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.

   $$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI$$

   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)

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

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

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

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:

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

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

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

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

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

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

- 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 formular of molasses can be represented by $C_{12}H_{22}O_{11}$ (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)

Step 1: $HCO_3^- = C_{12}H_{22}O_{11}$

Step 2: $HCO_3^- + H_2O + e^- = C_{12}H_{22}O_{11}$

Step 3: $12HCO_3^- + H_2O + e^- = C_{12}H_{22}O_{11}$

Step 4: $12HCO_3^- + e^- = C_{12}H_{22}O_{11} + 25H_2O$

Step 5: $12HCO_3^- + 60H^+ + e^- = C_{12}H_{22}O_{11} + 25H_2O$

Step 6: $12HCO_3^- + 60H^+ + 48e^- = C_{12}H_{22}O_{11} + 25H_2O$

Step 7: $\underline{\frac{1}{4}HCO_3^- + \frac{5}{4}H^+ + e^- = \frac{1}{48}C_{12}H_{22}O_{11} + \frac{25}{48}H_2O}$

2) Write the energy reaction, R_e, in an electron-equivalent form. How much grams of molasses are needed per g of NO_3-N consumed for the energy reaction? (20 points)

Solution)

R_a *(I-7):* $\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O$

$-R_d$: $\frac{1}{48}C_{12}H_{22}O_{11} + \frac{25}{48}H_2O = \frac{1}{4}HCO_3^- + \frac{5}{4}H^+ + e^-$

R_e: $\frac{1}{48}C_{12}H_{22}O_{11} + \frac{1}{5}NO_3^- = \frac{1}{10}N_2 + \frac{1}{4}HCO_3^- + \frac{1}{20}H^+ + \frac{19}{240}H_2O$

Molasses molecular weight: $12\times12+1\times22+16\times11=342$

g molasses needed/g NO₃-N consumed = $\dfrac{\frac{1}{48}\,mole\times342\;g\;molasses/mole}{\frac{1}{5}\,mole\times14\;g\;NO_3-N/mole}=2.54$

3) Write the cell formation half reaction, R_c, in an electron-equivalent form. Use the cell formula of $C_5H_7O_2N$ 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)

Step 1: $HCO_3^- = C_5H_7O_2N$

Step 2: $HCO_3^- + NO_3^- + H_2O + e^- = C_5H_7O_2N$

Step 3: $5HCO_3^- + NO_3^- + H_2O + e^- = C_5H_7O_2N$

Step 4: $5HCO_3^- + NO_3^- + e^- = C_5H_7O_2N + 16H_2O$

Step 5: $5HCO_3^- + NO_3^- + 34H^+ + e^- = C_5H_7O_2N + 16H_2O$

Step 6: $5HCO_3^- + NO_3^- + 34H^+ + 28e^- = C_5H_7O_2N + 16H_2O$

Step 7: $\frac{5}{28}HCO_3^- + \frac{1}{28}NO_3^- + \frac{17}{14}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{4}{7}H_2O$

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 NO_3-N consumed for the cell synthesis reaction? (20 points)

Solution)

R_c: $\frac{5}{28}HCO_3^- + \frac{1}{28}NO_3^- + \frac{17}{14}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{4}{7}H_2O$

$-R_d$: $\frac{1}{48}C_{12}H_{22}O_{11} + \frac{25}{48}H_2O = \frac{1}{4}HCO_3^- + \frac{5}{4}H^+ + e^-$

R_s: $\underline{\frac{1}{48}C_{12}H_{22}O_{11} + \frac{1}{28}NO_3^- = \frac{1}{28}C_5H_7O_2N + \frac{1}{14}HCO_3^- + \frac{1}{28}H^+ + \frac{17}{336}H_2O}$

g molasses needed/g NO_3-N consumed = $\underline{\dfrac{\frac{1}{48}\,mole \times 342\ g\ molasses/mole}{\frac{1}{28}\,mole \times 14\ g\ NO_3 - N/mole} = 14.25}$

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)

<u>(B)</u>

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 slowly growing state. Therefore, at a slowly growing state the amount of molasses needed to remove a gram of NO_3-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 NO_3-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)

$f_s = 0.05$, $f_e = 0.95$

f_sR_s: $0.001042C_{12}H_{22}O_{11} + 0.001786NO_3^-$

$= 0.001786C_5H_7O_2N + 0.003571HCO_3^- + 0.001786H^+ + 0.002530H_2O$

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

R: $0.02083C_{12}H_{22}O_{11} + 0.1918NO_3^-$

$= 0.001786C_5H_7O_2N + 0.2411HCO_3^- + 0.095N_2 + 0.04929H^+ + 0.07774H_2O$

i) molasses consumption

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

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,*

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

iii) biomass production

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