SES: Methods in Microbial Ecology, Fall 2017
Problem Set # 3 (*Due 5 Oct 2017*)[*]

As always, remember to show your calculations (note, Word has a good equation editor; ask if you can't find or don't know how to use).

1) For the bacterial productivity lab, report: A) volume of sample incubated, B) plot activity (DPM) of sample verse incubation times (0, 15, 30 min, or whatever incubation times you used), C) from the slope of the resulting regression line, calculate the bacterial production rate in **μmol C l^{-1} d^{-1}**. If the three points are not close to being co-linear, calculate the bacterial production after removing a point, etc., **but justify** why the point was removed or whatever you did. Note, the specific activity (SA) of the added leucine was 0.318 Ci $mmole^{-1}$.

2) From the measured activity of the working solution, calculate the specific activity (SA) of the added ^{14}C leucine. The leucine concentration of the working solution was 12 μM. How does your measured SA compare to that reported by the manufacturer (see Q. 1) above). If it differs from the manufactures, explain why you think it does. (If you didn't get any activity for your sample, show how you would calculate SA using a value of 170,000 DPM).

3) A) Report again the concentration of bacteria you measured using DAPI in the previous lab (use corrected values on graded problem set or posted on class web site). From the bacterial productivity in question 1 and the bacterial concentration from Lab #2, calculate the bacterial specific growth rate in units of d^{-1}. State any assumptions you make in your calculations. B) From the specific growth rate in (A), how long does it take for the bacterial population to double (i.e., doubling time)?

4) A) In the BP assay, we use low concentrations of ^{14}C-Leu (160 nM); why do we use such low concentrations? Or, what would happen if we used high concentrations of ^{14}C-Leu, say 1 mM? B) Why are the parameters used to convert leucine uptake into μmol C considered "pseudo constants"?

5) a) Besides killing the bacteria, what other purpose does the trichloroacetic acid (TCA) serve in the bacterial productivity (BP) method? B) Why did we need to rinse the microcentrifuge tubes with TCA and ethanol in the BP method?

6) A) In the bacterial productivity method all bacteria are killed in the "blank" (i.e., sample that is killed with TCA at time zero), so why is radioactivity detected in the "blank"? B) How do you know from your BP data that all the ^{14}C-leucine you added was **not** consumed after 30 min of incubation?

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[*] (There are more questions of the backside of this handout)

7) A) How does one remove or minimize the extracellular isotope dilution problem with the bacterial productivity method? B) Why isn't ^{14}C-glucose used to measure bacterial production?

8) a) What was the intracellular dilution factor for leucine that Simon and Azam measured? b) Briefly state the two ways Simon and Azam measured the intracellular isotope dilution.

9) Calculate the specific growth rate (in d^{-1}) of a bacterial culture that increases in population from 1.0×10^6 cells ml^{-1} to 3.0×10^6 cells ml^{-1} in 5.5 days. Assume exponential growth and that bacteria are not being preyed on (i.e., lab culture).

10) A) How much faster (or slower) will 1 L of bacteria 0.1 μm in diameter grow compared to 1 L of bacteria 1.0 μm in diameter? (volumes are total cell volumes, not media volumes) B) Will a *single* 1.0 μm diameter bacteria grow faster than a *single* 0.1 μm diameter bacteria? Explain, and think about the differences between growth rate and specific growth rate. Also, assume growth rate is solely proportional to the surface-area of a cell.