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Growth Curves of *S. elongatus* under salt stress and high carbon

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When liquid media is inoculated with bacteria and the cell population is counted at intervals, it is possible to plot a typical bacterial growth curve that shows the increase in the number of cells over time. Such growth curves show four distinct phases of growth:1

Lag phase: There is slow growth or lack of growth due to the physiological adaptation of cells to culture conditions or the dilution of exoenzymes (due to initial low cell densities).
Log or exponential phase: Optimal growth rates are seen in this phase. Cell numbers double at discrete time intervals known as the mean generation time.
Stationary phase: During this phase, the growth (cell division) and death of cells occur at the same rate, resulting in the number of cells being constant. The reduced growth rate is usually due to a lack of nutrients and/or a buildup of toxic waste constituents.
Decline or death phase: Here, the death rate exceeds the growth rate, resulting in a net loss of viable cells.

This is one of the simplest methods used to analyze trends in growth because it uses a spectrophotometer to track changes in the optical density (OD) over time. In other words, as the number of cells in a sample increases, the transmission of light through the sample will decrease.2

Growth curves for certain freshwater cyanobacterial species are carried out under salt stress to account for sucrose production in the particular strain. This is because sucrose is naturally produced intracellularly in these strains to balance the osmotic pressure of a saline environment.3

This experiment was carried out in two iterations: one at 0.04% of carbon dioxide - i.e, ambient carbon dioxide from the atmosphere - and the other with bicarbonate added to be equivalent to 0.6% of carbon dioxide. Find our results for these iterations here.

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1 Grow **100 mL** of culture in a **250 mL** flask in CoBG-11 medium.

2 To this add bicarbonate equivalent to 0.6% carbon dioxide., or 2 g/L of sodium bicarbonate in CoBG-11

3 12 hours later, take 3 **1 mL** samples from each flask in 2 microcentrifuge tubes

4 Use one set of samples to take the OD measurements

5 Take the other set of samples and centrifuge at **4000 rpm, 25°C, 00:07:00** 7m

6 Take the supernatant in fresh microcentrifuge tubes and store it at **-20 °C** , for later analysis. Discard the pellets.

7 Take the readings every twelve hours until the OD_{730} value reaches 1.0

8 Add the bicarbonate at regular intervals of initially once every 24 hours, increasing the frequency if the culture turns a dull yellow.

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