UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualification:-

M.Sc.

Biochem Eng G19: Bioprocess Synthesis and Process Mapping

COURSE CODE	:	**BENGEG19**
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). [10]

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. [5]

iv) The isotherms by definition are performed in a particular set of conditions. What conditions are most likely to be investigated to manipulate the isotherm and why? [5]

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.

PLEASE TURN OVER

	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. [7]

ii) Calculate throughput and productivity for the full cycle of operation. [6]

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? [6]

iv) Elution is carried out using a low ionic strength buffer. What does this imply about the mechanism of binding being utilised? [3]

v) The chromatography has revealed some possible product variants. Where might these derive from? [3]

3.

The following stoichiometric equation describes the production of a recombinant protein in a microorganism. Given a respiratory quotient (RQ) of 0.43, determine the coefficients a, b, c, d and e. [15]

Write assumptions made in deriving this equation [5]

$C_{16}H_{34}$+ a O_2+ b NH_3 ------> c $CH_{1.66}O_{0.27}N_{0.20}$(biomass)+ d CO_2 + e H_2O

CONTINUED

4.

i) Briefly describe the applications of distillation in the bioprocessing industries. [5]

ii) 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.

(a) Calculate the molar flowrates of distillate and bottoms products. [4]

(b) Calculate the minimum reflux ratio if the feed stream is a mixture containing equal molar quantities of saturated vapour and saturated liquid. [4]

(c) 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. [12]

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 [6]

(b) What is the minimum amount of water which must be used to dilute the ethanol to avoid acid inhibition [6]

(c) What is the composition of the fermenter off-gases [6]

(d) A new strain of *A. aceti* is developed to withstand up to 30% acetic acid. What is the new composition of the ethanol/water feed stream? [7]

PLEASE TURN OVER

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

6.

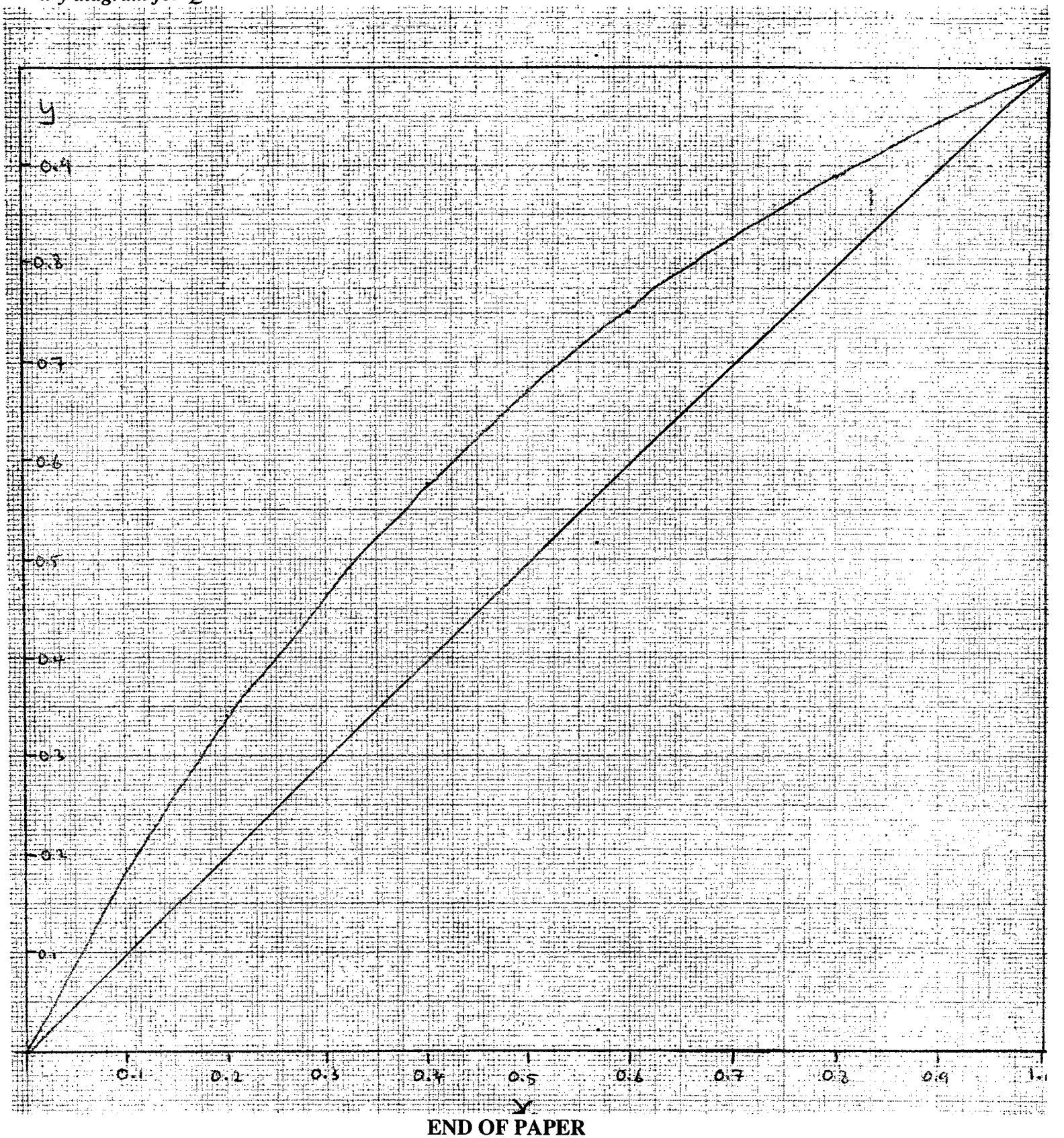
A process development team decided to use microfiltration to recover a therapeutic protein product from a fermentation broth of 1000 L. The product concentration is 1 g/L and the cell concentration is 120 g/L. The cell density may be assumed to be 1 g/mL. The membrane reject co-efficient for cell, product, and liquor are 1, 0.5 and 0 respectively. Both of concentration operation and dialfiltration operation can be utilised in the process development. However the maximum cell concentration in the retentate tank is 400 g/L and the maximum buffer volume available for diafiltration operation is 1000 L. Please design the membrane operation by selecting right concentration factor for concentration step so that the target yield of 85% is achieved. [25]

CONTINUED

VAPOUR-LIQUID EQUILIBRIUM DIAGRAM OF THE BINARY

SYSTEM A (MVC) - B

x-y diagram for Q4



END OF PAPER