UNIVERSITI SAINS MALAYSIA

First Semester Examination
2015/2016 Academic Session

December 2015 / January 2016

EKC 271 – Biotechnology for Engineers
[Bioteknologi untuk Jurutera]

Duration : 3 hours
[Masa : 3 jam]

Please check that this examination paper consists of SEVEN pages of printed material and ONE page of Appendix before you begin the examination.

[Sila pastikan bahawa kertas peperiksaan ini mengandungi TUJUH muka surat yang bercetak dan SATU muka surat Lampiran sebelum anda memulakan peperiksaan ini.]

Instruction: Answer **ALL** (4) questions.

*[**Arahan:** Jawab **SEMUA** (4) soalan.]*

In the event of any discrepancies, the English version shall be used.

[Sekiranya terdapat sebarang percanggahan pada soalan peperiksaan, versi Bahasa Inggeris hendaklah diguna pakai.]

Answer ALL questions.

1. [a] [i] What is the significance of K_M and V_{max}?

[ii] Why is the rate of an enzyme-catalyzed reaction proportional to the amount of *ES* complex?

[iii] Give 2 advantages of immobilized enzymes.

[5 marks]

[b] The data shown in Table Q.1.[b] are obtained for an enzyme reaction in the presence and absence of an inhibitor *Y*. Determine the type of inhibition that has occurred. Does the inhibitor *Y* combine with *E*, *ES* or both? Explain and show the mechanism.

Table Q.1.[b]

	V (mmol/min)	
[S] mM	Without *Y*	With *Y*
0.2	5.0	2.0
0.4	7.5	3.0
0.8	10.0	4.0
1.0	10.7	4.3
2.0	12.5	5.0
4.0	13.6	5.5

[10 marks]

[c] An enzymatic assay was carried out under two different sets of conditions using a pure substrate, *S*. The results are tabulated in Table Q.1.[c]:

Table Q.1.[c]

	V (mmol/min)	
[S]/10^{-5} M	Condition *A*	Condition *B*
2.5	8.2	11.6
5.0	12.9	18.9
7.0	15.3	23.0
10.0	17.8	28.0
15.0	20.5	32.6
20.0	22.2	36.0

[i] Plot the data using the Lineweaver-Burke plot.

[ii] Calculate the values of V_{max} and K_M for both sets of conditions.

[iii] Suggest 2 reasons why the two sets of results might be different.

[10 marks]

Jawab <u>SEMUA</u> soalan.

1. [a] [i] *Apakah kepentingan* K_M *dan* V_{mak}*?*

[ii] *Kenapakah kadar tindakbalas bermangkin-enzim berkadaran dengan jumlah kompleks* ES*?*

[iii] *Berikan 2 kelebihan enzim tak boleh gerak.*

[5 markah]

[b] *Data yang ditunjukkan di dalam Jadual S.1.[b] diperolehi dari satu tindakbalas enzim dengan kehadiran dan tanpa kehadiran perencat* Y*. Tentukan jenis perencatan yang telah berlaku. Adakah perencat* Y *bergabung dengan* E, ES *atau kedua-duanya sekali? Terangkan dan tunjukkan mekanisma tersebut.*

Jadual S.1.[b]

	V *(mmol/min)*	
[S] mM	*Tanpa* Y	*Dengan* Y
0.2	*5.0*	*2.0*
0.4	*7.5*	*3.0*
0.8	*10.0*	*4.0*
1.0	*10.7*	*4.3*
2.0	*12.5*	*5.0*
4.0	*13.6*	*5.5*

[10 markah]

[c] *Satu ujian enzim dilakukan di bawah dua set keadaan yang berbeza menggunakan substrat tulen,* S*. Keputusan ujian diberikan di dalam jadual S.1.[c]:*

Jadual S.1.[c]

	V *(mmol/min)*	
[S]/10^{-5} M	*Keadaan A*	*Keadaan B*
2.5	*8.2*	*11.6*
5.0	*12.9*	*18.9*
7.0	*15.3*	*23.0*
10.0	*17.8*	*28.0*
15.0	*20.5*	*32.6*
20.0	*22.2*	*36.0*

[i] *Plotkan data tersebut menggunakan plot Lineweaver-Burke.*

[ii] *Kirakan nilai* V_{mak} *dan* K_M *bagi kedua-dua set keadaan.*

[iii] *Cadangkan 2 sebab kenapa kedua-dua set keputusan mungkin berbeza.*

[10 markah]

...4/-

2. [a] Sketch the structure and describe the functions of each of the organelles:

[i] mitochondria

[ii] chloroplast

[iii] endoplasmic reticulum

[6 marks]

[b] Sketch a general growth curve for a bacterial cell grown under batch fermentation and describe the four main phases on the curve.

