22CGP069

Advanced Biochemical Engineering

Semester 2 2022/23 | 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 Question 1 (**SECTION A**) and then ATTEMPT any **TWO** of the remaining three questions (**SECTION B**).

Candidates should show full working for calculations and derivations.

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

SECTION A
COMPULSORY (Attempt this question)

1. *Halomonas* species are a type of halophilic bacteria, meaning that they require high salt concentrations to survive and hence are often found in salt-water habitats. Some *Halomonas* species may also be able to degrade hydrocarbons such as those found in crude oil. A potential industrial use for these bacteria would therefore be in the remediation of oil spills in marine environments. Imagine you are completing a research project that has isolated a new *Halomonas* species, denoted HA-1. You are now running experiments to understand the optimal conditions under which it replicates and degrades crude oil. In all experiments, HA-1 has been seeded into a 250 ml Erlenmeyer (conical shake) flask containing a basal medium (0·5 g KH_2PO_4, 0·4 g NH_4Cl, 0·33 g $MgCl_2$ $6H_2O$, 0·05 g $CaCl_2$ $2H_2O$, 0·5 g yeast extract and 1 ml trace element solution) supplemented with 100 g L^{-1} NaCl. Crude oil was added as specified in Figures Q1 A), B) and C). Flasks were cultured in a shaking incubator at 37°C. Samples were taken every 24 hours and analysed using optical density (for organism growth) and gas chromatography mass spectrometry (GC-MS). GC-MS is used to monitor crude oil degradation by identifying breakdown products and hence calculate the % biodegradation that has occurred. Acquired data is shown in Figures Q1 A), B) and C).

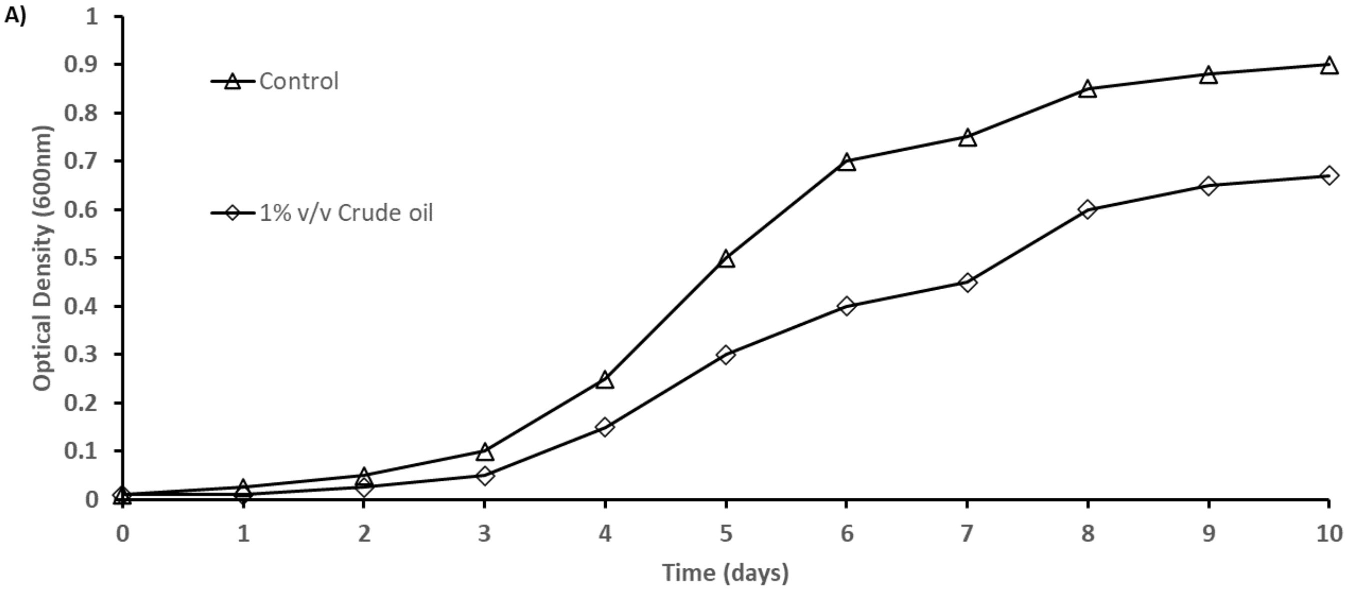

Fig Q1: A) Growth of the HA-1 species with (Crude oil) and without (control) 1% v/v crude oil added to basal medium supplemented with 100 g L^{-1} NaCl. Data represents the mean of 3 experimental runs.

