Loughborough University

23CGP069

Advanced Biochemical Engineering

Semester 2 2023/24 In-Person Exam paper

This examination is to take place in-person at a central University venue under exam conditions. The standard length of time for this paper is **2 hours**.

You will not be able to leave the exam hall for the first 30 or final 15 minutes of your exam. Your invigilator will collect your exam paper when you have finished.

Help during the exam

Invigilators are not able to answer queries about the content of your exam paper. Instead, please make a note of your query in your answer script to be considered during the marking process.

If you feel unwell, please raise your hand so that an invigilator can assist you.

You may use a calculator for this exam. It must comply with the University's Calculator Policy for In-Person exams, in particular that it must not be able to transmit or receive information (e.g. mobile devices and smart watches are **not** allowed).

Part A carries 25 marks and Part B carries 50 marks.

You **MUST** attempt all questions

Candidates should show full working for calculations and derivations.

Graph paper will be provided.

Relevant equations are listed on the final page of this exam paper.

Section A: all questions are compulsory

1. Adipose-derived stem cells (ASCs) have the capacity to differentiate into a variety of cell types. There has therefore been interest from the cultured meat research community in using ASCs isolated from, for example cows, as a starting material for their products as these cells could form both the muscle tissue and fat that meat is typically composed of. However, a key challenge for the research community is in the optimisation of the culture to produce sufficient cells for further processing as it is anticipated that up to $8x10^{12}$ cells may be needed to generate 1 kg of protein. In a recently completed study, Hanga *et al* (2020) cultured bovine (i.e. cow) ASCs on microcarriers and generated the data shown in Table Q1. Note that spinner flasks have an impeller but no online control. For medium exchanges, stirring was stopped, microcarriers allowed to settle to the bottom and a percentage volume (%) of the culture medium removed and replaced with fresh medium.

Table Q1: Growth kinetics for bovine ASCs cultured on microcarriers in 100 ml spinner flasks when different medium exchange strategies were trialled. Data is presented as mean +/- SD of n=5 cultured over 9 days. All other conditions such as culture temperature and total time in culture were kept the same. Data taken from DOI: 10.1002/bit.27469.

% medium exchanged	80%	65%	50%
Fold increase	28.01 ± 2.59	17.02 ± 1.9	10.49 ± 2.95
Specific growth rate (h^{-1})	0.0154 ± 0.0004	0.0131 ± 0.001	0.0107 ± 0.001
Doubling time (h)	45 ± 1.28	52.31 ± 2.03	65.15 ± 7.15

(a)

(i) What do you anticipate the fold increase to be if no medium exchange was done (a range rather than specific value is acceptable). [1 mark]

(ii) Discuss your reasoning, referring to cellular processes and data trends. [3 marks]

(iii) Comment on what additional data you would need to collect to confirm your hypothesis. [2 marks]

(b) Of the medium exchange profiles tested in Table Q1:

(i) which would you take forward and why? [1 mark]

(ii) Discuss what additional data you would need to confirm your decision. [6 marks]

2. (a) You have been provided with a strain of *E. coli* which has been transfected with a plasmid containing the gene for human glucagon-like peptide 1. Gene expression is under control of an inducing agent, IPTG.

(i) Briefly sketch a typical *E. coli* growth curve ensuring all phases of growth are clearly labelled and indicate on this at what point you would add IPTG to induce peptide expression. Justify your answer. [3 marks]

(ii) How will the biosafety or biohazard level of this genetically modified E. coli be established? [2 marks]

(b) Following the glucagon-like peptide 1 project you are now tasked with making a small, picogram, quantity of a newly identified human protein to enable structure crystallography to be carried out using a mammalian cell line. Would you choose a transient or constitutive expression system to generate this protein in the lab? Justify your answer.

