Remediation of soil and groundwater
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Problem set #2: microbial processes and monitoring
Solution

Problem 1:

k=18 µmol/(day.mg protein)
In order to calculate the minimum concentration of VC that will support growth (C_{min}), we need to determine b, the decay rate, Ks, the half-velocity coefficient, and Y, the growth yield coefficient.

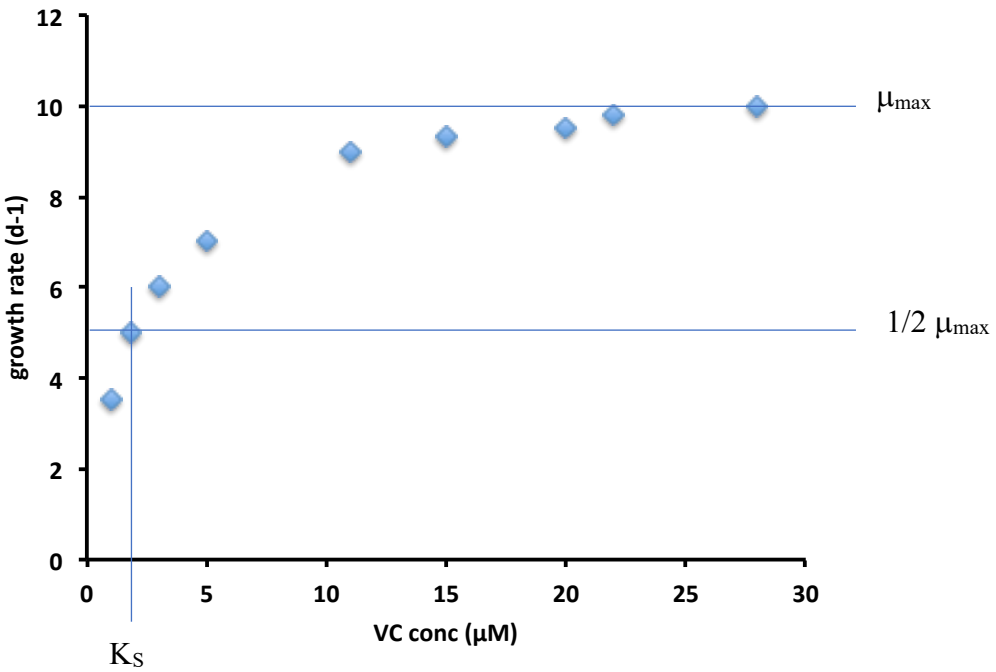
According to the following equation, those values would allow the calculation of C_{min}

$$C_{\min} = \frac{bK_S}{Yk - b}$$

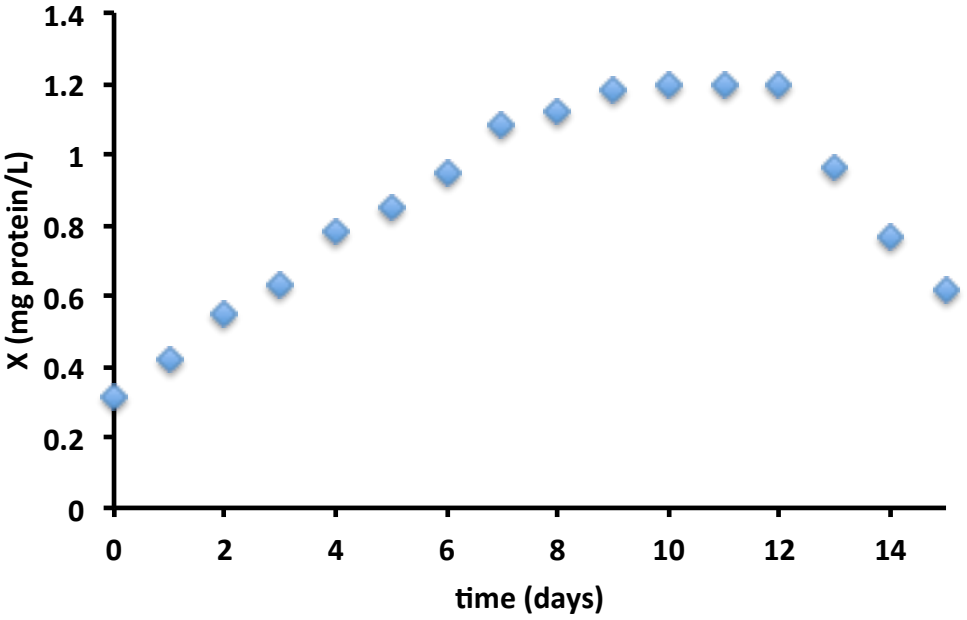
The data provided are the growth rate vs. VC concentration and the microbial biomass as a function of time.
From the former, we can obtain K_S and Y

We plot the data and observe that K_S=1.8 µM and µmax=10 d^{-1}. We can calculate Y because $Y=\mu_{max}/k$

so Y=10 day^{-1} /18 µmol/(day.mg protein)= 0.55 mg protein/µmol VC



From the second data set, we can estimate b. Let's plot the data.



