B3 Group Design Project

Cultured Beef Production

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Contents

MAINLY TALK ABOUT WHY YOUR CHOICE LED TO A BETTER ACHIEVEMENT OF THE DESIGN OBJECTIVES.

The generic structure of a technical report:

1. Introduction: Provide context, motivation, and background information

What is the big-picture problem? Why is it a problem? Who cares?

What have others done to solve the problem? What is still left to do? What did you do?

2. Methods: Explain what you did Provide enough detail for someone else to reproduce your results.

For each aspect, the level of detail should be commensurate with the level of novelty.

3. Results: Show and explain what you found. Provide figures and other qualitative and quantitative evidence for your conclusions.

Explain and interpret what you are showing. Not "What does the data look like?", but "What does the data mean?"

4. Discussion: Consider the implications and limitations of your results

5. Conclusions: Connect your results with the original problem.

What have you actually achieved here? What should be done next?

Tip 1. Emphasise results, interpretation, and discussion.

Tip 2. Technical writing needs to be precise, concise, objective, careful

Tip 3. Every sentence must: (i) make a point (ii) form a logical link between the previous sentence and the next

1 Example

Intro (unnumbered)

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Figure 1: An example image Cannon [2]

1.1 Example NUMBERED subsection (no * in environment)

Example equation 1.

$$beef_{moo} = fear^{10} \tag{1}$$

Example list:

- $\boldsymbol{X} \in \mathbb{R}^{n \times p}$, a matrix containing the scrutinised data-points $\boldsymbol{x} \in \mathbb{R}^p$ from each sample,
- *regression_targets*, known as $\bar{\boldsymbol{y}} \in \mathbb{R}^n$, is a vector containing the regression targets for the samples (individually known as $\bar{y}$), and
- *class_labels* $\in \{1, \ldots, p\}$ which keeps track of which Gaussian each sample came from (a

target for classification).

Example table 1

learning_rate	mse_train	mse_val
1e-05	1.1254	1.1747
0.0001	0.31296	0.3012
0.001	0.095492	0.088045
0.01	0.046835	0.052905
0.1	0.046534	0.052771
1	NaN	NaN

Table 1: Table of Mean Squared Difference from different learning rates.

Example aligned equation:

$$
\begin{aligned}
\mathcal{L}_c &= -ylog(\bar{y}) - (1-y)log(1-\bar{y}) \\
&= ylog(1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}) - (1-y)log(\frac{e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}}{1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}}) \\
&= (y-y)log(1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}) - (1-y)log(e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}) + log(1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}) \\
&= (1-y)\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta} + log(1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}) \\
\Delta_{\boldsymbol{\theta}}\mathcal{L}_c &= (1-y)\hat{\boldsymbol{x}} - \frac{e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}}{1+e^{-\hat{\boldsymbol{x}}^{\boldsymbol{T}}\boldsymbol{\theta}}}\hat{\boldsymbol{x}} \\
&= (1-y)\hat{\boldsymbol{x}} - (1-\bar{y})\hat{\boldsymbol{x}} \\
&= \hat{\boldsymbol{x}}(\bar{y}-y)
\end{aligned}
$$

END OF TEMPLATE

2 Medium

2.1 Introduction

The cell culture medium is an essential part of cultivated meat production as it replicates in vivo conditions necessary for cell growth. The medium is supplemented with vital components necessary for proliferation and differentiation, alongside chemicals to regulate the physiological conditions within the bioreactors.

A substance known as Foetal Bovine Serum (FBS) is commonly added to the medium to enrich it with key proteins and growth hormones. However, researchers are in search of alternatives and aim to phase out its use in the near future ([15]).

This section provides further details on the media composition (refer to paragraph 4.1) and explores why FBS should be excluded (refer to paragraph 4.2).

2.2 Medium composition

The medium traditionally consists of two main components: the basal media and the serum.

The **basal medium** contains many nutrients such as glucose, glutamine, amino acids, and vitamins, necessary for cell survival. Additionally, inorganic salts are supplemented to maintain the fluid at the right osmolarity, while buffering agents like HEPES stabilise the pH levels. To reduce costs, the buffering agents can be substituted with natural buffering processes with CO2 and HCO3 to reduce costs ([17]). Traces elements such as copper or zinc can also be found in the medium. Various media formulations are commercially available and provide different nutrient compositions tailored to specific cell types. Examples include MEM, Ham's F-10, DMEM/F-12 (used for proliferation – section 5.2), Leibovitz's L-15 and Neurobasal (used for differentiation – section 6.3).

