

Homework #1 - SOLUTIONS

Due: Oct 05, 23:59

1. Derive an equation that describes the rate of an enzyme reaction (v) under uncompetitive inhibition as a function of v_m , K_M , $[S]$, $[I]$, and K_I' where

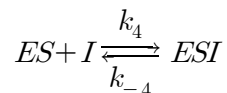
v_m = maximum enzyme reaction rate under no inhibition

K_M = half-velocity constant under no inhibition

$[S]$ = substrate concentration

$[I]$ = uncompetitive inhibitor concentration

$K_I' = k_{-4}/k_4$, where k_4 and k_{-4} are forward and backward reaction rate constants for the formation of the enzyme-substrate-inhibitor complex (ESI) from the enzyme-substrate complex (ES) and uncompetitive inhibitor (I), as illustrated in the following.



Use the equation you derived to explain how the maximum enzyme reaction rate and half-velocity constant changes due to the presence of an uncompetitive inhibitor.

(50 points)

Solution)

$$\text{from } [ES] = \text{constant: } k_1[E][S] + k_{-4}[ESI] = k_{-1}[ES] + k_2[ES] + k_4[ES][I] \quad (1)$$

$$\text{similarly, by assuming } [ESI] = \text{constant: } k_4[ES][I] = k_{-4}[ESI]$$

$$[ESI] = \frac{k_4}{k_{-4}} [ES][I] = \frac{1}{K_I'} [ES][I], \text{ where } K_I' = \frac{k_{-4}}{k_4}$$

Because eq (1) reduces to $k_1[E][S] = k_{-1}[ES] + k_2[ES]$, therefore

$$[E] = K_M \frac{[ES]}{[S]}$$

Enzyme mass balance: $[E]_{total} = [E] + [ESI] + [ES]$

The rate of enzyme reaction under uncompetitive inhibition:

$$\begin{aligned} v &= v_m \frac{[ES]}{[E]_{total}} = v_m \frac{[ES]}{K_M \frac{[ES]}{[S]} + \frac{1}{K_I'} [ES][I] + [ES]} \\ &= v_m \frac{[S]}{K_M + \left(1 + \frac{[I]}{K_I'}\right) [S]} = v_m \left(1 + \frac{[I]}{K_I'}\right)^{-1} \frac{[S]}{\left(1 + \frac{[I]}{K_I'}\right)^{-1} K_M + [S]} \end{aligned}$$

$$\text{from } [ES]=\text{constant: } k_1[E][S] + k_{-4}[ESI] = k_{-1}[ES] + k_2[ES] + k_4[ES][I] \quad (2)$$

$$\text{similarly, by assuming } [ESI]=\text{constant: } k_4[ES][I] = k_{-4}[ESI] \quad (3)$$

$$[ESI] = \frac{k_4}{k_{-4}} [ES][I] = \frac{1}{K_I'} [ES][I], \text{ where } K_I' = \frac{k_{-4}}{k_4}$$

From (3), we find (2) reduces to $k_1[E][S] = k_{-1}[ES] + k_2[ES]$. Therefore,

$$[E] = K_M \frac{[ES]}{[S]}$$

Enzyme mass balance: $[E]_{total} = [E] + [ESI] + [ES]$

The rate of enzyme reaction under uncompetitive inhibition:

$$v = v_m \frac{[ES]}{[E]_{total}} = v_m \frac{[ES]}{K_M \frac{[ES]}{[S]} + \frac{1}{K_I'} [ES][I] + [ES]}$$

$$= v_m \frac{[S]}{K_M + \left(1 + \frac{[I]}{K_I'}\right)[S]} \quad (4)$$

(4) can be rearranged to:

$$v_m \left(1 + \frac{[I]}{K_I'}\right)^{-1} \frac{[S]}{\left(1 + \frac{[I]}{K_I'}\right)^{-1} K_M + [S]} = v_m^* \frac{[S]}{K_M^* + [S]}$$

