

**Experiment (01) Title: Estimation of Settling Time of *E. coli* Cells in a Bench-top Laboratory Centrifuge**

**Principle:** Settling time of bacterial cells is directly proportional to time of centrifugation

**Materials required:**

- i) A bench-top centrifuge having a fixed-angle rotor
- ii) A fixed-angle rotor that can hold 2.0 mL centrifuge tubes
- iii) 2.0 mL centrifuge tubes
- iv) Overnight grown *E. coli* cell suspension
- v) Spectrophotometer

**Procedure:**

- i) *E. coli* DH5 $\alpha$  cells were inoculated (3%) in 100 ml LB (Luria-Bertani) media and kept for overnight incubation at 37°C at 120 rpm
- ii) The overnight grown culture was then checked for OD at 600nm. It will come around 1.0
- iii) After this an aliquot of 1.5 mL of *E. coli* DH5 $\alpha$  cell suspension was taken in 10 separate 2.0 mL centrifuge tubes and subjected to centrifugation
- iv) The centrifuge was operated at 1500 rpm and the cells were monitored as they travelled from the top of the suspension (1.5 mL mark in a graduated 2.0 mL centrifuge tube) to a reference point at 0.5 mL mark in the graduated centrifuge tube
- v) The centrifuge tubes (Tube No. 1-10) were each subjected to centrifugation for various time periods.
- vi) Following centrifugation for the respective time periods, a 1.0 mL aliquot of the sample was aspirated from the reference point of each tube without disturbing the liquid and the optical density of the drawn sample was measured at 600 nm using Luria-Bertani (LB) medium as blank

The centrifugation time and the final OD<sub>600</sub> (estimated after considering dilution factor) obtained for each sample aspirated from the reference point is indicated in the table shown below:

Sample	Sample Volume (mL)	Centrifugation Speed (rpm)	Centrifugation Time (Minute)	Final OD <sub>600</sub>
Control	1.5 mL	1500	0	
1			5	
2			7	
3			9	
4			11	
5			14	
6			17	
7			20	
8			23	
9			26	
10			29	

Draw a plot indicating OD<sub>600</sub> vs. centrifugation time for the samples. Based on the plot, report the settling time for *E. coli* DH5 $\alpha$  cells obtained in the current experiment.



**Problem 1.** In the context of problem 1, consider that the distance between the axis of rotation and top of cell suspension in the 2.0 mL centrifuge tube is  $R_0$  and the distance between axis of rotation to the marked reference point in the centrifuge tube till which the cells settle upon centrifugation is  $R$ . The ratio  $R/R_0$  is 1.05. The density of *E. coli* DH5 $\alpha$  cells and the density of the suspending medium of the cells is 1030 kg m<sup>-3</sup> and 1000 kg m<sup>-3</sup>, respectively. The viscosity of the suspending medium of the cells is  $1.25 \times 10^{-3}$  kg m<sup>-1</sup> s<sup>-1</sup>. Based on the settling time for *E. coli* DH5 $\alpha$  cells calculated in problem 1 and given that the centrifuge is operated at 1500 rpm, estimate the radius of *E. coli* DH5 $\alpha$  cells (in meter).

**Note:** All the steps or assumptions or hypothesis required for the solution of the numerical problem should be clearly indicated. The final answer should be clearly mentioned along with the required units. Incomplete steps or answers shown as fraction will not be considered.

**Problem 2.** In a separate experiment, overnight grown cells of *E. coli* DH5 $\alpha$  were again subjected to centrifugation at 6000 rpm. The centrifugation time and the final OD<sub>600</sub> (estimated after considering dilution factor) obtained for each sample aspirated from the reference point in a 2.0 mL graduated centrifuge tube is indicated in the table shown below:

Sample	Sample Volume (mL)	Centrifugation Speed (rpm)	Centrifugation Time (Minute)	Final OD <sub>600</sub>
Control	1.5 mL	1500	0	2.52
1			5	1.71
2			8	0.79
3			11	0.61
4			14	0.71
5			17	0.50
6			19	0.59
7			20	0.68
8			21	0.37
9			22	0.37
10			23	0.37

- Draw a plot indicating OD<sub>600</sub> vs. centrifugation time for the samples and report the settling time for *E. coli* cells obtained in the experiment.
- Provide a plausible explanation for the OD<sub>600</sub> profile obtained in the experiment.