Although researchers commonly add **antibiotics** to mitigate the risk of microbial infections, their use conflicts with the project's principles and, therefore, will not be considered for the mediums.

The **serum** component provides hormones (e.g. insulin), proteins (e.g. albumin and transferrin) and growth factors (e.g. fibroblast growth factor (FGF) and epidermal growth factor (EGF)) and various other chemicals that ensure cell growth ([14]). Historically, foetal bovine serum (FBS) has been the predominant serum used for bovine cell culture. Alternative options have been found but many are less effective.

2.3 Moving away from FBS

Researchers are striving to eliminate the use of FBS due to numerous concerns, particularly ethical ones. One major issue is the ethical implications surrounding the production of FBS, as depicted in Figure 1. Foetal Bovine Serum is harvested after the slaughter pregnant cows that are further along in gestation than the first trimester. To ensure low clotting and a large presence of growth factors, blood is extracted from the beating heart of the foetus before its eventual death. The blood is then left to clot and the clotted elements are filtered out, yielding the Foetal Bovine Serum ([12]).

The ethical concerns stem from the contradiction between this practice and the mission of cultivated meat to reduce animal suffering and minimise reliance on cattle in the meat industry. Furthermore, there are concerns that the foetus may experience pain and distress during the extraction procedure, as it is still alive at that stage ([15]).

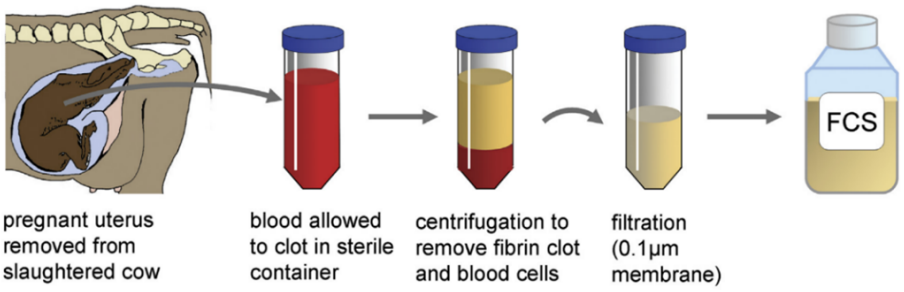

Figure 2: Process of FBS, also known as foetal calf serum FCS, ([11])

Another significant disadvantage of FBS is its complexity. Many of its components remain unidentified and some may even act as growth-inhibiting factors or harbour microbes. Moreover, the composition of FBS varies from one calf to another, depending on factors such as the mother's diet and geographical location. This variability results in inconsistent performance and high batch-to-batch variability, ultimately leading to reduced growth rates.

Additionally, FBS is the most costly component of the medium, with prices exceeding $1000 USD per litre in 2019 ([16]). This high cost adds financial burden to cultivated meat production.

Consequently, there are overwhelming reasons to move away from the use of FBS. This is however a difficult task because of the complexity of the serum that needs to be replicated.

2.4 Consideration

Many leading cultured meat companies, such as Mosa Meat and Future Meat, have claimed to have serum-free processes ([9],[13]). However, due to the competitive advantage it provides them, the

medium formulations have been kept secret. We will consequently consider media available on academic platforms, which are likely to be less efficient.

2.5 Summary

For the reasons listed above, this study only considers serum-free media, which then consist of the basal media and serum-alternatives. Since proliferation and differentiation are different processes, different mediums are considered for each. Refer to section 5.2 for further details on the growth medium selection and section 6.3 for the differentiation medium selection.

3 Proliferation seed train

3.1 Growth medium selection

The growth medium's purpose is, as its name suggests, to support a rapid cell growth. Selecting the right medium is essential as it affects the viability and growth rate of cells and consequently the whole process (e.g., production time, initial number of cells needed, etc.). The ideal medium should i) mimic the in vivo environment, ii) promote cell proliferation, iii) maintain the cell identity, iv) align with the team's ethos (environmental-friendly and ethical practices), v) be cost-effective and easily reproducible. A multi-criteria analysis of several commercially available options is conducted below to choose the optimal medium.

