Comparing Microvolume and Cuvette Based Measurements of Microbial Cell Cultures

Introduction

Using a spectrophotometer such as a Thermo Scientific NanoDrop 2000c to monitor growth of bacterial cultures by measuring the optical density at 600 nm (OD_{600}) is a central technique in microbiology. Optical density measurements, however, often contain very little true chemical absorbance. Instead, optical density measurements are actually representative of the amount of light scattered away from the instrument's detector by the bacterial suspension. The pedestal and cuvette options found on a NanoDrop™ 2000c will give different OD values when measuring light scattering samples. Two important aspects to consider when making light scattering measurements: 1) the linear range of the measurement systems and 2) the use of a conversion factor for the two measurement systems.

Linear Range Determination

When measuring OD values of scattering samples using a spectrophotometer, it necessary to determine the linear range, as this may not be the same as that observed for traditional absorbance measurements. Establishing the linear range is important because in some cases the OD_{600} of the culture may exceed the linear range of the instrument prior to reaching stationary phase (fig 1). The linear range depends largely on the optical configuration, and therefore will not be the same for the pedestal and cuvette on a NanoDrop 2000c.

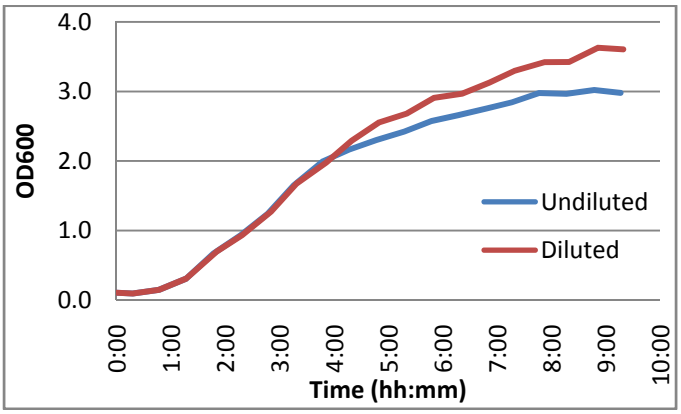

Figure 1: *A 9.5h batch culture of* E. coli *measured at 30 min intervals, diluted and undiluted. The divergence between the two lines occurs when the undiluted sample exceeds the linear range of the spectrophotometer. Note that this occurs before the culture has reached stationary phase.*

To determine the linear dynamic range, a series of dilutions performed from a young overnight culture (~16hr) of the microbial strain can be measured and OD_{600} graphed against the dilution factor. The limits of detection can be determined by identifying the OD_{600} at which there ceases to be a linear correlation between the dilution factor and the measured OD_{600}.

Measurement Conversion

To compare OD readings between the pedestal and cuvette systems found on a NanoDrop 2000c, a conversion factor can be calculated as follows:

$$\frac{\text{Cuvette OD}}{\text{Pedestal OD}} = \text{Conversion Factor}$$

This conversion factor can then be used to compare measurements using cuvette and pedestal options (fig. 2). The method for calculating a conversion factor can also be used when comparing OD_{600} measurements between different spectrophotometers.

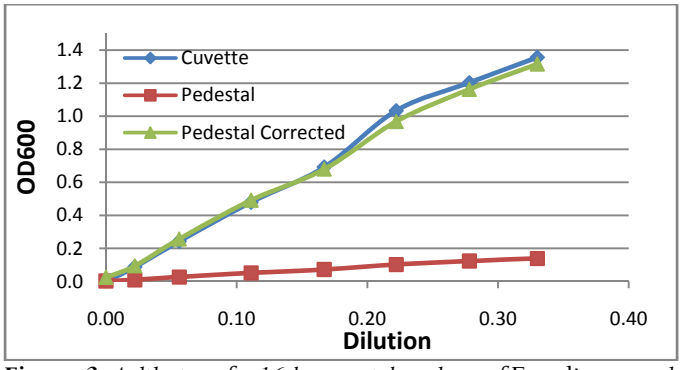

Figure 2*: A dilution of a 16 hr overnight culture of* E. coli *measured using the NanoDrop 2000c pedestal option (red) and cuvette option (blue). Applying the conversion factor to the pedestal data (green) facilitates comparison between the two measurement methods.*

Summary

When comparing the OD_{600} measurements made using the cuvette and pedestal options on a NanoDrop 2000c, the following points should be remembered:

- Ensure that the OD_{600} at the growth point of interest falls within the linear range of each system.
- To compare between pedestal and cuvette, a conversion factor is necessary, and should be based on measurements made close to the target OD.

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