BITS, PILANI – DUBAI CAMPUS
Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Comprehensive Examination

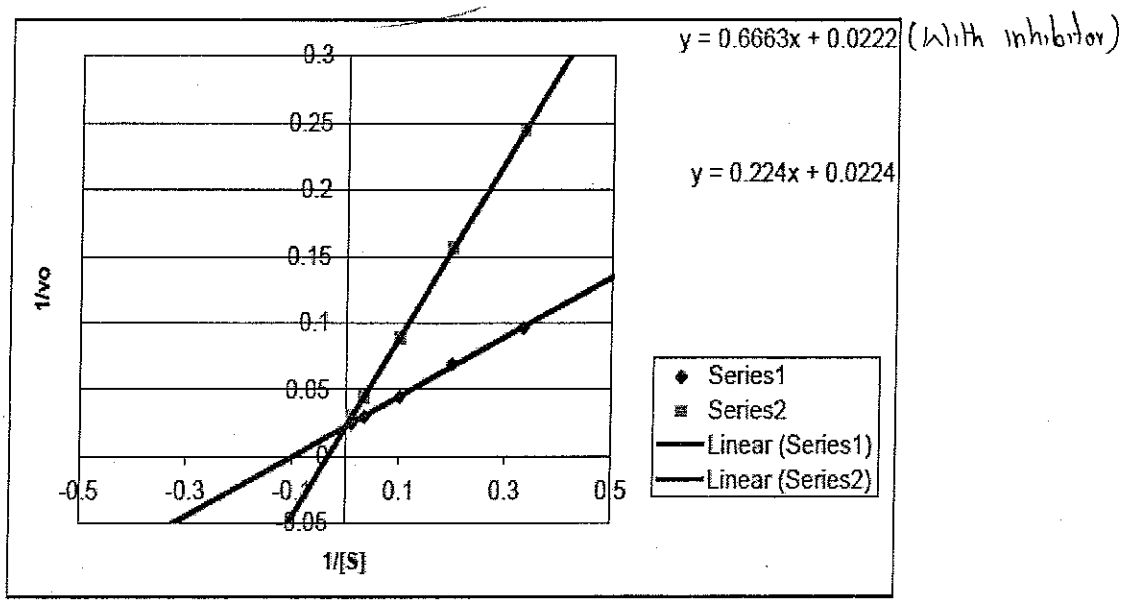
Max.Marks:40 **Date: 3-6-2013(FN)**

Weightage: 40 % **Time: 3h**

---

1. (a) An enzyme catalyzes a reaction at a velocity of 20 μmol/min when the concentration of substrate (S) is 0.01 M. The *K*m for this substrate is 1×10^{-5} M. Assuming that Michaelis-Menten kinetics are followed, what will the reaction velocity be when the concentration of S is 1×10^{-5} M? .**(2)**

   (b) What type of inhibition is shown in the plot? Justify. **(2)**



2. Explain Gel entrapment enzyme immobilization with an example. **(4)**

3. (a) What are saccarifying enzymes? Give an example for the same. **(2)**

   (b)What are cofactors? Give an example. **(2)**

4. Explain the condition for reaction limited regime and diffusion limited regime in immobilization reaction kinetics. **(4)**

5. (a) A strain of mold was grown in a batch culture on glucose. Calculate the doubling time for the cell mass using the data obtained,

Time (h)	Cell concentration(g/L)
0	1.25
40	40

(2)

(b)List the environmental factors that influence the kinetics of cell growth. **(2)**

6. The growth of baker's yeast (*S.cerevisiae*) on glucose may simply be described as,

$$C_6H_{12}O_6 + a\,O_2 + 0.48\,NH_3 \rightarrow b\,\underset{\text{Yeast}}{C_6H_{10}NO_3} + d\,H_2O + eCO_2$$

(i)Determine the yield coefficients $Y_{x/s}$ and $Y_{x/02}$

(ii)Find the respiratory quotients. **(4)**

7. (a) The probability of contamination required for a 10,000 dm^3 vessel containing 10^8 MO /cm^3 is one in 1000.The calculated $\nabla_{heating}$ **and** $\nabla_{cooling}$ are 9.5 and 10.1 respectively. If the specific death rate of *B.stearothermophilus* spores is 2.54 min^{-1} calculate the holding time. **(2)**