[10 marks]

[c] A single continuous-stirred-tank bioreactor (chemostat) operates with a sterile feed. Assuming that Monod growth kinetics applies, show that the cell concentration x_{ss} at steady-states is given as;

$$x_{ss} = Y_{\frac{X}{S}}\left\{s_{in} - \frac{DK_S}{\mu_{max} - D}\right\}$$

where;

$Y_{\frac{X}{S}}$ = yield factor

D = dilution rate

K_S = Monod constant

μ_{max} = maximum growth rate

s_{in} = substrate inlet

[5 marks]

The same chemostat is used to carry out the fermentation of *Lactobacillus acidophilus* and is operating at a dilution rate, D of 0.7 h^{-1} with a sterile feed containing 15 g/L of limiting substrate. The growth parameters for the system are given below;

Monod constant = 1.1 g/L
Maximum growth rate = 0.8 h^{-1}
Yield factor = 0.3 $g_{cell}/g_{substrate}$

[i] calculate the steady-state cell and nutrient concentrations

[ii] calculate the maximum possible dilution rate.

[4 marks]

2. [a] *Lakarkan struktur dan nyatakan fungsi-fungsi setiap organel berikut:*

[i] *mitokondria*

[ii] *kloroplas*

[iii] *endoplasmik retikulum*

[6 markah]

[b] *Lakarkan secara umum lengkuk pembiakan bagi sel bakteria yang membiak di bawah pembiakan kelompok dan nyatakan empat fasa utama pada lengkuk tersebut.*

[10 markah]

[c] *Satu bioreaktor tangki-teraduk-berterusan (kemostat), kinetik pembiakan beroperasi pada suapan steril. Dengan mengandaikan bahawa model Monod digunakan, tunjukkan bahawa kepekatan sel,* x_{ss} *pada keadaan mantap diberi sebagai;*

$$x_{ss} = Y_{\frac{X}{S}} \left\{ s_{in} - \frac{DK_S}{\mu_{\max} - D} \right\}$$

di mana;

$Y_{\frac{X}{S}}$ = *Faktor alah*

D = *Kadar pencairan*

K_S = *Pemalar Monod*

$\mu_{\max}$ = *Kadar pembiakan maksimum*

s_{in} = *Kemasukan substrat*

[5 markah]

Kemostat yang sama telah digunakan untuk menjalankan fermentasi Lactobacillus acidophilus *dan beroperasi pada kadar pencairan,* D $0.7\ j^{-1}$ *dengan suapan steril mengandungi 15 g/L substrat terhad. Parameter-parameter pembiakan bagi sistem tersebut diberi di bawah;*

Pemalar Monod = 1.1 g/L
Kadar pembiakan maksimum = $0.8\ j^{-1}$
Faktor alah = $0.3\ g_{sel}/g_{substrat}$

[i] *kirakan kepekatan-kepekatan sel dan nutrien pada keadaan mantap.*

[ii] *kira kadar pencairan maksimum yang mungkin.*

[4 markah]

3. [a] Write short notes on the following macromolecules:

[i] Carbohydrate

[4 marks]

[ii] Lipid

[4 marks]

[iii] Protein

[4 marks]

[b] Aerobic metabolism and anaerobic metabolism are involved during running and strength training, respectively. Explain the differences between these two types of metabolism.

[13 marks]

4. [a] What method would you use to sterilize the following solutions? Give the reasons and describe the chosen method.

[i] Glucose and amino acid stock solutions for growing *Escherichia coli*

[6 marks]

[ii] Macronutrient stock solution containing NH_4NO_3, KH_2PO_4, $MgSO_4$ and $CaCl_2$ for plant tissue culture.

[6 marks]

[b] A penicillin production medium is to be continuously sterilized at a flow rate of $2m^3/h$ in a sterilizer by direct steam injection in Figure Q.4. The holding section of the sterilizer is a tube, 0.15 m in the internal diameter. The temperature of the holding section is maintained at 120°C, and the time for heating and cooling can be neglected. The bacterial count of the entering medium, 2×10^{12} per m^3, must be reduced to such an extent that only one organism can survive during 30 days of continuous operation. The specific death rate of bacterial spores in the medium is 121 h^{-1} at 120°C. Compute the required length of the holding section of the sterilizer.

