UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For the following qualifications :-

M.Sc.

M7: Biochemical Engineering

COURSE CODE	:	**CENG00M7**
DATE	:	**20-MAY-02**
TIME	:	**14.30**
TIME ALLOWED	:	**3 hours**

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UNIVERSITY OF LONDON

Biochemical Engineering

M7

Answer 4 questions, at least one from each section.
Marks distributed as shown [ ]

SECTION A

1. a) Yeast is to be produced in continuous culture using glucose as the limiting substrate.

i) Describe the main features of a continuous operation using a chemostat. [3]

ii) Derive the following equation for the steady-state biomass concentration (X) in a well-mixed chemostat with single nutrient limitation:

$$X = Y_{x/s}(S_0 - S)$$

where $Y_{x/s}$ is the biomass yield on substrate, S_0 is the input substrate concentration and S is the steady-state substrate concentration. Clearly state all assumptions made. [10]

iii) Calculate the steady-state substrate and biomass concentrations given that the input rate of glucose, F = 20 kg h^{-1} and the input substrate concentration, $S_0 = 40$ kg m^{-3}. [7]

You can assume that the chemostat has a working volume of 100 m^3, cell growth can be adequately described by the Monod equation and that the organism has the following specific characteristics:

Substrate affinity constant: $K_s = 0.1$ kg m^{-3}
Maximum specific growth rate: $\mu_{max} = 0.4$ h^{-1}
Biomass yield on glucose: $Y_{x/s} = 0.4$

b) Industrial fermentations are often run in fed-batch mode. Describe the features of a fed-batch operation and give examples of its application. [5]

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2. a) Explain the mechanism by which extensive mixing in mechanically agitated bioreactors can cause shear damage to microorganisms. [8]

b) A 5 L stirred tank reactor (70% working volume) is used for the production of a growth hormone by recombinant CHO cells grown on microcarrier beads with a diameter of 120 μm. The cell culture is agitated using a paddle impeller (6 cm diameter) and the stirrer speed is set at 120 rpm. Air and carbon dioxide are supplied by flow through the reactor headspace. The microcarrier suspension has a density of ca. 1010 kg m^{-3} and a viscosity of $1.3x10^{-3}$ Pa s.

i) Estimate the Reynolds number and comment on the type of flow in the reactor. [2]

ii) Calculate the average energy dissipation in the reactor assuming that the power number is constant and can be approximated to 2. [4]

iii) Estimate the microscale of turbulence in the impeller region of the reactor and comment on the value obtained. Clearly state any assumptions made. [6]

c) Discuss the effects of shear forces on the morphology of filamentous microorganisms. [5]

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3. a) The destruction of microorganisms by steam can be described as a first-order chemical reaction as follows:

$$N_t/N_0 = e^{-kt}$$

where N_0 is the number of viable organisms present at the start of the sterilisation, N_t is the number of viable organisms present after a treatment period of time, t, and k is the reaction rate or specific death rate constant.

Derive the following relationship that relates the Del factor (∇) to the Arrhenius constant (A) and the activation energy (E):

$$\nabla = A\,t\,e^{-(E/RT)}$$

where R is the gas constant and T is the absolute temperature. [5]

b) A fermentation process requires 100 m^3 batches of complex medium to be steam sterilised at 120 °C. Assuming:

(1)the medium before sterilisation contains $5x10^6$ bacterial spores mL^{-1};
(2)the rate of sterilisation below 100 °C is insignificant;
(3)a probability of non-sterility after sterilisation is 1 in 1000;
(4)the amount of sterilisation (∇) during heating from 100 °C to 120 °C is 10;
(5)The death rate constant k at 120 °C is 1.8 min^{-1};

i) Calculate the holding time at 120 °C if the rate of heating from 100 °C to 120 °C is 1.5 °C min^{-1} and the rate of cooling from 120 °C to 100 °C is 2.5 °C min^{-1}. [8]

ii) Justify the use of assumptions (2) and (3) above in your calculations. [5]

c) Discuss the advantages and disadvantages of a continuous sterilisation process as compared to a batch sterilisation. [7]

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4. A 700 L scale (working volume) batch fermentation process produces 20 g L^{-1} of a recombinant *E. coli* strain when grown on a glucose-based medium. Using the data below calculate the amount of glucose required and hence the yield coefficient, $Y_{X/S}$ (w/w), for biomass on substrate.
Clearly state any assumptions made. [25]

Biomass composition	=	$CH_{1.8}O_{0.5}N_{0.3}$
Ash content	=	6.5 % w/w
Nitrogen source	=	NH_4OH
Yield of biomass on oxygen	=	1.45 g (dcw) g $(O_2)^{-1}$

5. a) Briefly outline the design and operating characteristics of two types of impellers used in industrial fermentation processes. [6]

b) You have been asked to design a 500 L pilot scale stirred-tank fermenter to be fitted with a mixed impeller system. This comprises a Rushton turbine impeller (N_P = 5.7) and a Lightnin A315 up-pumping impeller (N_P = 2.5). Specify the dimensions of the vessel and the size and location of each impeller. Clearly state, and justify, any assumptions made. [11]

c) Your vessel is to be used for the cultivation of a filamentous microorganism that attains a maximum broth viscosity of 0.03 Ns m^{-2}. If the maximum impeller speed to be used is 500 rpm specify the size of the motor required. Clearly state any assumptions made. [8]

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SECTION B

6. a) Produce a table listing the main differences between microbial and mammalian cells which affect their capacity for bioprocessing. [5]

b) Describe the types of protein products made by mammalian cell culture systems and the features of these products that mean they can only be made in mammalian cell systems. [3]

c) Compare and contrast systems used for growth of attached cells and suspension cells. [5]

d) Discuss the major features of the design and operation of industrial scale mammalian cell bioreactors. Include in the discussion, the distinguishing features from microbial bioreactors, the two main operational modes used in industry, the challenges remaining in their optimisation and some of the factors involved in deciding which mode is appropriate for a certain product. [12]

7. This question concerns the implementation of biocatalytic processes.

a) What is the role of biocatalysis in industrial organic synthesis? [5]

b) Give an example of one industrial biocatalytic process and describe its key features. [5]

c) List the advantages and disadvantages of biocatalysis compared to chemical catalysis. [5]

d) Draw a typical flowsheet for a bioprocess using an immobilised isolated enzyme. Indicate as appropriate the input and output streams for each operation. [10]

END OF PAPER