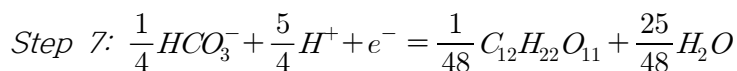
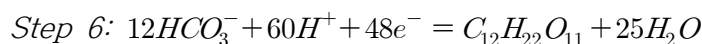
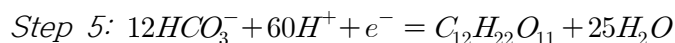
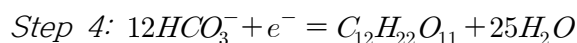
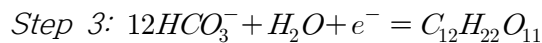
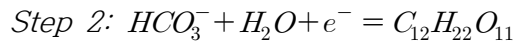
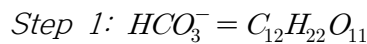
$$\text{where } v_m^* = \left(1 + \frac{[I]}{K_I'}\right)^{-1} v_m, \quad K_M^* = \left(1 + \frac{[I]}{K_I'}\right)^{-1} K_M$$

- Effect: decreased v_m and K_M (the graph shifts down and to the left)

2. You want to develop an eco-friendly and cost-effective process for removal of nitrate (NO_3^-) from groundwater. Your plan is to supply molasses, a byproduct of sugar manufacturing, as an e^- donor to enhance denitrification in groundwater. Assuming that the molecular formula of molasses can be represented by $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ (same as that for sugar), answer the following.

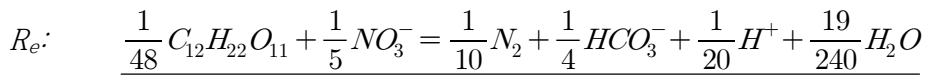
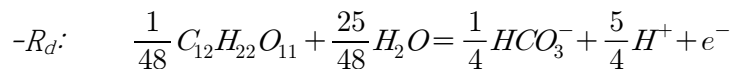
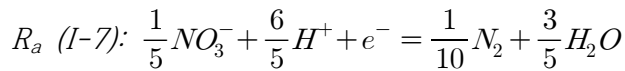
1) Write the electron donor half reaction, R_d , in an electron-equivalent form. Use HCO_3^- as an only form of an oxidized carbon species. (20 points)

Solution)



- 2) Write the energy reaction, R_e , in an electron-equivalent form. How much grams of molasses are needed per g of $\text{NO}_3\text{-N}$ consumed for the energy reaction? (20 points)

Solution)

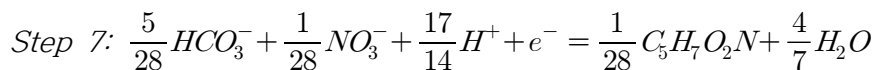
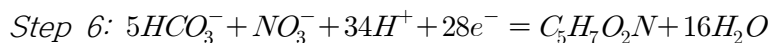
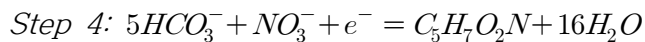
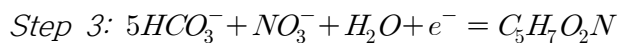
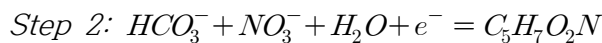
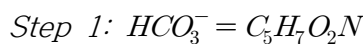


Molasses molecular weight: $12 \times 12 + 1 \times 22 + 16 \times 11 = 342$

$$g \text{ molasses needed/g } \text{NO}_3\text{-N consumed} = \frac{\frac{1}{48} \text{ mole} \times 342 \text{ g molasses/mole}}{\frac{1}{5} \text{ mole} \times 14 \text{ g } \text{NO}_3\text{-N/mole}} = 2.54$$

- 3) Write the cell formation half reaction, R_c , in an electron-equivalent form. Use the cell formula of $\text{C}_5\text{H}_7\text{O}_2\text{N}$ and NO_3^- as a source of nitrogen (not NH_4^+). Also use HCO_3^- as an only form of oxidized carbon species. (20 points)