[13 marks]

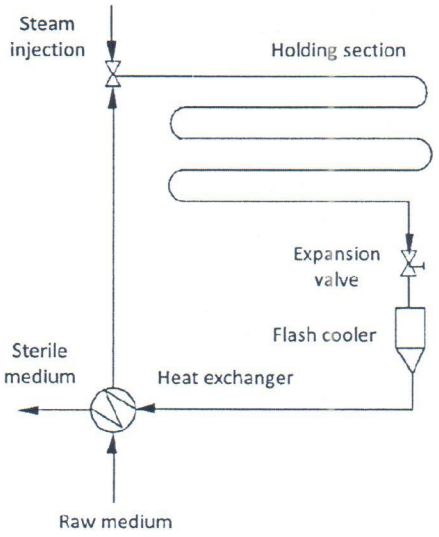


Figure Q.4. Heat exchanger and continuous steam injection

3. [a] *Tulis nota ringkas mengenai makromolekul-makromolekul berikut:*

[i] Karbohidrat

[4 markah]

[ii] Lipid

[4 markah]

[iii] Protein

[4 markah]

[b] *Metabolisma aerobik terlibat semasa berjalan manakala metabolisma anaerobik terlibat semasa latihan kekuatan. Terangkan perbezaan di antara kedua-dua jenis metabolisma.*

[13 markah]

4. [a] *Apakah kaedah yang akan anda gunakan untuk mensterilkan larutan-larutan berikut? Beri sebab-sebab dan terangkan mengenai kaedah yang dipilih.*

[i] Larutan stok glukosa dan asid amino untuk pembesaran Escherichia coli.

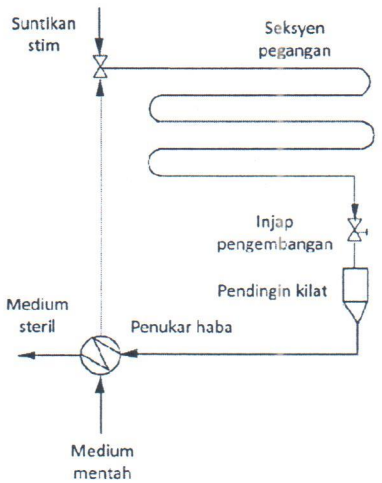
[6 markah]

[ii] Larutan stok makronutrien yang mengandungi NH_4NO_3, KH_2PO_4, $MgSO_4$ dan $CaCl_2$ untuk kultur tisu tumbuhan.

[6 markah]

[b] *Medium pengeluaran penisilin akan disterilkan secara berterusan pada kadar aliran $2m^3/j$ di dalam pensteril melalui suntikan langsung stim di Rajah S.4. Seksyen pegangan pensteril adalah tiub dengan diameter dalaman 0.15 m. Suhu seksyen pegangan dikekalkan pada 120°C dan masa untuk pemanasan dan penyejukan boleh diabaikan. Kiraan bakteria pada medium masuk, 2×10^{12} per m^3, perlu dikurangkan sehingga ke tahap bahawa hanya satu organisma boleh hidup sepanjang 30 hari operasi berterusan. Kadar kematian khusus spora bakteria di dalam medium adalah $121j^{-1}$ pada 120°C. Kira panjang seksyen pegangan pensteril yang diperlukan.*

[13 markah]



Rajah S.4 Penukar haba dan suntikan stim berterusan

- oooOooo -

Appendix

$$-\frac{dn}{dt} = k_d n$$

$$k_d = k_{d0} e^{-E_a/RT}$$

$$\ln\frac{n}{n_0} = -k_{d0}\int_0^t e^{-E_a/RT}\,dt$$

$$\ln\frac{n_f}{n_0} = \ln\frac{n_{heat}}{n_0} + \ln\frac{n_{hold}}{n_{heat}} + \ln\frac{n_f}{n_{hold}}$$

$$\ln\frac{n_0}{n_f} = k_{d0} e^{-E_a/RT}\tau_{hold}$$

$$\tau_{hold} = \ln\frac{n_0}{n}/k_d$$

$$(Pe) = (vL/E_{Dz})$$

slope of regression line

$$m = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\Sigma(x_i - \bar{x})^2}$$

$$b = \bar{y} - m\bar{x}$$

Easier Form of Least Squares Equations

$$m = \frac{\Sigma x_i y_i - [(\Sigma x_i \Sigma y_i)/n]}{\Sigma x_i^2 - [(\Sigma x_i)^2/n]}$$

- n is the number of data points