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Q1 Continued/…

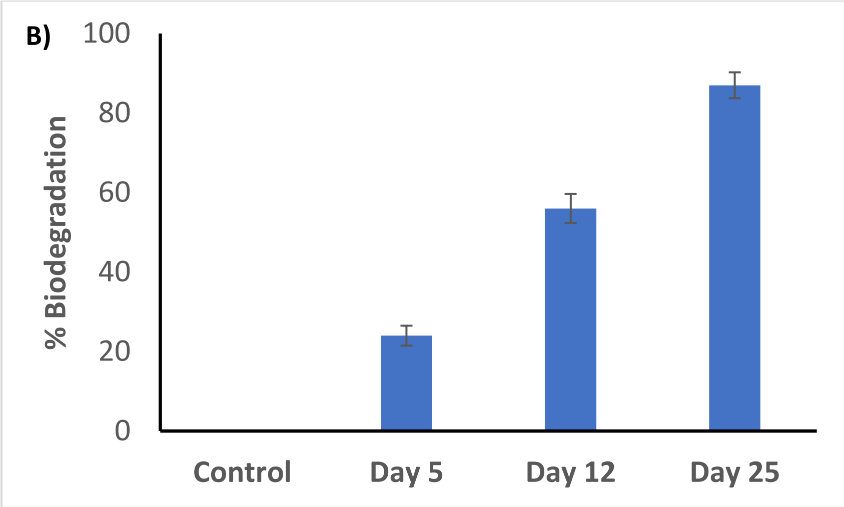

Fig Q1 B) Percentage biodegradation of 1% v/v crude oil when added to the HA-1 culture for up to 30 days. Data represents the mean of 3 experimental runs +/- standard deviation.