(b) Is it possible to operate a Chemostat with a dilution rate greater than the maximum growth rate? Justify **(2)**

8. Explain the need for a Fed Batch Reactor using any one industrial application **(4)**

9. (a) Give the different strategies to recover and purify bio-products.**(2)**

(b)Write a note on the mechanical method used for cell disruption of frozen cell paste. **(2)**

10. What are the problems encountered in the recovery of citric acid from the fermentation broth? Explain the ways it was overcome in a commercial production. **(4)**

x----------x

BITS Pilani
Dubai Campus

II Semester 2012-13.

BIOT C441 Biochemical Engineering

3/6/12 (FN) Comprehensive Examination. Max. Mark: 40.

Answer Key

1 (a). $V = 20 \mu mol\ min^{-1}$; $S = 0.01M$; $k_M = 1 \times 10^{-5} M$.

According to M.M kinetics, $V = \frac{V_{max} S}{k_m + S}$.

When $S >> k_m$. $k_m + S \approx S$.

$\therefore V = V_{max} = 20 \mu mol\ min^{-1}$.

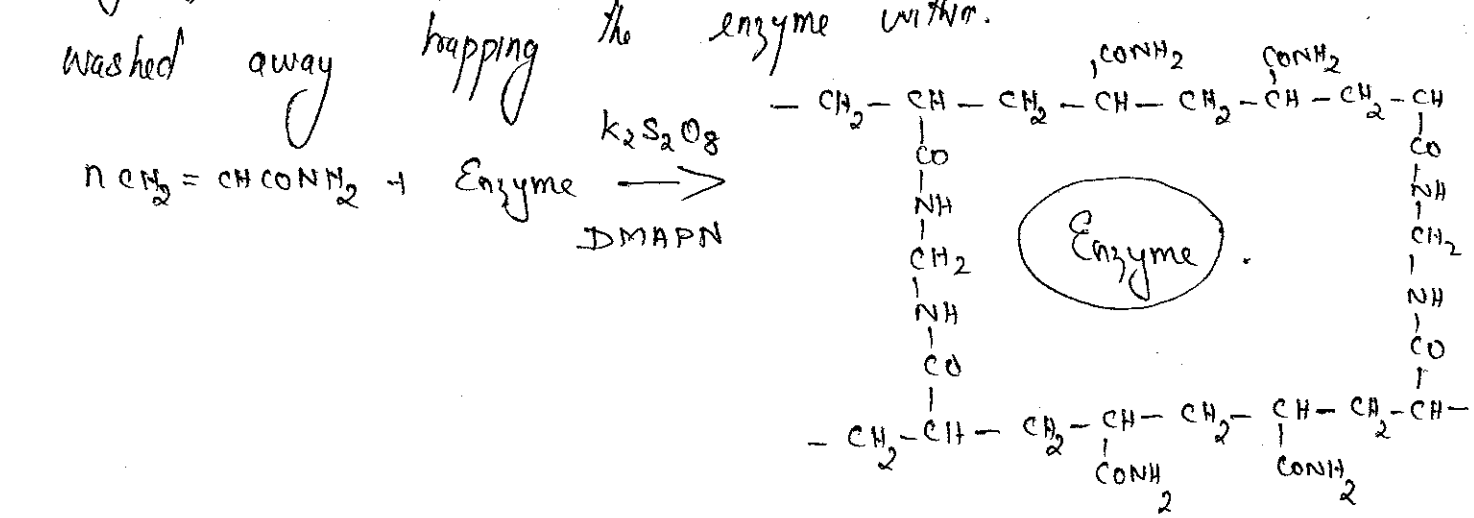
at $S = 1 \times 10^{-5} M$,

$$V = \frac{20 \mu mol\ min^{-1} \times 1 \times 10^{-5}}{2(1 \times 10^{-5})} = 10 \mu mol\ min^{-1}.$$

(b). The Lineweaver - Burk Plot gave the intercept to be same ie $1/V_{max}$ is same.

slope = k_m/V_{max} as the slope for the m with inhibitor increases, it clearly indicates it is a Competitive inhibition.

