GROWTH RATE AND GENERATION TIME DETERMINATIONS

Growth curves are prepared from cell density data obtained with a hemocytometer or electronic particle counter on cultures sampled at intervals, such as once per day, depending on the growth rate of the alga. Plots of number of cells against time (in days) (Fig. 1), and from these curves specific growth rate or growth constant ($\mu$), division or generation time ($T_g$) and $Y_{max}$ can be calculated.

![Growth Curve](image)

**Fig. 1 Growth curve for a typical algal batch culture.**

1. Lag Phase
2. Exponential Phase
3. Declining Growth Phase
4. Stationary Growth Phase
5. Death Phase

**Determination of Specific Growth rate ($\mu$)**

Use the data obtained from the last lab to prepare growth curves for each nutrient concentration.

After the graphs of growth curves have been plotted, the next step is to calculate the specific growth constant ($\mu$) of the exponential growth phase. Since the curve of the exponential growth phase is constantly increasing it is difficult to find a "straight" line portion to measure. One solution is to calculate the natural logarithm ($ln$) or the common logarithm (log) of each of the average daily cell densities and then

- plot these logarithmic values against time in days. The exponential part of the curve will now be a straight line.
- Identify the portion of the graph that is linear which represents the **exponential growth phase**.
- Fit, by eye, a straight line through the points at this phase.
From this line, chose any two points which lie within the exponential growth phase (Fig. 2). The first of these points represents the \( \ln \) of the cell number at time zero \((t_0)\), ie. \((\ln N_0)\) and the second number represents the \( \ln \) of cell number at time "one" \((t_1)\), ie. \((\ln N_1)\). If the points which correspond to the exponential growth phase are scattered, it may be necessary to fit a straight line through the points by performing a "linear regression". From the straight line fit you can calculate the \( \ln \) values for the \( t_0 \) and \( t_1 \) in days.

![Example of estimation of data points.](image)

Once you have determined the values of \((\ln N_0)\) and \((\ln N_1)\), the specific growth rate \((\mu)\) is then calculated using the formula:

\[
\mu = \frac{\ln N_1 - \ln N_0}{t_1 - t_0} \quad \text{or} \quad \mu = \frac{\ln \frac{N_1}{N_0}}{t_1 - t_0}
\]

where \(N_1\) and \(N_0\) are number of cells at times \(t_1\) and \(t_0\).

**Determination of Doubling Time "G".**

While the specific growth rate represents a measure of the ability of the organism to grow under a given set of environmental conditions, doubling times are more easily understood or meaningful. The doubling time is simply the time [in days] required for the cells to divide. A large doubling time value means slow growth, while a small doubling time value means rapid growth. Doubling time \((G)\) can be calculated using the following formula:

\[
T_g = \ln(2) \times \mu^{-1}
\]

As \(\ln(2)\) is equal to 0.6931, generation time can be calculated in days with the following equation:

\[
T_g = \frac{0.6931}{\mu}
\]

To calculate in hours, you may multiply the above formula by 24 i.e.

\[
T_g = \left(\frac{0.6931}{\mu}\right)24
\]
Estimation of "Maximum Yield" (Y max)

In any closed system, such as these culture flasks, exponential growth cannot be maintained indefinitely. Factors such as depletion of nutrients, shading, production of toxic waste products or simply overcrowding will lead to a decrease in the rate of growth of the population of algae in the culture by about day 6. At some point a maximum yield (Ymax) will be attained. This is the time in the growth of the culture when the number of cells dying is equal to the number of cells being produced, therefore the total cell number does not change. You may estimate the maximum yield directly from the original growth curve, LN cell number vs. time in days. When reporting the estimate of Ymax, convert the LN cells number back (antilog) to a simple cells value i.e. take the $e^{\text{LN value}}$ where $e = 2.718$.

This Ymax is actually the "carrying capacity" of that culture (environment). That is it is the number of individuals that can be supported by the resources available in that environment. This is one of the most important concepts in ecology.

It is often useful to summarize your results to this point in a summary table:

**Summary of Growth Results**

<table>
<thead>
<tr>
<th>Species</th>
<th>Specific Growth Constant (µ)</th>
<th>Doubling Time (G) (hours)</th>
<th>Ymax (cells/field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td></td>
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<tr>
<td>25%</td>
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<td>50%</td>
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<tr>
<td>100%</td>
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</tbody>
</table>

**Questions**

1. Which of the cultures produced the least amount of growth? The most growth?
2. What do you think would happen if you added even more nutrient to the media?
3. Do you think there would be an upper limit to the number of cells that could be grown in these cultures, even with more nutrients? Why?