UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualifications:-

B.Eng. *M.Eng.*

Bioprocess Engineering

COURSE CODE	:	**BENG1004**
UNIT VALUE	:	**0.50**
DATE	:	**12-MAY-06**
TIME	:	**10.00**
TIME ALLOWED	:	**3 Hours**

TURN OVER

Answer FOUR QUESTIONS. ALL questions carry a total of 25 MARKS each, distributed as shown [ ]. Graph paper is provided. Only the FIRST FOUR ANSWERS will be marked.

1.

Using a pure solution of protein X and an Ion Exchange matrix Langmuir adsorption isotherm parameters of Q_{max} – 100mg/ml and K_D – 0.1mg/ml were determined.

i) Replot sufficient data on the graph paper provided to allow you to plot a line of best fit (y-axis 0-100mg/ml & x-axis 0-2mg/ml). [12]

ii) On the same graph plot the data below which has been performed for protein X in an impure mixture from the first step of the purification process for protein X, and sketch a line of best fit. [5]

c* (mg/ml)	Q* (mg/ml)
0	0
0.25	35
0.5	40
1	37
1.5	35
2.0	32

iii) Give possible reasons for the differences between the two lines. [8]

2.

The table below refers to the initial chromatography capture stage of a protein product from the host organism, *Pichia pastoris*. Shown are data for the different phases of the chromatographic procedure, volumes for each stage are shown, as is the product concentration and absorbance area measured with an optical density detector placed directly after the column (detector pathlength – 0.2 cm, protein extinction coefficient 2.0 $L*g^{-1}*cm^{-1}$). The column is 15 cm deep.and 2.9 cm in diameter and is operated at flow rate of 40 column volumes$*h^{-1}$ throughout its operation.

	Volume (L)	Product Concentration (g/L)	Absorbance area at 280nm (AU*L)
Feed	1.0	0.34	2
Equilibration	0.2	0	0
Load Pool	1.1	0.02	1.3
Wash Pool	0.3	0.02	0.025
Elution Pool	0.25	1.2	0.65
CIP Pool	0.2	Not measured	0.025

i) Calculate the yield and purity of product over the step. [9]

ii) Calculate throughput and productivity for the full cycle of operation. [8]

iii) By mass balance with respect to product determine the expected product concentration in the CIP Pool. What is the likely reason for it not being measured? [8]

3.

The following data are given for a batch fermentation of a microbial system grown on a defined medium

Time from inoculation (hours)	Biomass concentration (g/L)
0.0	0.1
0.5	0.1
1.0	0.12
1.5	0.15
2.0	0.21
2.5	0.30
3.0	0.50
3.5	0.82
4.0	1.35
4.5	2.22
5.0	3.66
5.5	6.03
6.0	9.94
6.5	16.38
6.6	18.10
6.7	19.25
7.0	19.25

Calculate:

a) The maximum specific growth rate [12]

b) The doubling time during exponential growth phase. [4]

c) How does the value obtained in b) compare with that expected for an animal cell and why? [4]

CONTINUED

4.

The x-y diagram provided represents the liquid-vapour equilibrium of the binary solvent system A-B, where A is the more volatile component. A feed stream of 80 $kmol.h^{-1}$ containing 0.45 mole fraction A is to be separated to produce distillate and bottoms product containing 0.95 and 0.05 mole fraction A respectively.

(i) Calculate the molar flowrates of distillate and bottoms products. [5]

(ii) Calculate the minimum reflux ratio if the feed stream is a mixture containing equal molar quantities of saturated vapour and saturated liquid. [5]

(iii) Find the number of theoretical stages required for the separation if the column has a partial reboiler and a total condenser and is operated with a reflux ratio of 3.5. [15]

An x-y diagram for the A-B system is supplied

5.

Acetobacter aceti bacteria convert ethanol to acetic acid under aerobic conditions. A continuous fermentation production is proposed using a non-viable *A. aceti* cells immobilised on the surface of gelatine beads which will be retained within the fermenter during operation. The production target is 2 kg h^{-1} acetic acid; however, the maximum acetic acid concentration tolerated by the cells is 12%. Air is pumped into the fermenter at a rate of 200 mol h^{-1}.

(a) What is the minimum amount of ethanol required [8]

(b) What is the minimum amount of water which must be used to dilute the ethanol to avoid acid inhibition [8]

(c) What is the composition of the fermenter off-gases [9]

Additional Data:

Molecular weights: Ethanol = 46
Acetic acid = 60
O_2 = 32
N_2 = 28
H_2O = 18
Composition of air: 21% O_2, 79% N_2

PLEASE TURN OVER

6.