We observe a decay in the protein concentration after 12 days corresponding presumably to depletion of the substrate. To evaluate the value for b, we will assume that the substrate is depleted. Hence,

$$\frac{dX}{dt} = -bX$$

so, we can rewrite this expression as

$$b = \frac{\ln(X_0) - \ln(X)}{t}$$

Hence, b=ln(1.2)-ln(0.61)/(15-12)= 0.22 day^{-1}

Now, we can calculate C_{min}

$$C_{min} = \frac{0.22\ (day^{-1}) * 1.8\ \mu M}{0.55\ mg\ protein/\mu mol * 18\ \mu mol/(day.mg\ protein) - 0.22\ day^{-1}} = 0.04\ \mu M$$

This is a reasonable if you consider that we are looking for a growth rate of zero which can be extrapolated from the first graph.

Problem 2:

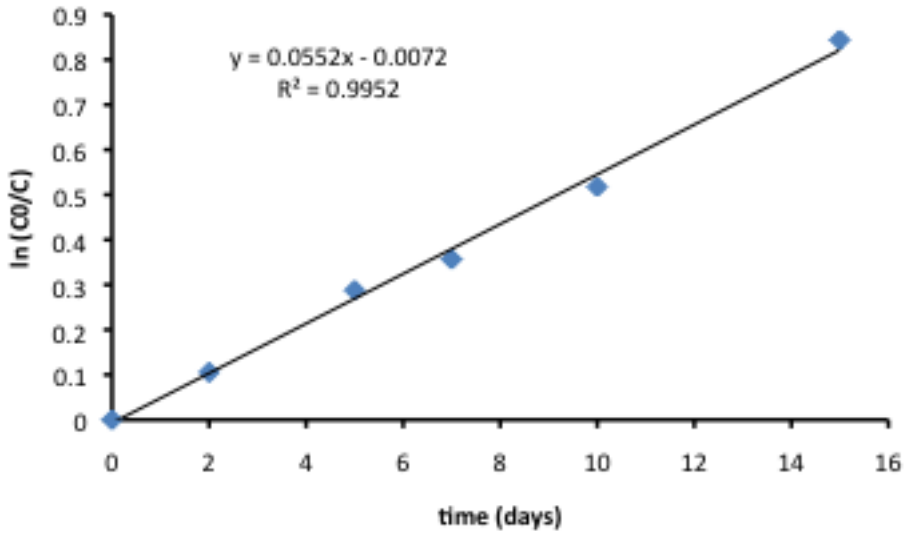
a- For first-order kinetics:

$$C = C_0 e^{-kt}$$

A linearized form of the equation is

$$ln\frac{C_0}{C} = \frac{\mu_{max} B_0}{Y K_S}\ t = kt$$

A plot of this function results in k, the slope of the curve. The value for k obtained is k=0.055/day



b- The half-life of phenanthrene is the time needed to degrade 50% of the contaminant initially present.

$$t = \frac{-1}{k} \ln\frac{C}{C_0} = -\ln\frac{0.5}{0.055} = 12.6\, days$$

Problem 3:

In this case, the goal is to determine whether toluene monooxygenase or dehalogenase is active.

This calls for RNA-based techniques since we are looking for the activity of microorganisms rather than their presence. Bacteria present in the subsurface could bear one or the other enzyme but not be active. Thus, a single measurement of DNA would not inform us on whether one or the other process is important. However, we could also follow quantitatively the concentration of the genes encoding either enzyme over time and if the concentration of one is increasing, we can conclude that the corresponding process is taking place.

The RNA based technique of choice would be: extracting RNA from groundwater followed by a reverse transcription and a quantitative PCR for each of the genes. Thus, we would know how much mRNA was present encoding each of the genes and we could compare the values.

The DNA based technique would require an evaluation of the concentration of the genes over time. So DNA would be extracted, and qPCR run with primers for each gene. An increase in the concentration of either gene would indicate growth of the corresponding bacteria and thus, indirectly, denotes that the process is taking place.