruption constant **(in min^{-1})**.

Hint: *Use the absorbance values, fold dilution and the calibration equation to obtain the amount of protein (in microgram) released from one mL of each sample.*