UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualification:-

B.Sc.

Biochemical Eng E105: Downstream Processing

COURSE CODE	:	**BENGE105**
UNIT VALUE	:	**0.50**
DATE	:	**20-MAY-03**
TIME	:	**10.00**
TIME ALLOWED	:	**3 Hours**

TURN OVER

*Answer **FOUR QUESTIONS.*** **Only the first four answers will be marked.**
*ALL questions carry a total of **25 MARKS** each, distributed as shown* [ ]

1. The following specification for a microfiltration membrane has been provided to you by a new supplier. Provide an engineering critique of this supporting your analysis by theory wherever possible showing how you would assess each element of the specification. i.e. membrane, module and performance data.
"The membrane material we recommend for you protein concentration step is our latest cellulose acetate material in the form of a hollow – fibre geometry. Typical fluxes will be in the range of 100–200 L/m^2/h. The membrane has a MW cut-off at 25,000 making it ideal for the total rejection of your product at 30,000 whilst ensuring high transmission of all species below this size." [25]

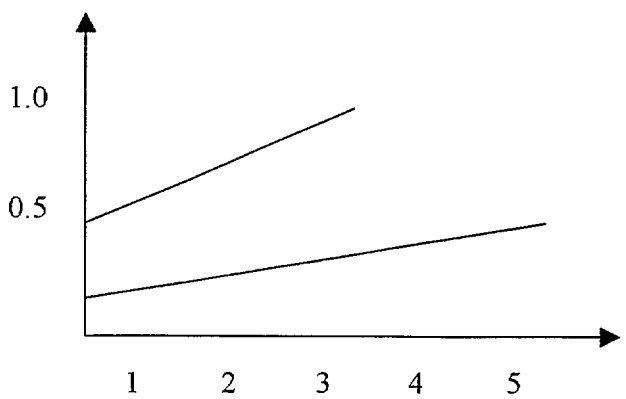
2. The figure below (Figure 1) shows two sets of protein release data obtained from pilot–scale fermentation trials but graph axes have not been defined.

i) Define the axes and the units of measurement. What form of equation may be used to fit such data? Explain the physical basis of the equation. [5]

ii) What reasons can you provide for the differences between the two fermentation samples? [5]

iii) Why does the data not pass through the origin? [5]

iv) In the light of the above what method of cell harvest prior to disruption would you recommend? [10]



(Figure 1)

PLEASE TURN OVER

3.

i) Explain the significance of controlling the distribution of fluid flow during low–pressure chromatography. Your answer should include an analysis of all the components of a chromatography system and the influence of each on the separation performance. [15]

ii) What is the basis of gel filtration as a separation mechanism? [5]

iii) Detail five essential features of expanded bed separation that distinguishes it from conventional packed bed chromatography. [5]

4.

The table below shows an experiment in which a 15kDa antibody fragment (Fab) and a 60kDa monoclonal antibody (MCA) were compared as immunoaffinity chromatography ligands for antigen X.

	Fab column	MCA column
Silica used (g)	1.0	1.0
Ligand used (mg)	4.0	4.0
Ligand coupled (mg)	3.4	2.0
Capacity for antigen X (mg)	0.80	0.15
Antigen X recovered (mg)	0.62	0.12

i) Comment on and compare the quantity of ligand coupled in each column. [5]

ii) Comment on and compare the capacity for antigen X in each column. [5]

iii) Calculate the % maximum recovery in each case. [3]

iv) Comment on and compare the % recovery and total yield of antigen X in each column in terms of accessibility of the antigen-binding surface of each ligand. [6]

v) What characteristics constitute an ideal support for affinity chromatography? [6]

5. A pharmaceutical intermediate, AX-415, is to be recovered from a bioconversion medium by liquid-liquid extraction. Following removal of the biocatalyst, the aqueous medium contains 85 g kg^{-1} of AX-415 and can be provided at a mass flow rate up to 400 kg h^{-1}. Laboratory tests have shown that hexane is the best extraction solvent giving an equilibrium distribution coefficient of 12.

CONTINUED

a) If the extraction process is to be operated counter-currently derive an operating line equation for the process clearly stating any assumptions made. [10]

b) Calculate the number of theoretical stages required to recovery greater than 95% w/w of AX-415 if the flow rate of hexane to be used is 300 kg h^{-1}. [8]

c) Laboratory tests have also shown that AX-415 is rather unstable and hence it will be necessary to use centrifugal phase contactors for the process. Briefly describe the reasons for this choice. [7]

A sheet of graph paper is supplied.

6. The broth from a $10m^3$ (total volume) fermenter is to be clarified by continuous centrifugation. The resulting clarified broth will be treated by precipitation and centrifugation to concentrate up an extracellular product. You are required to put in place a laboratory-based strategy to evaluate the proposed design and specification (see below) of the industrial scale operation. This strategy is to include:

i) a flowsheet to define the full large scale process sequence. [5]

ii) the design of a 100 m L batch scale precipitation reactor with specifications for its operation. [15]

iii) an evaluation of the potential interactive effects between precipitate formation and precipitate recovery which would need to be examined at the laboratory scale.
Credit will be given for prioritisation of these interactive effects to be studied. [5]

Process specification:

Clarified fermentation broth (viscosity 0.003 N s m^{-2}, density 1000 kg m^{-3}) is fed to a $10m^3$ stirred tank vessel (vessel height = diameter; impeller diameter is one third vessel diameter, impeller speed = 50 rpm) Precipitant ($2m^3$ ethanol) is added to the broth to effect precipitation (final total volume $9m^3$). After 1 h the precipitant suspension is to be recovered in a continuous disc stack centrifuge operating at $1.5m^3$/h. Precipitate is to be stored and subsequently redissolved and filtered prior to chromatographic separation. Final product is to be used for therapeutic purposes (repeat injectables).

PLEASE TURN OVER

7. A spray drier is currently processing 1 L/min of a dilute protein solution. The final moisture content of the powder is 0.1 kg / kg dry solid and the outlet temperature of the drier is 70 °C. The resultant protein powder is found to be made up of partly inactive protein and it is suggested that this may be corrected by reducing the outlet air temperature to 60 °C. You are required to evaluate the consequences of this change:

i) by maintaining the same dried moisture content of the powder [9]

ii) by maintaining the same feed rate to the drier. [9]

Provide an explanation, with diagrams showing moisture and temperature profiles, for why this reduction in temperature may lead to an increase in active protein. What other reasons may there be for loss of protein activity? [7]

Inlet air humidity = 10 g / kg dry air.
Moisture sorption isotherms and air water enthalpy humidity charts are provided.

END OF PAPER