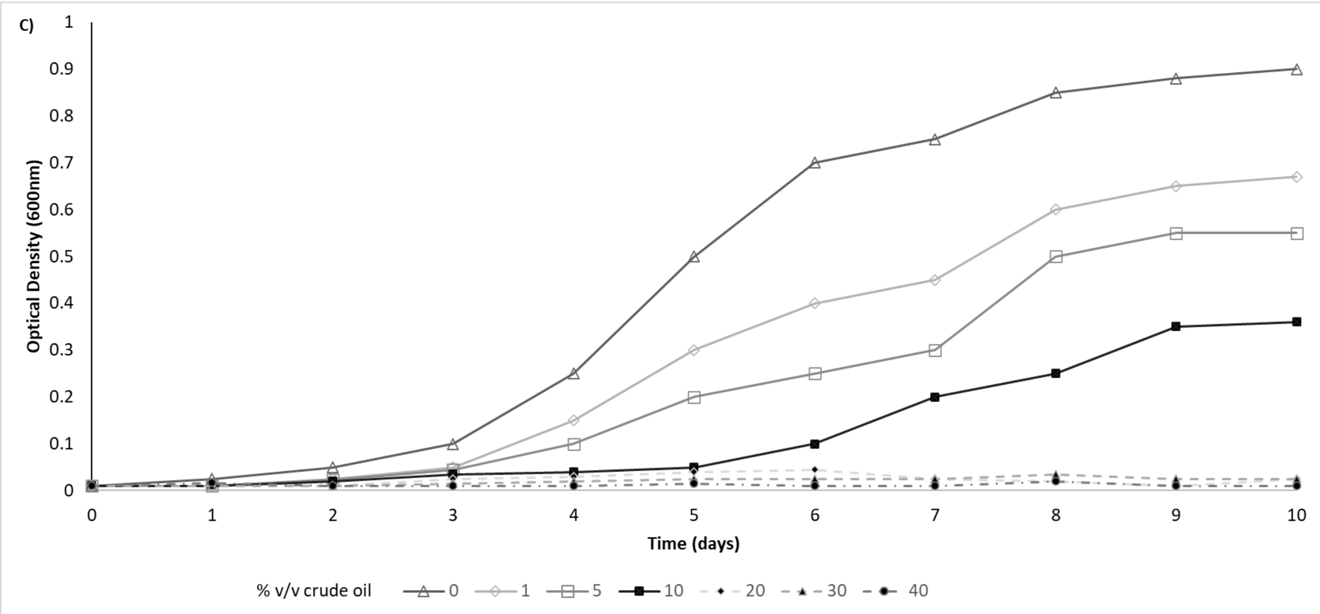

Fig Q1 C) Growth of the HA-1 species with up to 40% v/v crude oil added to basal medium supplemented with 100 g L^{-1} NaCl. Data represents the mean of 3 experimental runs.

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Q1 Continued/…

(a) Describe the cell cycle of how bacteria replicate. You may use an annotated diagram if you wish. [3 marks]

(b) Based on the data in Figures Q1 (A), (B), (C):

(i) At what concentration does the crude oil become toxic to the HA-1 species? Justify your answer. [3 marks]

(ii) Explain what additional experiments or data you might need to confirm your answer to Q1(b)(i) and why. [5 marks]

(c) Based on the information provided above about *Halomonas* and your knowledge of bacteria and bioprocessing, what additional culture parameters would you suggest are studied in order to identify the optimal conditions under which this new species replicates and degrades crude oil. For each parameter, include why you think it needs to be studied and provide a broader reasoning as to why it is important to understand how this new species will behave under a range of conditions. [8 marks]

(d) Given its ability to grow in a marine (i.e. salt water) environment, why do you think other researchers are exploring how different *Halomonas* species may be engineered in order to produce biofuels or bioplastics? [4 marks]

(e) If the researchers in part 1 (c) genetically modified *Halomonas*, the resulting organism would be considered a genetically modified organism. However, if a human is treated with a gene therapy, they are not considered to be genetically modified. Why is this? [2 marks]

SECTION B

Attempt **TWO** out of the three questions

2. (a) Explain two factors that would affect the rheology of a cell culture. [6 marks]

(b) Explain through two examples how the rheology will have an impact on the design and scale up of an anaerobic bioprocess taking place in a mechanically agitated bioreactor. [4 marks]

(c) An aerobic fermentation process is run at two different sites of a company. The mechanically agitated bioreactors at sites A and B are of the same dimensions. These are cylindrical, have a flat base and the tank diameter is $T = 3.0$ m. Liquid height is the same as the tank diameter, $H = T$, and both tanks are fully baffled. Measurements performed at smaller scales have determined the following relationships in the turbulent and transitional regimes:

$$k_l a = 5.1 \times 10^{-2} \times \left(P_g/V\right)^{0.43} \times v_{sg}^{0.55} \qquad \text{Turbulent regime}$$

$$k_l a = 4.7 \times 10^{-2} \times \left(P_g/V\right)^{0.41} \times v_{sg}^{0.43} \times \mu^{0.68} \qquad \text{Transitional regime}$$

The density and viscosity of the medium are ρ= 1090 kg m^{-3} and μ= 0.09 Pa s respectively and these do not change significantly during the process.

At site A, the impeller used is a concave bladed disc turbine which has an ungassed power number of 2.3. This is of a diameter of $D = T/2$ and operated at a speed of 120 rpm. At site B, an up pumping large blade hydrofoil is used at a speed of 180 rpm. This impeller is of a diameter of $D = T/3$ and has an ungassed power number of Po= 0.9.

Gas is supplied at 1.0 vvm (volume of gas per volume of liquid per minute) in fermenter at site A and 1.5 vvm at site B. The power reduction due to gassing is around 3% for the concave blade impeller at site A and 5% for the large blade hydrofoil at site B.

