UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2010/2011 Academic Session

April/May 2011

IBG 311 – ENZYME TECHNOLOGY
[TEKNOLOGI ENZIM]

Duration: 3 hours
[Masa: 3 jam]

Please check that this examination paper consists of EIGHT pages of printed material before you begin the examination.

[Sila pastikan bahawa kertas peperiksaan ini mengandungi LAPAN muka surat yang bercetak sebelum anda memulakan peperiksaan ini.]

Instructions: Answer **ALL (5)** questions. You may answer the questions either in Bahasa Malaysia or in English.

*[**Arahan**: Jawab **SEMUA (5)** soalan. Anda dibenarkan menjawab soalan sama ada dalam Bahasa Malaysia atau Bahasa Inggeris.]*

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].

1. Enzyme inhibition can be either irreversible or reversible.

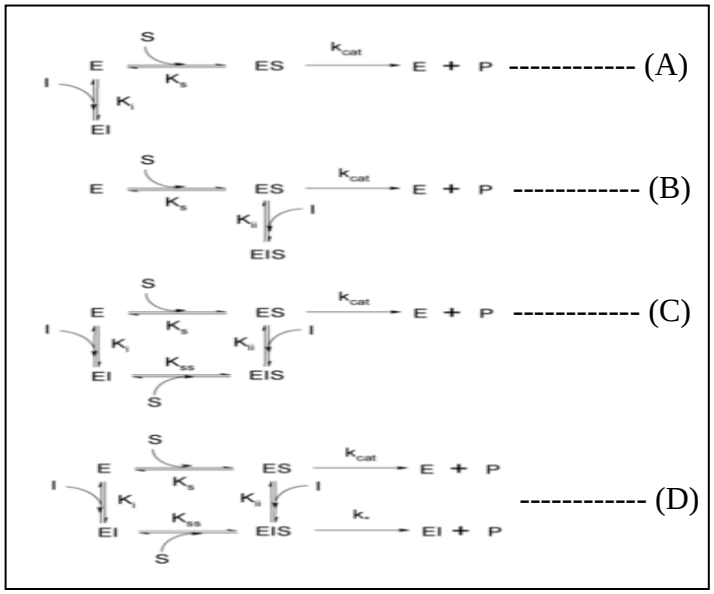

Figure 1: Types of inhibition as classification by W.W. Cleland

(a) With reference to figure1, list and elaborate the different types of reversible inhibition.

(10 marks)

(b) Enzymes can show up to five different degrees of specificity. List All the different degrees of specificity and give an example of enzyme for each.

(10 marks)

2. Enzyme reaction can be affected by several factors which include temperature, pH, enzyme concentration, substrate concentration, salt concentration and the presence of any inhibitors or activators. In addition, different enzymes have different optimum temperature and pH they catalyzed.

(a) Explain in detail why enzyme reactions are slow at low and below the optimum temperature?

(10 marks)

(b) How does pH affect enzyme reaction?

(5 marks)

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(c) With reference to figure 2; explain why the product concentration from an enzyme reaction is increasing from point A to B but become constant (stationary) from point B to C?

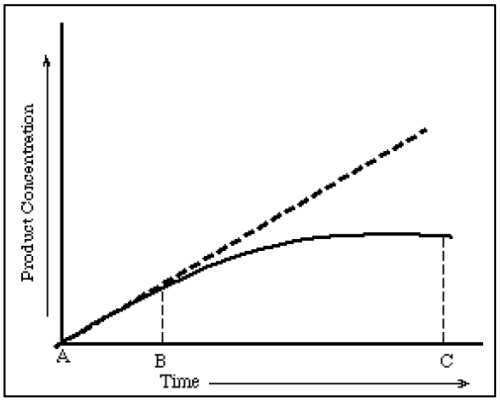


Figure 2: Product formation at different enzyme reaction time

(5 marks)

3. Elaborate the principles of four immobilization methods.

(20 marks)

4. Answer all parts in this question.

Table 1: Enzyme catalyzed reaction (S ⟶ P)

Substrate [S] (M)	**Velocity (*V*) (nmoles x liter^{-1}x min^{-1})**
6.25 x10^{-6}	15.00
7.50 x10^{-5}	56.25
1.00 x10^{-4}	60.00
1.00 x10^{-3}	74.90
1.00 x10^{-2}	75.00

(a) Estimate the V_{max} and K_m.