2. Gel entrapment is the localization of enzyme within the interstitial spaces of cross linked water insoluble polymer gels. It is a physical method of immobilization with a typical pore size of 100-400nm. (eg) immobilization using polyacrylamide. Any substrate larger than the pore size get washed away trapping the enzyme within.



3 (a). Saccharifying enzymes targets the α-1,4 glycosidic bonds in polysaccharides only on the non reducing end there by producing simple sugars. eg: β-amylase amyloglucosidase etc.

(b). Cofactors is a non protein compound which combines with otherwise inactive protein (apoenzyme) to give a catalytically active complex.

(eg) Co^{+2} in glucose isomerase; Mn^{+2} in Arginase.

4. Rn limited Regime ⇒ situation where in the max. enzymatic rn rate is lesser than mass transfer to the surface.

Diffusion limited Regime ⇒ Max. rate of substrat consuption is more than the max. rate of substrate diffusion.

5) a $\ln \frac{x}{x_0} = \mu_{net} \cdot t$.

$\ln \frac{40}{1.25} = \mu_{net} \cdot 40$.

$\frac{3.466}{40} = \mu_{net}$.

$\therefore \mu_{net} = 0.087 h^{-1}$.

$\tau_d = \frac{\ln 2}{\mu_{net}} = \frac{\ln 2}{0.087} = 7.967 h$.

b) Environmental factors that influence the kinetics of cell growth are temperature, pH, D.O, DCO_2, redox potential, ionic strength of the fermentation media etc.

(6). $C_6H_{12}O_6 + a\,O_2 + 0.48\,NH_3 \rightarrow b\,C_6H_{10}NO_3 + d\,H_2O + e\,CO_2$

$M_s = 180$ $\qquad$ $M_x = 144$.

<u>for C</u> $\quad 6 = 6b + e$ — (1) $\qquad$ <u>for H</u> $\quad 12 + 3(0.48) = 10b + 2d$ — (2)

<u>for O</u>

$6 + 2a = 3b + d + 2e$ — (3)

<u>for N</u>

$\boxed{0.48 = b}$ — (4).

Sub (4) in (1) $\qquad 6 = 6(0.48) + e \quad \boxed{\therefore e = 3.12}$ → (5)

~~Sub (5) & (4) in~~

Sub (4) in (2) $\qquad 12 + 3(0.48) = 10(0.48) + 2d$.

$\boxed{d = 4.32}$ — (6).

Sub 6, 5 & 4 in (3).

$6 + 2a = 3(0.48) + 4.32 + 2(3.12)$

$\therefore \boxed{a = 3}$.

(i) $Y_{x|s} = \dfrac{0.48 \times 144}{180} = 0.384\ g^x/g_s$.

(ii) $Y_{x|O_2} = \dfrac{0.48 \times 144}{3 \times 32} = 0.72\ g^x/g_{O_2}$.

(iii) $R.Q = \dfrac{e}{a} = \dfrac{3.12}{3} = 1.04$.

7 a $N_0 = 10^8 \times 10^3 \times 10^4 = 10^{15}$

$N_t = 10^{-3}$.

$$\nabla_{overall} = \ln N_0/N_t = \ln\left(10^{15}/10^{-3}\right) = 41.446$$

$$\nabla_{heating} = 9.5 \; ; \quad \nabla_{cooling} = 10.1.$$

$$\therefore \nabla_{holding} = \nabla_{overall} - \nabla_{heating} - \nabla_{cooling} = 41.446 - 9.5 - 10.1 = 21.9$$