[3 marks]

(c) Cell-free synthesis of human proteins is now possible. Identify two challenges that are associated with generating human proteins specifically that mean we need to explore alternatives such as cell-free synthesis. In each instance, specify whether cell free synthesis overcomes the challenge not at all, in part or wholly and why. [4 marks]

Section B: all questions are compulsory

3. Insulin, a polypeptide hormone predominantly secreted by pancreatic β cells within the islets of Langerhans, plays a pivotal role in regulating various physiological processes. Given its significance in the onset and progression of numerous chronic diseases, the synthesis and levels of insulin are of paramount importance. Large-scale production of insulin is essential for various clinical applications worldwide. Currently, several methods are employed for insulin production: (1) Insulin extraction from the pancreas of diverse animal species involves acid-ethanol extraction, subsequent gel filtration, and crystallization processes. (2) The complete human insulin gene has been successfully cloned and inserted into microbe such as an *Escherichia coli* (*E. coli*) expression system, enabling the large-scale production of recombinant human insulin. (3) Utilization of mammalian cells, such as Chinese hamster ovary (CHO) cells, is also employed for the production of recombinant human insulin. These methodologies represent pivotal approaches in insulin production, each offering unique advantages in the biomedical field.

(a) Analyse the differences between the 1st method and the 2nd and 3rd methods.

[8 marks]

(b) Assess the advantages of cultivating mammalian cells, such as CHO cells, as opposed to utilizing animal models. [9 marks]

(c) In the third method, which involves culturing anchorage-dependent CHO cells in a stirred tank bioreactor, what is the key additional equipment or resource required for this culture?

[1 mark]

(d) Propose appropriate procedures for inoculating CHO cells into the stirred tank bioreactor as part of the third method for insulin production. [7 marks]

4. (a) An aerobic fermentation process will be carried out at a batch size of 300 litres. The viscosity changes slightly during fermentation from around 0.250 to 0.325 Pa s. State what type of bioreactor you would recommend for this fermentation and provide justifications for your recommendation. [5 marks]

(b) Explain why it is important to operate under complete dispersion conditions in an agitated, aerated bioreactor. [3 marks]

(c) The rheology of a fermentation broth at the start and end of the fermentation is given in the table below.

(i) Describe the rheological behaviour at the start and end of the process based on the constitutive equations you have obtained, using the graph paper if required.

[5 marks]

t=0	
$\dot{\gamma}$ (s^{-1})	τ (Pa)
0	0
10	0.50
20	1.00
40	2.00
50	2.50
75	3.75
100	5.00
110	5.50
150	7.50

End	
$\dot{\gamma}$ (s^{-1})	τ (Pa)
0	0
10	5.68
20	8.31
40	12.17
50	13.76
75	17.20
100	20.14
110	21.23
150	25.18

(ii) This process is carried out in a fully baffled, cylindrical stirred tank which has a diameter of T= 3.0 m using an impeller of a diameter of D= T/3. This impeller has a power number of Po= 0.3 over the turbulent and transitional flow regimes. The impeller is rotated at a speed of N= 120 rpm. The density of the medium, ρ, increases from 1075 to 1190 kg m^{-3} from the start to the end of the process. Based on your calculations, state how this change in the physical properties of medium would affect the operating costs, i.e. the power requirement. Also show your calculations relating to the flow regime.

[6 marks]

(d) A fermentation broth of 331 litres is prepared in a fully baffled, cylindrical, mechanically agitated bioreactor. The vessel base can be assumed to be flat. The liquid height is the same as the tank diameter ($H = T$) and the impeller diameter is half the tank diameter
($D = T/2$). It is rotated at a speed of 120 rpm. The viscosity of the broth is μ = 0.70 Pa s and density is ρ = 1100 kg m^{-3}.

The fermentation is carried out using cells attached onto microcarrier beads. These are of a diameter of d_p = 110 μm and have a density of ρ_s = 1800 kg m^{-3}. Solids mass concentration is 10%. "s" value for this impeller configuration can be taken as 5.5.

Having made relevant calculations, comment whether the fermentation process can be run successfully.

[6 marks]

Relevant Equations and data

Power number: $Po = \frac{P}{\rho N^3 D^5}$

Reynolds number: $Re = \frac{\rho N D^2}{\mu}$

k_s can be taken as 11

Zwietering correlation for just suspension speed:

$$N_{JS} = s\nu^{0.1}\left[\frac{g\Delta\rho}{\rho_L}\right]^{0.45} {d_p}^{0.2} X^{0.13} D^{-0.85}$$

END OF PAPER

Prof K Coopman, Dr NG Özcan-Taşkın, Dr T Sun