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Escherichia coli Calibration of OD_{600} vs Colony Forming Units

Objective:

The goal of the experiment was to produce a calibration curve with moderately apt regression. This curve can then be utilized to determine the approximate *Escherichia coli* (*E. coli*) concentrations in our cultures by analysing only the OD_{600}.

Materials and Methods:

The strain of *E. coli* used was DH5α and contains ampicillin-resistant genes as well as green fluorescent proteins. Nutrient broth and nutrient agar plates were prepared at the producer's specifications. Both media were made with an added working concentration of 100ng/mL of ampicillin, allowing more specific growth of our bacterium. The bacterium was inoculated from frozen beads into a culture of nutrient broth and a nutrient agar plate. Another culture was inoculated with a single isolated colony of this bacterium and allowed to incubate at 37.0°C for 16 hours. This culture was then used as our starter culture in log phase for the experiment.

Using the prepared log phase culture, a small volume of 1.000mL was inoculated into 100mL of nutrient broth in an Erlenmeyer flask. This culture was allowed to incubate at 37.0°C and the OD_{600} was monitored periodically using a spectrophotometer. Seven points of OD_{600} were used in our experiment. At each point, serial dilutions were done and plated onto prepared nutrient agar plates to determine the amount of colony forming units per milliliter.

Results:

Table 1. OD_{600} of different points and their respective concentrations.

Point	OD_{600}	CFU/mL
1	0.095	4.80E+05
2	0.209	8.70E+06
3	0.444	1.45E+07
4	0.320	6.20E+06
5	0.423	7.50E+06
6	0.510	1.89E+07
7	0.589	1.80E+07

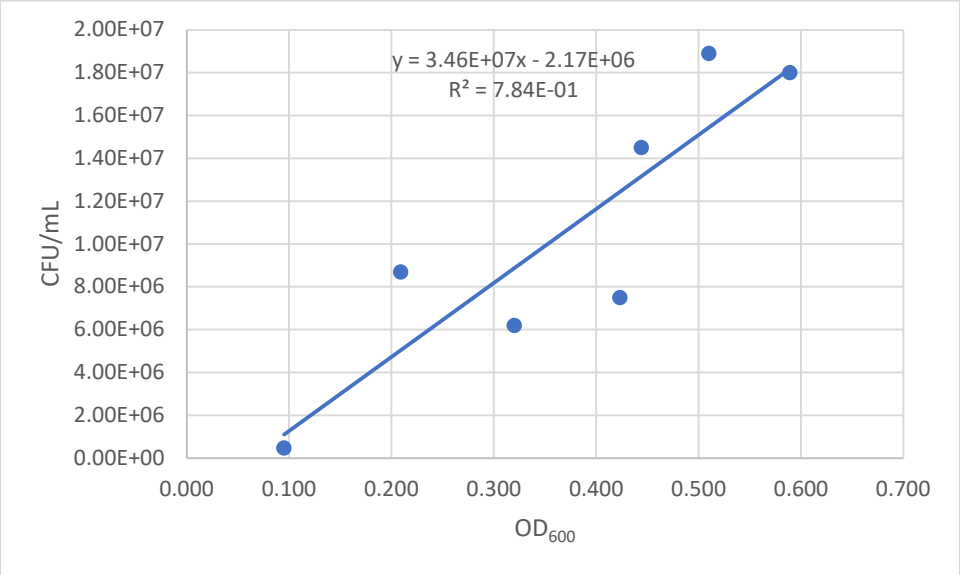

Figure 1. Calibration curve of CFU/mL plotted against OD_{600} of *Escherichia coli*.

Discussion:

Since this experiment was only run once with only one trial, the precision of this technique was not determined. Additionally, there is some error that accompanies these numbers since each cell in the diluted solution represents millions of cell in the undiluted culture. However, there is still a relatively straight curve that demonstrates a trend to be used to approximate the concentrations if needed. In the future, starting with a greater inoculation would be ideal since it took the bacteria several hours to get up to around 0.100 absorbance. This experiment allows for the determination of the approximate concentration of *E. coli* solely by determining the OD_{600}.