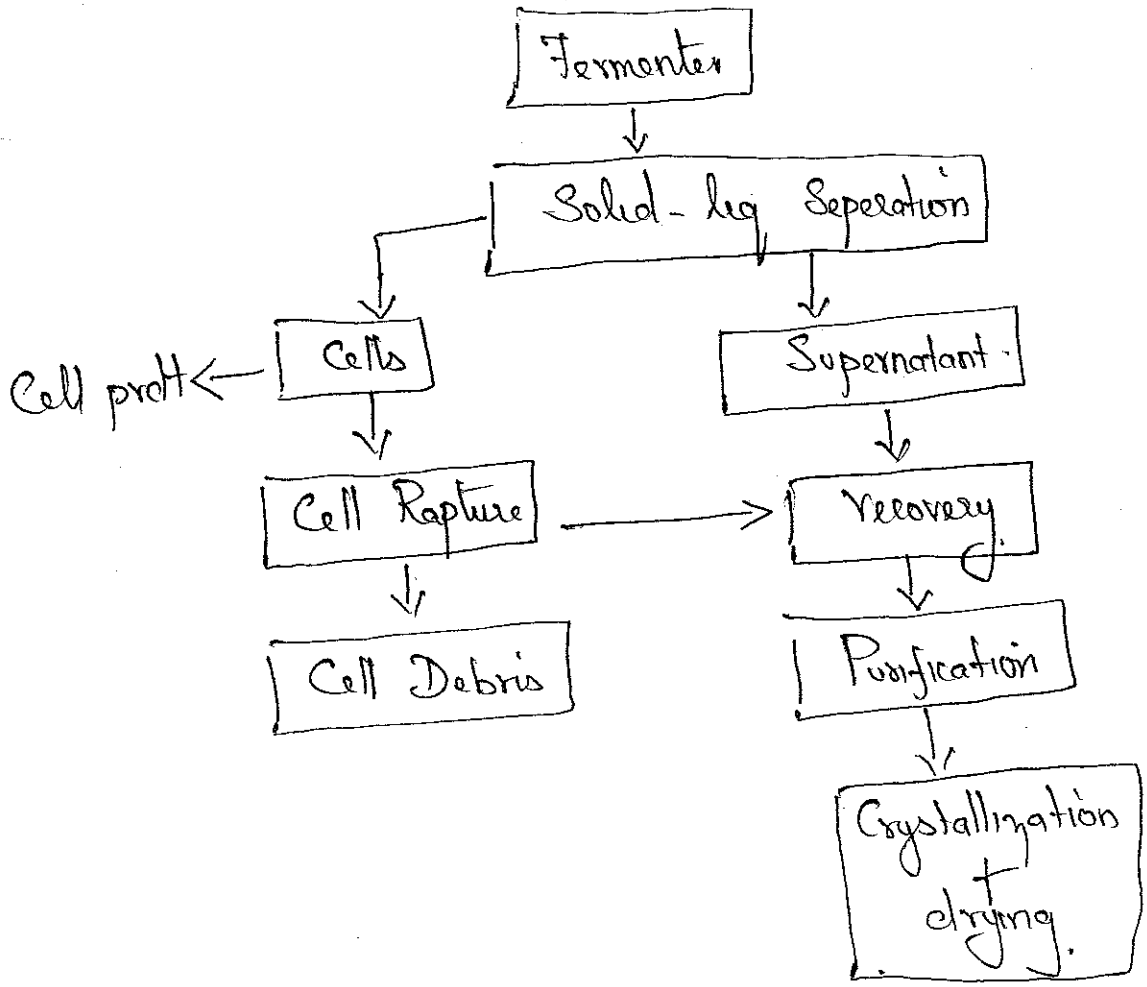
as $\nabla = kt$ $\quad t = \frac{\nabla}{k} = \frac{21.9}{2.54} = 8.6$ min.

2) If the dilution rate is greater than the maximum growth rate then the cell growth cannot keep up with the cells removed from the reactor and after some time the cells would wash out of the reactors.

3). Fed batch culture is important for E. Coli fermentations to make proteins from recombinant DNA technology. To make a high concentration of prdt, ⇒ max growth of biomass is attained if E coli is given excess of glucose growth rate will be good by acetic acid formed will inhibit the growth hence the glucose is supplied in a

fed batch to maintain the growth rate to be slightly less than maximum. In the next stage, biomass is induced to produce target protein.

7(a). Strategies to recover and purify bioproducts



b). For a solid medium such as frozen cell paste or cells attached to or within a solid matrix, Ball mill is preferred for large scale processes. In ball mill, cells are agitated in suspension with small abrasive particles. Cells break because of shear forces to release biomolecules

(10) Fermentation broth for citric acid production is Colloidal in nature hence starting from the filtration moderation is required to recover citric acid. Mycelial mats retain 15% of the prdt hence it is repeated washed till it contains less than 0.2% citric acid.