Based on your calculations, state whether there would be a difference in the performance of the two fermenters. [11 marks]

Continued/…

Q2 Continued/…

(d) A plant cell culture is used to produce citrus oil for the cosmetic industry. The cells are known to be sensitive to agitation, therefore, shear damage needs to be avoided to minimise any detrimental effects on oil production. The bioreactor used is flat based, has a diameter of $T = 3.6$ m and the liquid height is the same as the tank diameter: $H = T$. It is equipped with a pitched blade turbine of a diameter of $D = T/3$, rotated at 120 rpm. The power number of the impeller is Po= 1.4. Whilst the process is run under gassed conditions, the change in power input due to aeration is negligible. The density of the medium is ρ= 1025 kg m^{-3} and viscosity is $\mu = 3.0 \times 10^{-3}$ Pa s. Cells are of a size of approximately 80 microns. Would you conclude that there is shear damage in this bioprocess? Show your calculations to justify your conclusion.

Based on your calculations and conclusion state whether you would recommend any changes to how the process is operated. [4 marks]

3. Therapeutic peptides are a unique class of pharmaceutical agents composed of a series of well-ordered amino acids, usually with molecular weights of 500-5000 Daltons (Da). Since the synthesis of the first therapeutic peptide, insulin, in 1921, remarkable achievements have been made resulting in the approval of more than 80 peptide drugs worldwide. Therapeutic peptides can be produced via microbial fermentation or the culture of mammalian cells such as Chinese hamster ovary (CHO) cells.

   (a) Evaluate the bioprocessing challenges when microbes or mammalian cells are selected as the biological systems for therapeutic peptide production. [7 marks]

   (b) If a stirred tank reactor (STR) has been selected for the culture of anchorage dependent CHO cells in a bioprocess for therapeutic peptide production, analyse the specific requirements of this STR in comparison with the STRs usually used for fermentation. [4 marks]

   (c) Apart from the STR, what else will be needed for the cell seeding and culturing of these adherent CHO cells? Assess two possible methods to inoculate the CHO cells into the STR. [6 marks]

   (d) In addition to the production of therapeutic peptides, mammalian cell cultures also have other applications. Compared with animal models, evaluate the benefits associated with the cultured mammalian cells. [8 marks]

4. Stem cells are an active and growing area of basic science and clinical research due to their ability to self-renew and differentiate into mature cell types. Current clinical applications for stem cells include treatments for neurological and cardiovascular diseases, autoimmune disorders, cancer, wound healing, disease modelling and drug screening. Stem cells require specialized, high-quality media and expert culture techniques for propagation from small to large scales.

   (a) If it is your responsibility to select the suitable stem cell type for the manufacture of certain cell therapy products, briefly explain the issues associated with the selection of stem cells. [4 marks]

   (b) Based on the evaluation of the culture medium, supporting matrix and scale up issues associated with mammalian cell cultures, explain why the bioprocesses developed for microbial fermentations are not suitable for large-scale culture of human stem cells. [9 marks]

   (c) For the successful culture of stem cells, supporting cells are usually used. Propose a simple scale-down cell culture model to investigate the interactions between the stem cell and the supporting cell using a conventional phase-contrast microscope (PCM) during cell cultures. Explain how both stem and supporting cells are seeded and cultured in the system, and how PCM is integrated with the culture system for live cell imaging. [5 marks]

   (d) Using stem cell processing as an example, briefly discuss the general scale-up challenges biochemical engineer usually have when they design the bioprocess for the manufacture of stem cells from small to moderate and industrial scale. [7 marks]

END OF PAPER

K Coopman, NG Özcan-Taşkın, T Sun

List of equations

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

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

Kolmogoroff microscale of turbulence (λ_k)

$$\lambda_k = ({\nu^3}/{\varepsilon})^{1/4}$$

where ν (m s^{-2}) is the kinematic viscosity and

ε (W kg^{-1}) is power per unit mass.