A disc-stack centrifuge is used to recover antibody product from a mammalian cell culture. The processing volume of fermentation broth is 5000 L with 250g/L cell concentration and 4 g/L antibody concentration. The cell density may be assumed to be 1 g/mL. The centrifuge has a solid carry-over of 5% and a dewatering level of 50%. The target yield is 90%. It is know that a single stage centrifugation is not sufficient and a washing stage is necessary.

1. Please calculate the amount of buffer needed in the washing stage in order to reach total 90% yield. [15]

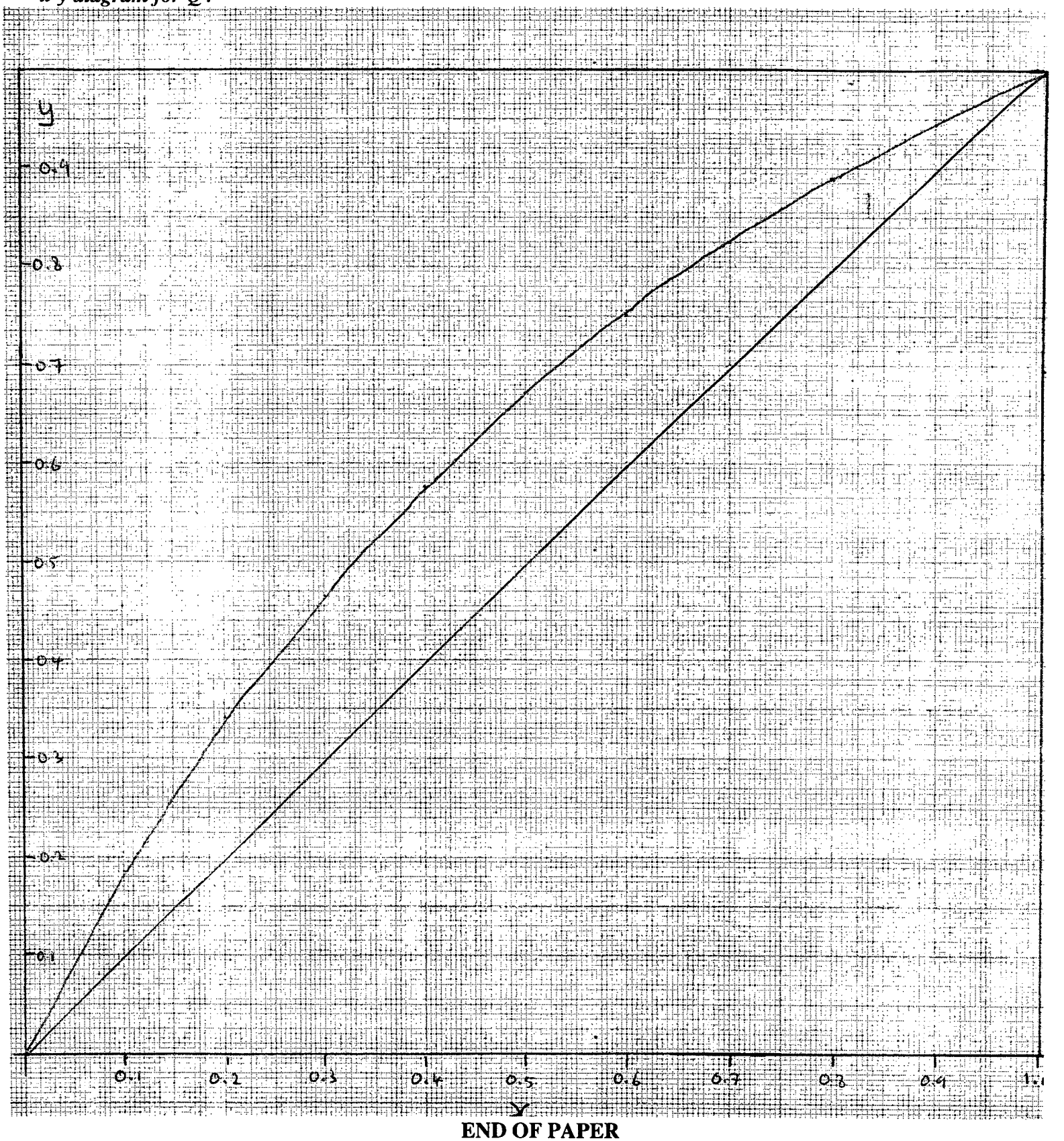
2. List at least 5 assumptions (other than mentioned above). [10]

CONTINUED

VAPOUR-LIQUID EQUILIBRIUM DIAGRAM OF THE BINARY

SYSTEM A (MVC) - B

x-y diagram for Q4



END OF PAPER