Oxalic acid is also produced as a by product of fermentation along with citric acid. hence it should be selectively removed using lime in the pH range of 2.7-2.9. After the removal of Cal. oxalate, citric acid is ppted as cal. citrate using lime in the pH of about 7. The ppted cal. citrate is treated with sulphuric acid to get the citric acid. But this process, ppts gypsum which need to be filtered and washed repeatedly to recover as much citric acid as possible. The commercial biochemical method of citric acid require large quantity of lime and sulphuric acid and it also produces equal quantity of gypsum.

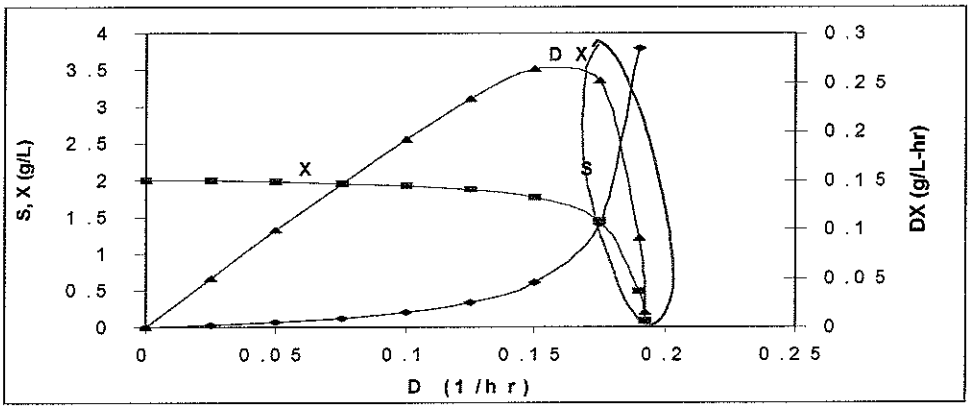
BITS, PILANI – DUBAI CAMPUS
Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Test 2 [Open Book]

Max.Marks:20 **Date: 6-5-2013**

Weightage: 20 % **Time: 50min**

---

1. What is the physical meaning of the Monod constant? **(2)**
2. A chemostat operating in steady-state at a dilution rate of 0.20 h^{-1} sets a limiting nutrient concentration of 0.6 micromoles l^{-1}. Determine the Monod constant in suitable units if μ_{max} for the organism is 0.25 h^{-1} **(3)**
3. Mention any two advantages and disadvantages over choosing a chemostat instead of a batch reactor for bioreactions? **(2)**
4. Explain when the circled observation will happen in a chemostat. **(2)**



5. Pilot sterilization is carried out in a 1000dm^3 vessel with a medium containing 10^9 organisms / cm^3.At the end of the sterilization process, a probability of 1 in 100 contaminations is required. If the fermentation broth is heated from 100° C to 121 °C in 45 min and cooled from 121°C to 100°C in 15 min. Find the holding time *if a value of 12.549 is obtained for B.stearothermophilus assuming a rate of temperature change of 1° min^{-1} and negligible spore destruction below 100°C. The specific death rate of B.stearothermophilus spores at 121°C is 2.54 min^{-1}* **(5)**

6. *Pseudomonas putida* with μ_m =0.5 h^{-1} is cultivated in a continuous culture under aerobic conditions where D=0.28 h^{-1} .The carbon and energy source in the feed is lactose with a concentration of S_0=2g/l .the effluent lactose concentration is desired to be S=0.1g/L.If the growth rate is limited by oxygen transfer by using the following information: **$Y_{x/s}^{M}$ =0.45 gX/gS, $Y_{x/o2}^{M}$ =0.25gX/gO2 and C*=8mg/L**
(a) Determine the steady state biomass concentration (X)**(b)** Determine the specific rate of oxygen consumption (q_{02})(c) What should be the oxygen transfer coefficient (k_la) in order to overcome oxygen transfer limitation(if C_l =2mg/L) **(6)**

Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Test 2 [Open Book]

