

## SES: Methods in Microbial Ecology, Fall 2019

### Problem Set 4 (Due 10 Oct 2019)

- 1) For each enzyme that was assayed, make a table that includes: time, MUF concentration of the measured sample, sample dilution, and actual MUF concentration. *That is, make sure you account for dilution* (we started with 5x dilution, but you may have done further dilutions). Make a plot of *actual* MUF concentration versus time. Include proper axes and graph labels and units (**graphs will be graded for proper presentation**). Use linear regression to determine  $V^{\text{MAX}}$  for each enzyme assayed. *Please plot the data with regression regardless of their quality or if they produce negative slopes*; however, data points can be removed to determine  $V^{\text{MAX}}$  **if justified**. Report  $V^{\text{MAX}}$  in units of  $\mu\text{mol l}^{-1} \text{d}^{-1}$  (15 points).
- 2) A) What is the difference between extracellular enzymes and ectoenzymes? (2 points) B) Briefly explain how you might modify the enzyme assay to measure activity of extracellular enzymes only (4 points). C) What is an apoenzyme (2 pts)? D) Name the three main metabolic pathways responsible for synthesis of building block compounds (2 pts).
- 3) A) Why is the fluorescence of the MUF fluorogenic substrate measured at high pH (2.5 points)? B) What does an enzyme (or catalyst) do that allows a reaction to proceed? (2.5 pts)
- 4) A) Why do bacteria produce extracellular and ecto- enzymes? (5 pts) B) What is the advantage of producing ectoenzymes? (2 pts) C) What is the advantage of producing extracellular enzymes? (3 pts)
- 5) A) Why are the MUF fluorogenic substrates added at high concentrations in the enzyme assays? (5 pts) B) What is "high concentration" in reference to? (5 pts) *For both parts, assume that there is no natural substrate present in the sample.*
- 6) Give the expression for Michaelis-Menten kinetics. A) How do enzyme kinetics proceed when the substrate is at high concentration relative to  $K_M$  (*show equation*), versus B) the kinetics when the substrate is at low concentration relative to  $K_M$  (*show equation*)? C) What assumption must be invoked to derive the Michaelis-Menten equation? D) What is  $V^{\text{MAX}}$  proportional to? (each 2.5 pts)
- 7) Why doesn't the reaction rate measured in the assay reflect the actual rate that is occurring in the sampled system? Assume the fluorogenic substrates are perfect analogs for the natural substrates. (10 pts)
- 8) How would you modify the enzyme assay so that the Michaelis-Menten parameter,  $K_M$ , could also be determined? Briefly describe. (10 pts)
- 9) Say you measure high activity of phosphatase. What might this imply about the ecosystem sampled? Briefly explain. (10 pts)
- 10) We did not run a control (sometimes called a blank) in our enzyme assay method. Describe the purpose of running a control, and briefly how you might conduct such a control. (6 pts).
- 11) What are the six major internationally recognized classes of enzymes? (4 pts)