4 Control systems

4.1 Introduction

Homeostasis, defined as the internal regulatory functions of the body to maintain certain conditions constant [10, 1], is extremely crucial to living organisms. For example, for humans, the blood pH outside the range between 7.35 and 7.45 can cause death [4]. In addition to its significance in maintaining an existing life, homeostasis has great importance in creating a new life: Mammalian cell culture.

In a bioreactor, homeostasis can be achieved by solving the classical problem of tracking the reference signal $r(t)$ in control theory, as shown in Figure 3. A thoroughly designed bioreactor and its constituent control systems will lead to better achievement of the design objectives, which are: (i) How can one achieve the production rate of 100 kg/month? (ii) How can one produce a better quality of meat?

The design of the control system mainly answers the latter question. The former question is rather answered through the overall process diagram, number and sizes of bioreactors, mass inflows and outflows, so will not be tackled in this chapter. Bioreactor control, however, addresses the formation and maintenance of the optimal environment to produce the best quality of meat. Thus, in this chapter, the design of temperature (T), dissolved oxygen (DO) and acidity (pH) control systems are discussed.

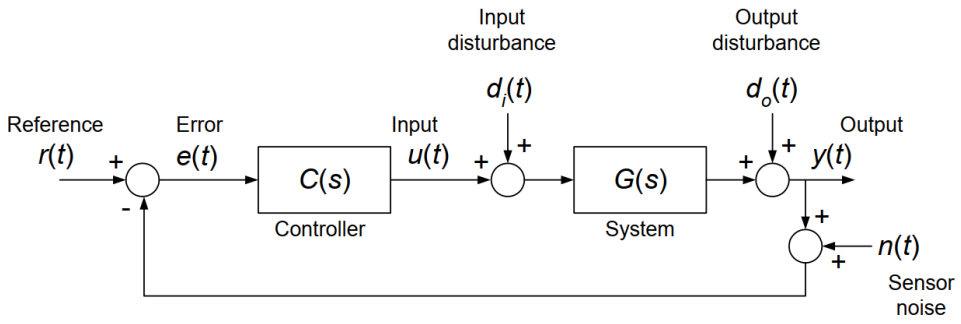

Figure 3: A schematic of a negative feedback control system Cannon [2]

4.2 Temperature control

4.2.1 Introduction

The body temperature of beef cattle should be maintained at $39.6 \pm 0.1°C$ [8]. To do so, different physical setups of the heat exchanger around the bioreactor will be compared to find the optimal one. Differential equations will be constructed to derive the plant transfer function. Design criteria will be posed with appropriate justification. Different control strategies will be compared to find the optimal one. The design criteria introduced will be used to find control parameters. MATLAB simulations will

be used to confirm the validity of the step and impulse responses.

4.2.2 Methods

Various types of heat exchangers used to control the heat in and out of the bioreactor are shown in Figure 4. The best design choice is (a), the jacketed bioreactor. The logic is as follows: (c) and (d) have the heat exchange inside the bioreactor, causing an intervention in the rotational pathway of the impeller used to stir the meat, and thus adding unnecessary complexity to the design to avoid this; (e) involves taking the meat out of the bioreactor, which firstly may harm the meat cells by pumping and pressurising them above the maximum stress that they they can resist, and secondly has a potential issue of fouling in the pump. One is now left with (a) and (b), but for better heat transfer it is more efficient for the working fluid to cover the entire bioreactor, and for more evenly distributed heat transfer it is better if the inlet and the outlet temperatures of the heat exchanger do not vastly differ. Hence, (a) is the best option.

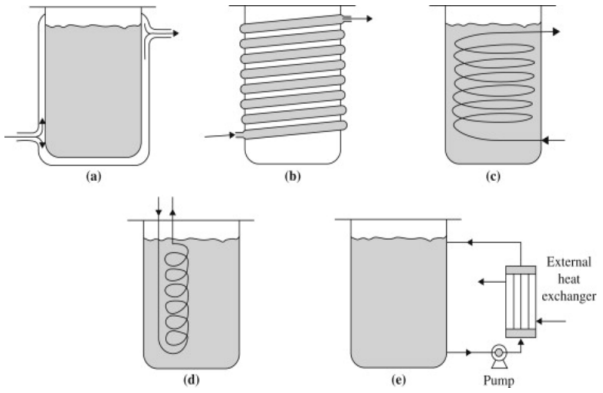

Figure 4: Heat exchanger configurations for a bioreactor [5]

Theoretically, the heat exchanger's working fluid temperature T_{fluid} controls the bioreactor's internal temperature T. Practically, the observer is implemented using a temperature sensor, and the controller is implemented digitally using a computer system, as shown in Figure 5.