Max.Marks:20 **Date: 6-5-2013**

ANSWER KEY

Weightage: 20 % **Time: 50min**

---

1. What is the physical meaning of the Monod constant? **(2)**
   The Monod constant is a substrate concentration at which the growth rate of the biomass of microbial cells participating in the reaction is half the maximum growth rate.
2. A chemostat operating in steady-state at a dilution rate of 0.20 h^{-1} sets a limiting nutrient concentration of 0.6 micromoles l^{-1}. Determine the Monod constant in suitable units if μ_{max} for the organism is 0.25 h^{-1} **(3)**

$$\mu_g = D = \frac{\mu_m S}{k_s + S} \Rightarrow k_s + S = \frac{\mu_m S}{D} \quad \therefore k_s = \frac{\mu_m S}{D} - S$$

$$= \frac{0.25 \times 0.6 \mu M L^{-1}}{0.20} - 0.6$$

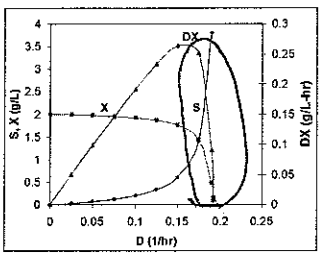
$$\therefore k_s = 0.15 \ \mu M L^{-1}.$$

3. Mention any two advantages and disadvantages over choosing a chemostat instead of a batch reactor for bioreactions?

Adv CHEMOSTAT : Better for growth associated prdts. time wasted in batch. Maintaining Condition is better in chemostat.

Disadv : No flexibility ; Sterilization may be difficult. **(2)**

4. Explain when the circled observation will happen in a chemostat. **(2)**



$\Rightarrow$ Washout

If $D > \mu_m$, the culture cannot reproduce quickly enough to sustain.

5. Pilot sterilization is carried out in a 1000dm^3 vessel with a medium containing 10^9 organisms / cm^3.At the end of the sterilization process, a probability of 1 in 100 contaminations is required. If the fermentation broth is heated from 100° C to 121 °C in 45 min and cooled from 121°C to 100°C in 15 min. Find the holding time if a Δ value of 12.549 is obtained for *B.stearothermophilus* assuming a rate of temperature change of 1° min^{-1} and negligible spore destruction below 100°C. $k = 2.54\ min^{-1}$. **(5)**

$V = 1000\ dm^3$. No. of organism $= 10^9 / cm^3$.

$\therefore$ for $1000\ dm^3 \Rightarrow 10^9 \times 10^3 \times 10^3 = 10^{15} = N_0$.

$N_t = 10^{-2}$ $\therefore \nabla = \ln(N_0 / N_t) = \ln(10^{15} / 10^{-2})$

$\boxed{\nabla_{overall} = 39.14}$

$$\nabla_{heating} = \frac{12.549 \times 45}{21} = 26.89$$

$$\nabla_{cooling} = \frac{12.549 \times 15}{21} = 8.96$$

$$\therefore \nabla_{holding} = \nabla_{overall} - \nabla_{heating} - \nabla_{cooling}$$

$$= 39.14 - 26.89 - 8.96 = 3.35$$

$$\nabla = kt \quad \therefore t = \nabla / k = \frac{3.35}{2.54} = \boxed{1.32\ min.}$$

6. *Pseudomonas putida* with $\mu_m = 0.5\ h^{-1}$ is cultivated in a continuous culture under aerobic conditions where D=0.28 h^{-1}.The carbon and energy source in the feed is lactose with a concentration of S_0=2g/l .the effluent lactose concentration is desired to be S=0.1g/L.If the growth rate is limited by oxygen transfer by using the following information: **$Y_{x/s}^M$ =0.45 gX/gS, $Y_{x/o2}^M$ =0.25gX/gO2 and C*=8mg/L**

(a) Determine the steady state biomass concentration (X)**(b)** Determine the specific rate of oxygen consumption (q_{02})(c) What should be the oxygen transfer coefficient (k_la) in order to overcome oxygen transfer limitation(if C_l =2mg/L) **(6)**

$\mu_m = 0.5 h^{-1}$ $D = 0.28 h^{-1}$. $S_0 = 2 g/l$ $S = 0.1 g/L$.

$Y^M_{x/s} = 0.45 \, g x / g s$ $Y^M_{x/o_2} = 0.25 \, g x / g.o_2$ $c^* = 8 \, mg/L$.

