Plotting of the Growth Curve and Calculation of Growth Rate

Once you have determined the cells/field for each nutrient concentration each day of your experiment, plot the cells/field against time in days. (Fig. 1). For the purpose of calculation of μ, G, and Ymax, you should plot a separate graph of the cells/field vs. time, for each nutrient concentration. For the final report you may wish to show all growth curves on one graph.

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

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

**Determination of Growth Rate**

**Determination of Specific Growth Constant (μ)**

After the graphs of growth curves have been plotted, the next step is to calculate the specific growth constant (μ) 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 to Log10 of each of the average daily cells / field estimates and plot these log values against time. The exponential part of the curve will now be a straight line.

Now, identify the portion of the graph that is linear and 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 Log10 of the cell number/field at time zero (t0), ie. (Log10 N0) and the second number represents the Log10 of cell number/field at time "one" (t1), ie. (Log10 N1). 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 Log10 values for the t0 and t1 in days.

Fig. 2 Example of estimation of data points.

Once you have determined the values of (Log10 N0) and (Log10 N1), the specific growth constant (µ) is then calculated using the formula:

$$\mu = \text{Log10 } N_1 - \text{Log10 } N_0/t_1 - t_0$$

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

While the specific growth constant 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 hours] 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:

$$G = \left(\frac{\text{Log10 } 2}{\mu}\right) \times 24$$

or

$$G = \left(\frac{0.301}{\mu}\right) \times 24$$

**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, log10 cell number vs. time in days. When reporting the estimate of Ymax, for each herbicide concentration, convert the log10 cells/field. number back to a simple cells/field value.
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 Dose Response Growth Results**

**Species:** ____________________________

<table>
<thead>
<tr>
<th>[Nutrient]</th>
<th>Specific Growth Constant (µ)</th>
<th>Doubling Time (G) (hours)</th>
<th>Ymax (cells/field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 drops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 drop</td>
<td></td>
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<tr>
<td>4 drops</td>
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<td></td>
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<tr>
<td>8 drops</td>
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<tr>
<td>16 drops</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?