Absorbance Method

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1 Introduction

Measurement of absorbance is used in cell biology to study bacterial growth curves, thus estimating the phase of growth of bacteria and its population. The aim of the experiment, was to check whether the ratio of the concentration in co-culture of *E. coli* and *B. cereus* could be characterized with Absorbance measurements. The experiment was a simplistic measurement of absorbance spectra of the bacteria at different concentration at different wavelengths followed by data analysis for complete characterization.

2 Theory

The theoretical framework lies behind Beer-Lambert's Law. Beer-Lambert's Law can be effectively written in one equation as stated below:

$$A(\lambda) = \sum_{i=1}^{n} \epsilon_i(\lambda) C_i l \qquad (1)$$

Here, $C_i's$ are an arbitrarily chosen linear measures of concentrations.

The basic idea underpinning the equation is that, the absorbance of individual components add up. Thus, for a two component system, i.e., that of *E. coli* and *B. cereus:*

$$A(\lambda) = \epsilon_1(\lambda) C_1 l + \epsilon_2(\lambda) C_2 l \qquad (2)$$

Now, suppose one is able to find two wavelengths λ_1 and λ_2 such that the above linear equations in C_1 and C_2 are linearly independent, then we can use those two linear equations to obtain the values of C_1 and C_2 by solving the the two linear equations:

$$A(\lambda_1) = \epsilon_1(\lambda_1) C_1 l + \epsilon_2(\lambda_1) C_2 l \qquad (3)$$
$$A(\lambda_2) = \epsilon_2(\lambda_2) C_2 l + \epsilon_2(\lambda_2) C_2 l \qquad (4)$$

Now, we know that the condition for linear independance of linear equations is:

$$det \begin{pmatrix} \epsilon_1(\lambda_1) l & \epsilon_1(\lambda_2) l \\ \epsilon_2(\lambda_1) l & \epsilon_2(\lambda_2) l \end{pmatrix} \neq 0$$

Solving this, we get:

$$\frac{\epsilon_1(\lambda_1)}{\epsilon_2(\lambda_1)} \neq \frac{\epsilon_1(\lambda_2)}{\epsilon_2(\lambda_2)} \tag{5}$$

So, we just need two wavelengths for which the function $\frac{\epsilon_1(\lambda_1)}{\epsilon_2(\lambda_1)}$ gives different values

3 Experiment

We ran a spectrum run for both the bacterium at specific concentrations, and therefore we got the values of the following:

1) $\epsilon_1(\lambda)C_{1_i}l$, where λ varied from 300 nm to 700nm, and varied values of C_{1_i}
2) $\epsilon_2(\lambda)C_{2_i}l$, where λ varied from 300 nm to 700nm, and various values of C_{2_I}

Now:

$$\frac{\partial \epsilon_j(\lambda)C_{j_i}l}{\partial C_{j_i}} = \epsilon_j(\lambda)l \qquad\qquad j \in \{1,2\}$$

Using this relation, we calculated the values of $\frac{\epsilon_1(\lambda_1)}{\epsilon_2(\lambda_1)}$ for all lambda between 300 nm and 700 nm.

4 Observations

We observed that the graph of the function $\frac{\epsilon_1(\lambda_1)}{\epsilon_2(\lambda_1)}$ is a constant function, as shown below.

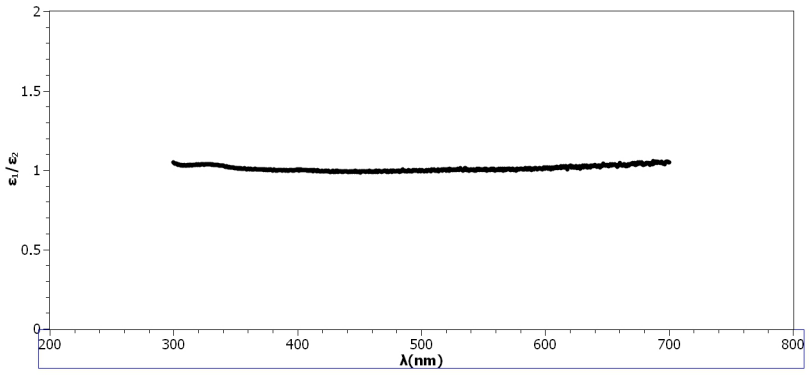

Figure 1: Plot of $\frac{\epsilon_1}{\epsilon_2}$ vs λ

5 Result

The use of OD at two wavelengths to calculate the ratio in a co-culture is not possible.

6 Conclusion and further research

The presence of this functional form was discussed in the paper cited below[1]. Moreover, this forced us to look for alternate experimental methods for determining concentrations of bacteria in co-cultures.

7 References

[1] C. Waltham, J. Boyle, B. Ramey, and J. Smit, *Light scattering and absorption caused by bacterial activity* in water. Appl. Opt. 33, 7536-7540 (1994).