(a). at steady state with growth limited by one substrate

$$X = Y^M_{x/s} (S_0 - S) \Rightarrow 0.45 (2 - 0.1) = 0.855 \, g x / L$$

(b) OUR = OTR. $\Rightarrow q_{o_2} X = \frac{\mu_g X}{Y_{x/o_2}}$.

$$\therefore q_{o_2} = \frac{\mu_g}{Y_{x/o_2}} \qquad \mu_g = D.$$

$$\therefore q_{o_2} = \frac{0.28 h^{-1}}{0.25} = 1.12 \, g.o_2 / g.x.h.$$

(c). $$\frac{\mu_g X}{Y_{x/o_2}} = k_L a (c^* - c_L) = q_{o_2} . X.$$

$$\therefore k_L a = \frac{q_{o_2} . X}{c^* - c_L} = \frac{(1.12)(0.855)}{(8 - 2)}.$$

$$= 0.1596 \times 10^{-3} h^{-1}.$$

4th pm

BITS, PILANI – DUBAI CAMPUS
Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Test 1 [Closed Book]

Max.Marks:25 **Date: 13-3-2013**

Weightage: 25 % **Time: 50min**

---

1. The enzyme fumarase follows a mechanism,

$$E + S \underset{k-1}{\overset{k1}{\rightleftharpoons}} ES \overset{k2}{\rightarrow} P + E$$

where $k_1 = 1\times10^{-9}$ M^{-1} s^{-1}, $k_{-1} = 4.4 \times10^{4}$ s^{-1} and $k_2 = 1\times10^{3}$ s^{-1}.

   a. Derive the rate expression w.r.t substrate concentration using steady state principle**(8)**

   b. What is the value of Michaelis constant for the given enzyme? **(2)**

   c. At an enzyme concentration of 10^{-6} M what will be the rate of product formation when the substrate concentration is 10^{-3}M **(5)**

2. A pesticide inhibits the activity of a particular enzyme, how is it possible to determine if the pesticide is competitive or non competitive inhibitor **(4)**

3. An enzyme with k_m of 1×10^{-3} M was assayed using an initial concentration of 3×10^{-5} M .After 2 min,5% of the substrate was converted, find the V_{max} if the reaction follows Michaelis –Menten Mechanism.? **(4)**

4. Mention an advantage and a disadvantage of chemical method of immobilizing an enzyme **(2)**

TEST-1 Biochemical Engineering ANSWER KEY 13.3.13.

(1) The enzyme fumarase follows a mechanism,

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E.$$ Where $k_1 = 10^9\ M^{-1} s^{-1}$

$k_{-1} = 4.4 \times 10^4\ s^{-1}$ and $k_2 = 10^3\ s^{-1}$

(a) What is the value of the Michaelis Const k_m for this enzyme?

(b) At an enzyme concn of 10^{-6} M, what will be the rate of prdt formn at a substrate concentration of 10^{-3} M?

$$\frac{dP}{dt} = k_2 [ES].$$

Apply S.S.A to ES (ie) $\frac{d[ES]}{dt} = 0$.

$$k_1 [S][E] - k_{-1}[ES] - k_2 [ES] = 0.$$

$$[E] = [E_0] - [ES]$$

$$\therefore\ k_1 [S][E_0] - k_1 [S][ES] - k_{-1}[ES] - k_2 [ES] = 0.$$

$$\frac{k_1 [S][E_0]}{k_1 [S] + (k_{-1} + k_2)} = [ES]$$

$$\frac{dP}{dt} = \frac{k_2 k_1 [S][E_0]}{k_1 [S] + k_{-1} + k_2} = \frac{k_2 [S][E_0]}{[S] + k_m}.$$

(b) $$k_m = \frac{k_{-1} + k_2}{k_1} = \frac{4.4 \times 10^4 + 10^3\ s^{-1}}{10^9\ M^{-1} s^{-1}}.$$

$$k_M = \frac{4.4 \times 10^4}{10^9} = 4.5 \times 10^{-5}\ M.$$

(E) $[E_0] = 10^{-6}\ M$

$[S] = 10^{-3}\ M.$

$$V = \frac{10^3 \times 10^{-3} \times 10^{-6}}{10^{-3} + 4.5 \times 10^{-5}} = 9.569 \times 10^{-4}\ M s^{-1}.$$

$$V_{max} = k_2 [E_0] = 10^3 \times 10^{-6} M = 10^{-3} M s^{-1}.$$

(2). In competitive inhibition, there will be a increase in the apparent value of km.