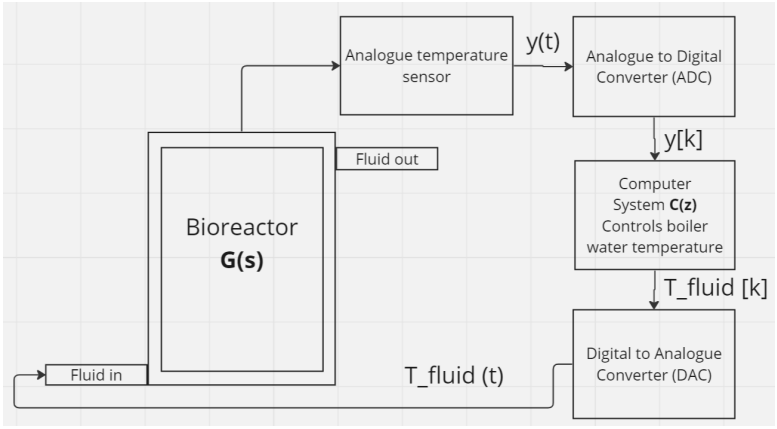

Figure 5: Practical implementation of the control system

The dynamics over time of temperature (T) can be described via the energy balance equation, where ρ is the wet cell density, V is the volume of the bioreactor, c_p is the specific heat capacity, Q_{met} is the metabolic heat generation rate, h is the heat transfer coefficient and A is the area of the bioreactor.

$$\rho V c_p \frac{\partial T}{\partial t} = Q_{met} V - hA(T - T_{fluid}) \tag{2}$$

The change of variables to perturbations $\Delta T = T(t) - T_{ss}$ and $\Delta T_{fluid} = T_{fluid}(t) - T_{fluid,ss}$ leads to Equation 3. During the entire culturing period ($t > 0$) the steady-state assumption ($\frac{\partial \Delta T}{\partial t} = 0$, $\Delta T \approx \Delta T_{fluid} \approx 0$) can be made because otherwise the cells will die due to the violence of homeostasis. This leads to Equation 4. Substituting Equation 4 into Equation 3 and taking the Laplace transform leads to the desired transfer function, as shown in Equation 5.

$$\rho V c_p \frac{\partial \Delta T}{\partial t} = Q_{met} V - hA(T_{ss} - T_{fluid,ss}) - hA(\Delta T - \Delta T_{fluid}) \tag{3}$$

$$Q_{met} V - hA(T_{ss} - T_{fluid,ss}) = 0 \tag{4}$$

$$G(s) = \frac{\Delta T(s)}{\Delta T_{fluid}(s)} = \frac{hA}{\rho V c_p s + hA} \tag{5}$$

4.2.3 Results

The wet cell mass of $3.5 \times 10^{-12}\ kg/cell$ and the cell diameter of $295 \times 10^{-6}\ m$ [7] are used to calculate the density so that $\rho = 0.26\ kg/m^3$. The bioreactor height of $2\ m$ and the bioreactor diameter of $2.1\ m$, are used to calculate the area and the volume of the bioreactor so that $A = 20.12\ m^2$ and $V = 6.93\ m^3$. The yield of the final product to the wet cell $\eta = 0.5$ is used along with $c_{p,cell} = 3.440\ kJkg^{-1}K^{-1}$ [6] and $c_{p,water} = 4.180\ kJkg^{-1}K^{-1}$ to linearly interpolate the specific enthalpy as shown in Equation 6. The heat transfer coefficient is assumed to be $h = 0.5\ kWm^{-2}K^{-1}$. Substituting these values in, the plant transfer function is derived as shown in Equation 7.

$$c_p = \eta c_{p,cell} + (1 - \eta) c_{p,water} = 3.810\ kJkg^{-1}K^{-1} \tag{6}$$

$$G(s) = \frac{10.06}{6.872s + 10.06} \tag{7}$$

The design of the controller is often done by setting the gain margin (GM) or the phase margin (PM) of $C(s)G(s)$ at a chosen frequency. The best design criterion is $PM = 60^\circ$ at $\omega = 4.16\ rad/s$.