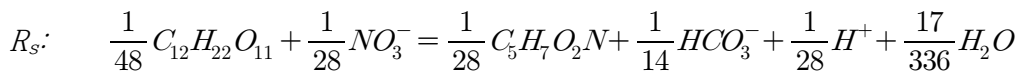
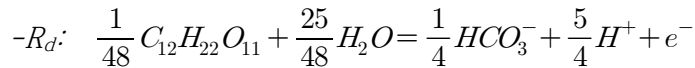
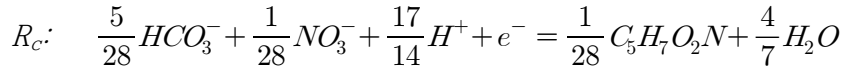
Solution)



- 4) Write the overall cell synthesis reaction, R_s , in an electron-equivalent

form. Use the R_d derived from 1) and the R_c derived from 3). How much grams of molasses are needed per g of $\text{NO}_3\text{-N}$ consumed for the cell synthesis reaction? (20 points)

Solution)



$$g \text{ molasses needed/g } \text{NO}_3\text{-N consumed} = \frac{\frac{1}{48} \text{ mole} \times 342 \text{ g molasses/mole}}{\frac{1}{28} \text{ mole} \times 14 \text{ g } \text{NO}_3\text{-N/mole}} = 14.25$$

- 5) From the calculations you did for 2) and 4), which growth state do you think is more favorable for efficient use of molasses? (A) a rapidly growing state or (B) a slowly growing state? Briefly describe the reason for your selection. (10 points)

Solution)

(B)

At a slowly growing state, the f_e value is greater, meaning that the overall stoichiometry is more weighted to R_e than it is for a rapidly growing state. Therefore, at a slowly growing state the amount of molasses needed to remove a gram of $\text{NO}_3\text{-N}$ will be greater (more efficient use of molasses).

- 6) You are planning to control the molasses supply rate and other environmental conditions relevant to the bacterial growth such that a f_s value of 0.05 is achieved. Write the stoichiometry of the overall reaction occurring at this condition. For 1 g of $\text{NO}_3\text{-N}$ consumption, i) how much molasses will be consumed (in g molasses), ii) how much alkalinity will be produced (in g as CaCO_3), and iii) how much biomass will be produced (in g biomass)? (30 points)

Solution)

$$f_s = 0.05, f_e = 0.95$$

$$\begin{aligned} f_s R_s: & 0.001042 C_{12}H_{22}O_{11} + 0.001786 NO_3^- \\ & = 0.001786 C_5H_7O_2N + 0.003571 HCO_3^- + 0.001786 H^+ + 0.002530 H_2O \end{aligned}$$

$$f_e R_e: 0.01979 C_{12}H_{22}O_{11} + 0.19 NO_3^- = 0.095 N_2 + 0.2375 HCO_3^- + 0.0475 H^+ + 0.07521 H_2O$$

$$\begin{aligned} R: & 0.02083 C_{12}H_{22}O_{11} + 0.1918 NO_3^- \\ & = 0.001786 C_5H_7O_2N + 0.2411 HCO_3^- + 0.095 N_2 + 0.04929 H^+ + 0.07774 H_2O \end{aligned}$$

i) molasses consumption

$$\frac{0.02083 \text{ mole} \times 342 \text{ g molasses/mole}}{0.1918 \text{ mole} \times 14 \text{ g } NO_3 - N/\text{mole}} = 2.65 \text{ g molasses/g } NO_3 - N$$

ii) alkalinity production

HCO_3^- : alkalinity-contributing species; H^+ : alkalinity-consuming species

Total alkalinity produced = $0.2411 - 0.04929 = 0.1918$ equivalent (for 1 e-equivalent reaction)

Per g NO_3-N basis,

$$\frac{0.1918 \text{ eq} \times 50 \text{ g as } CaCO_3}{0.1918 \text{ mole} \times 14 \text{ g } NO_3 - N/\text{mole}} = 3.57 \text{ g as } CaCO_3/\text{g } NO_3 - N$$

iii) biomass production

$$\frac{0.001786 \text{ mole} \times 113 \text{ g cells/mole}}{1918 \text{ moles} \times 14 \text{ g } NO_3 - N/\text{mole}} = 0.0752 \text{ g cells/g } NO_3 - N$$