(5 marks)

(b) What would ***V*** be at [S]=2.5 x10^{-5} M and at [S]=5.0 x10^{-5}M?

(5 marks)

(c) What would ***V*** be at [S]=5.0 x10^{-5}M if the enzyme concentration were doubled?

(5 marks)

(d) The ***V*** given in table 1 was determined by measuring the concentration of product that had accumulated over a 10 minute period. Verify that ***V*** represents a true initial velocity.

(5 marks)

5. Answer all parts in this question.

Table 2: Enzyme purification table

Purification step	Volume (ml)	Total Protein (mg)	Activity (Units)	Total Activity	Specific Activity (Units/mg)	Purification Fold	% Yield
Lysate	10.00	56.30	65897	658970	1170		
Ion exchange (DEAE)	12.50	25.36	42845	535563	1689		
Affinity (Ni-Agarose)	8.25	18.25	51481	424718	2820		
Gel filtration (S-200)	4.30	3.75	48239	207428	12863		
Affinity (Reactive Blue)	3.50	1.28	46578	163023	36389		

(a) Complete purification fold and % yield for each step of the purification in the table above.

(5 marks)

(b) In order to improve this purification process, you need to eliminate one of the purification steps. Which step might you choose? Why?

(5 marks)

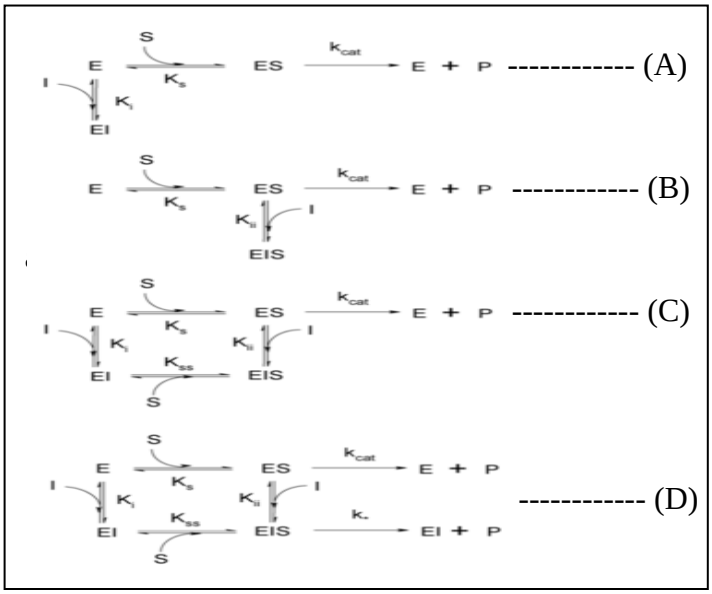
(c) Which step of the purification process gave the best purification? Why?

(5 marks)

(d) Elaborate the principles of gel filtration chromatography.

(5 marks)

1. *Perencatan enzim boleh secara berbalik atau tidak berbalik.*



Gambarajah 1: Jenis perencatan seperti yang diklasifikasikan oleh W.W. Cleland

(a) *Dengan merujuk kepada gambarajah 1, senarai dan huraikan jenis perencatan berbalik.*

(10 markah)

(b) *Enzim dapat menunjukkan sehingga lima tahap kekhususan yang berbeza. Senaraikan semua tahap kekhususan yang berbeza dan memberikan satu contoh enzim untuk setiap tahap kekhususan tersebut.*

(10 markah)

2. *Reaksi enzim boleh dipengaruhi oleh beberapa faktor meliputi suhu, pH, kepekatan enzim, kepekatan substrat, kepekatan garam dan kehadiran perencat atau pengaktif. Selain itu, setiap enzim mempunyai suhu dan pH optimum yang berbeza dimana mereka memangkin.*

(a) *Jelaskan secara terperinci mengapa reaksi enzim lambat pada suhu rendah dan di bawah suhu optimum?*