In non competitive inhibition, km remains constant but there will be a decrease in the Vmax value.

(3). According to M.M eqn, $\frac{ds}{dt} = -\frac{V_{max} S}{k_m + s}.$

$$\therefore \left(\frac{k_m}{s} + 1\right) ds = -V_{max}\, dt$$

at $t \Rightarrow s$; $t_0 \Rightarrow s_0$.

On integrating, $\ln [s]_{s_0}^{s} + [s]_{s_0}^{s} = -V_{max}\, t.$

$$\ln s/s_0 = s_0 - s - V_{max}\, t.$$

$S_0 = 3 \times 10^{-5} M$, $K_m = 1 \times 10^{-3} M$, at $t = 2 min$ $S = 0.95 S_0$

$$\therefore \quad V_{max}\ 2 = 1\times10^{-3}\,M \ \ln \frac{3\times10^{-5}}{3\times10^{-5}\times0.95} + (3\times10^{-5} - 3\times0.95\times10^{-5})$$

$$\therefore \quad V_{max} = 2.639\times10^{-5}\ M\ min^{-1}.$$

(4) <u>Adv</u>

Minimal enzyme leaching

<u>Disadv</u>

loss of active binding sites leads to ↓ activity.

BITS, PILANI – DUBAI CAMPUS
Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Quiz 2 [Closed Book]

Max.Marks:7 **Date: 23-4-2013**

Weightage: 7 % **Time: 20min**

Aerobic degradation of an organic compound by a mixed culture of organism in waste water can be represented by the following reaction

$$C_3H_6O_3 + a\ O_2 + b\ NH_3 \longrightarrow c\ C_5H_7NO_2 + d\ H_2O + eCO_2$$

I. Determine a,b,c,d and e if $Y_{x/s}$ =0.4g X/g S **(2.5)**
II. Determine $Y_{x/o2}$, **(1)**
III. Determine the fraction of available electron incorporated into the biomass **(3)**
IV. RQ for the organism**(0.5)**

BITS, PILANI – DUBAI CAMPUS
Second Semester 2012-13
Course: BIOT C441 Biochemical Engineering
Quiz 2 [Closed Book]

Max.Marks:7 Date: 23-4-2013

Weightage: 7 % Time: 20min

Aerobic degradation of an organic compound by a mixed culture of organism in waste water can be represented by the following reaction

$$C_3H_6O_3 + a\,O_2 + b\,NH_3 \longrightarrow c\,C_5H_7NO_2 + d\,H_2O + e\,CO_2$$

I. Determine a,b,c,d and e if $Y_{x/s}$ =0.4g X/g S **(2.5)**
II. Determine $Y_{x/o2}$, **(1)**
III. Determine the fraction of available electron incorporated into the biomass **(3)**
IV. RQ for the organism**(0.5)**

(I) $Y_{x/s} = 0.4\,g\,x/g\,s = \frac{c\,(113)}{90}$ $\therefore$ $\boxed{c = 0.32}$

for N $\boxed{b = c = 0.32}$

for O $2a + 3 = 2c + d + 2e$

$2a + 3 = 2(0.32) + 2.36 + 2(1.4)$

$\boxed{a = 1.38}$

for C $3 = 5c + e$ $\therefore$ $\boxed{e = 1.4}$

for H $6 + 3b = 7c + 2d$ $6 + 3b - 7c = 2d$

$\therefore$ $\boxed{d = 2.36}$

II $Y_{x/o_2} = \frac{c \times 113}{a \times 32} = \frac{0.32 \times 113}{1.38(32)} = 0.82\,g\,x/g\,o_2$

III

$$\varepsilon_b = c \times \gamma_b / \gamma_s$$

$$\gamma_s = 3 \times 4 + 6 - 6 = 12/3 = 4$$

$$\gamma_{bio} = 5 \times 4 + 7 - 3 - 4 = 20/5 = 4$$

fraction of e^- incorporated into the biomass $= \dfrac{c \cdot \gamma_{bio}}{\gamma_{sub}} = 0.32.$

V

$$RQ = \frac{e}{a} = \frac{1.4}{1.38} = 1.014$$