UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualification:-

Biochemical Eng E171: Design and Control of Biochemical Reactors

COURSE CODE	:	**BENGE171**
UNIT VALUE	:	**0.50**
DATE	:	**13-MAY-04**
TIME	:	**10.00**
TIME ALLOWED	:	**3 Hours**

TURN OVER

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

SECTION A

1.

a) In process scale-down, regime analysis is often used. Describe the purpose of regime analysis and the four steps in its application to scale-down of fermentation processes. [5]

b) A regime analysis of a batch process that produces a viscous compound was performed. The viscous product changes a variety of characteristic times as shown in the table below. Comment on the rate limiting regime(s) at the various time intervals shown and whether or not gradients will be established in the reactor. [15]

Batch time (h)	Mixing time = t_m (s)	Oxygen consumption time = t_{OC} (s)	Oxygen transfer time = t_{OT} (s)
0	10	380	1
20	10	340	3.0
40	18	260	6.5
60	26	114	11
80	65	60	22
100	100	15	68

c) Explain the use of a two-compartment model system in scale-down experiments and describe the set-up with the help of a diagram [15]

2.

a) Estimate the microscale of turbulence in the impeller region of a 150 L stirred tank reactor with 80% working volume. The fermenter is of standard configuration with a 3:1 aspect ratio and contains 3 Rushton turbine impellers with a ratio of tank to impeller diameter of 3. The fermenter is aerated and the agitation speed is set to 300 rpm. The broth has a viscosity of 0.001 Pa s and a density of 1010 kg m^3. [17]

b) Comment on the results of your calculations given that filamentous fungi are being grown under these conditions. [8]

In both parts of the question clearly state any assumptions made and comment on the validity of the results obtained.

PLEASE TURN OVER

3.

a) Calculate the holding time for a pilot-scale batch sterilisation process given the following information. [8]

- the probability of a contamination is 1 in 1000
- the initial spore concentration is 2×10^7 spores mL^{-1}
- sterilisation below 100°C is negligible
- heating from 100°C to 121°C is at 2.5°C min^{-1}
- cooling from 121°C to 100°C is at 5°C min^{-1}
- the death rate constant k at 121°C is 2.54 min^{-1}
- the Del factor for 121°C is 12.55
- broth volume is 700 L

b) Explain briefly how you would overcome the problems caused if the medium contained:

(i) vegetable oil and (ii) amino acids. [4]

c) The production scale process for the fermentation described in Part (a) operates by using a continuous steriliser. Steam is available at 130 °C and the medium needs to be produced at a rate of 5 m^3 per hour. Given that the death rate constant k at 130 °C is 17.524 min^{-1}, the diameter of the holding pipe is 40 mm and the acceptable risk of contamination is 1 organism surviving 60 days of operation, calculate for the system on the basis of 1 hour:

(i) the total Del factor
(ii) the holding pipe residence time
(iii) the length of the holding pipe

Clearly state all assumptions made. [7]

d) Describe the operation of a continuous steriliser with direct heating with the help of a diagram. [6]

4.

a) In a batch fermentation process explain why the average chemical composition of cultured *E. coli* cells will vary as a function of time and medium composition. [5]

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b) You are investigating the pilot scale production of *E. coli* ($CH_{1.79}O_{0.5}N_{0.2}$, ash content 7.1% w/w) in a 2 m^3, working volume, fermenter. The batch fermentation process is aerobic using glucose as a carbon and energy source and ammonium hydroxide as a simple source of nitrogen. Previous trials have shown the yield of biomass on oxygen to be 1.53 g (O_2) g (dry cell weight)$^{-1}$. If your target biomass concentration is 16.5 g (dry cell weight) L^{-1} calculate the minimum quantity of glucose required. Clearly state any assumptions made. [20]

5.

a) You have been charged with the design of a 15 m^3 stirred tank fermenter to be used for the production of an intracellular enzyme synthesised in *E. coli*. Clearly stating and justifying any assumptions made calculate:

- the dimensions of the tank
- the number, design and locations of your chosen impellers
- the unaerated and aerated liquid heights
- the number and width of any baffles fitted to the tank

[14]

b) Pilot scale fermentations have shown that *E. coli* broth typically attains a viscosity of 0.015 Ns m^{-2} and a density of 1005 kg m^{-3}. If the maximum impeller speed to be used in the 15 m^3 tank designed in Part (a) is 450 rpm calculate the unaerated and aerated power requirements clearly stating any assumptions made. Specify also the power rating of the motor to be fitted to the fermenter. [11]

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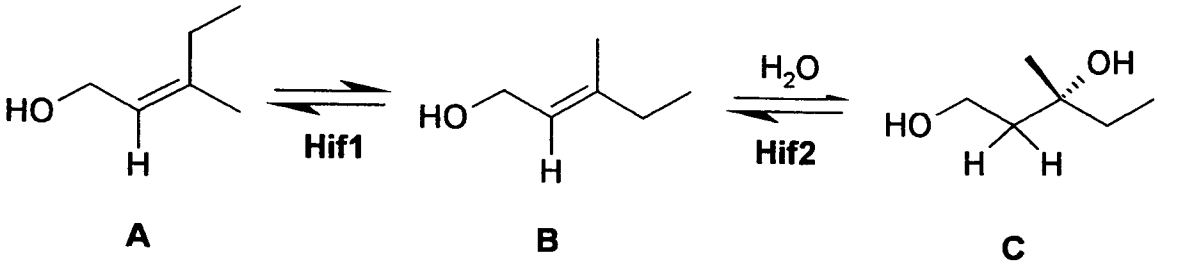
SECTION B

6.

The natural enzymes **Hif1** and **Hif2** have recently been isolated from an unknown organism and expressed from a plasmid in *E. coli*. **Hif1** and **Hif2** catalyse the following bioconversions:



A **B** **C**

a) What class of enzyme does **Hif1** belong to? [2]

b) Which stereoisomer of C, shown above, is made by **Hif2**? [3]

c) A Biochemical Engineer wishes to produce the stereoisomer of **C** shown above, at large scale, starting from compound **A**. Briefly list some of the problems that might be encountered in relation to the substrate (**A**) used, product (**C**) made, and the activity of the enzymes under the large-scale process conditions. [8]

d) Briefly describe a directed evolution experiment, to improve the use of the **Hif2** enzyme in the large-scale production of **C**. Include details of the methods that could be used to alter the amino-acid sequence of **Hif2**, and a short description of the overall directed evolution process. [12]

7.

Briefly describe each of the following:

a) Key features of mammalian cell cultures for the production of recombinant therapeutics. [5]

b) The importance of cell banking in cell culture development and manufacture. [5]

c) Characteristics of continuous cell lines and their relevance within a biopharmaceutical context. [10]

d) The current most popular manufacturing strategies for antibodies for use in human therapy. [5]

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