(10 markah)

(b) *Bagaimana pH boleh mempengaruhi reaksi enzim?*

(5 markah)

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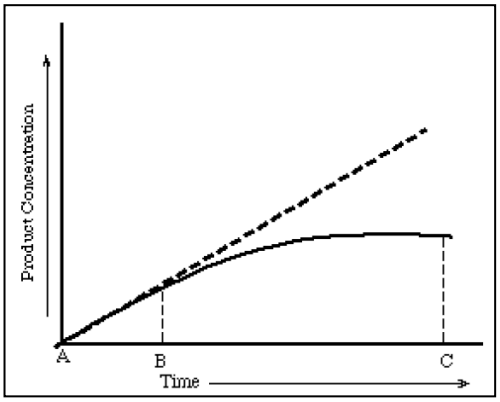
(c) *Dengan merujuk kepada gambarajah 2; jelaskan mengapa kepekatan produk hasil daripada reaksi enzim semakin meningkat dari titik A ke titik B tetapi menjadi malar (pegun) dari titik B ke titik C?*



Gambarajah 2: Pembentukan produk pada masa reaksi enzim yang berbeza

(5 markah)

3. *Hurai prinsip-prinsip empat kaedah penyekat gerak*

(20 markah)

4. *Jawab semua soalan di bahagian ini.*

Jadual 1: Tindak balas enzim bermangkin (S ⟶ P)

Substrate [S] (M)	***Velocity (V) (nmoles x liter^{-1}x min^{-1})***
6.25 x10^{-6}	*15.00*
7.50 x10^{-5}	*56.25*
1.00 x10^{-4}	*60.00*
1.00 x10^{-3}	*74.90*
1.00 x10^{-2}	*75.00*

(a) *Apakah nilai V_{max} and K_m.*

(5 markah)

(b) *Apakah nilai **V** apabila [S]=2.5 x10^{-5} M dan apabila [S]=5.0 x10^{-5}M?*

(5 markah)

(c) *Apakah nilai **V** apabila [S]=5.0 x10^{-5}M, jika kepekatan enzim diganda dua kali?*

(5 markah)

…7/-

(d) *Nilai **V** yang diberikan dalam jadual 1 ditentukan dengan mengukur kepekatan produk yang telah terkumpul selama 10 minit. Sahkan bahawa **V** merupakan kelajuan awal yang benar.*

(5 markah)

5. *Jawab semua soalan di bahagian ini.*

Jadual 2: Jadual penulenan enzim

Purification step	Isipadu (ml)	Jumlah Protein (mg)	Activiti (Units)	Jumlah Activiti	Specifik Activiti (Units/mg)	Ganda Penulenan	% Hasil
Lysate	10.00	56.30	65897	658970	1170		
Ion pertukaran (DEAE)	12.50	25.36	42845	535563	1689		
Afiniti (Ni-Agarose)	8.25	18.25	51481	424718	2820		
Penurasan gel (S-200)	4.30	3.75	48239	207428	12863		
Afiniti (Reactive Blue)	3.50	1.28	46578	163023	36389		

(a) *Lengkapkan bahagian ganda penulenan dan % hasil untuk setiap langkah penulenan dalam jadual di atas.*

(5 markah)

(b) *Untuk menambahbaik proses penulenan ini, anda perlu menyingkirkan salah satu langkah penulenan di atas. Langkah manakah yang anda akan pilih? Kenapa?*

(5 markah)

(c) *Langkah yang manakah dalam proses penulenan tersebut memberi penulenan yang paling baik? Kenapa?*

(5 markah)

(d) *Huraikan prinsip-prinsip kromatografi penurasan gel.*

(5 markah)

Formulas

1 $$v = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

2 $$\frac{1}{v} = \frac{K_{\mathrm{m}}}{V_{\max}} \frac{1}{[\mathrm{S}]} + \frac{1}{V_{\max}}$$

3 $$\frac{[\mathrm{S}]}{v} = \frac{[\mathrm{S}]}{V_{\max}} + \frac{[K_m]}{V_{\max}}$$

4 $$v = -K_m \frac{v}{[\mathrm{S}]} + V_{\max}$$