The logic is as follows: the rise time of the plant's step response, defined as the time taken from 10% to 90% of the steady-state value, is $\Delta t = 1.58 - 0.07 = 1.51\ s$, as it is also visible from Figure 6. The rise time can be viewed as the mean time taken for the bioreactor to respond to the heat exchanger, and hence the period. The operating frequency is then $\omega = 2\pi/\Delta t = 4.16\ rad/s$.

Practically, there is a higher chance of acceleration or delay in heating and cooling than a sudden overheating or underheating. Thus, one may be more concerned with the phase margin that relates to unexpected phase lags $\angle G(j\omega)$ than the gain margin that relates to unexpected magnitude deviations $|G(j\omega)|$. An acceptable rule of thumb is $PM = 60°$, so one reaches the posed criterion.

One of the most commonly used controllers in the process control industry is proportional-differentiator-integral (PID). The engineer can use the above criterion along with the condition that "the low-frequency asymptote of the Nyquist on the $M = 1$ line" [3], which means the unity D.C. gain $\frac{Y(s)}{R(s)} = 1$ and thus zero steady-state error. Through this, one can find the three controller gains in $C(s) = K_p + \frac{K_i}{s} + K_d s$.

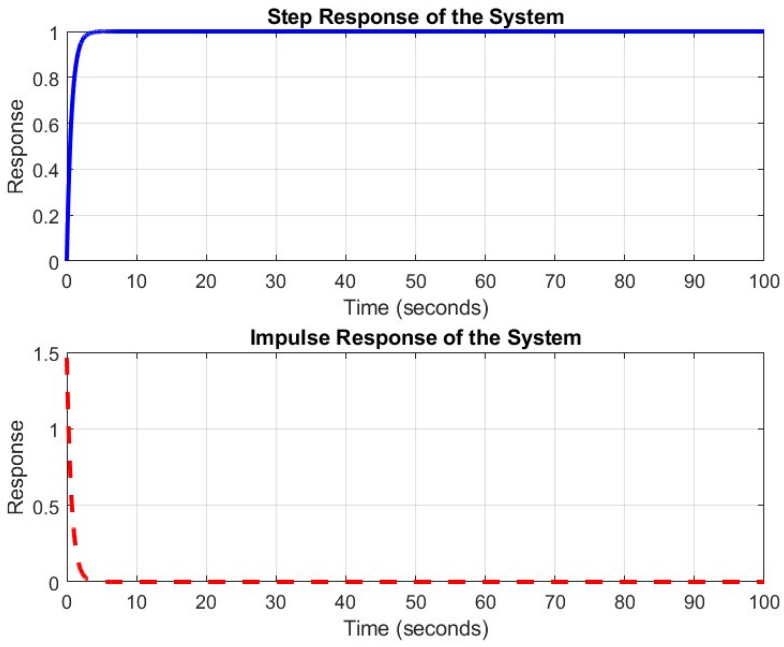

Figure 6: The step and impulse responses of the plant

4.2.4 Discussion

- (D) discussion of how my choices led to better achievement of the final objectives, compared to other control strategies

4.3 Oxygen control

4.4 Acidity control

5 Purification methods

5.1 Lactic acid purification

5.2 Ammonia purification

6 Final product formulation

References

[1] *Aging: From Fundamental Biology to Societal Impact*. Academic Press, 2022. ISBN: 978-0-12-823761-8. DOI: https://doi.org/10.1016/C2020-0-00576-1.

[2] Mark Cannon. *A2 Introduction to Control Theory: Lectures 1-4*. Department of Engineering Science, University of Oxford, Michaelmas Term. 2022.

[3] Mark Cannon. *A2 Introduction to Control Theory: Lectures 5-8*. Department of Engineering Science, University of Oxford, Michaelmas Term. 2022.

[4] Andrea E. Donaldson and Iain L. Lamont. "Biochemistry Changes That Occur after Death: Potential Markers for Determining Post-Mortem Interval". In: *PLOS ONE* 8.11 (2013). Published online 2013 Nov 21., e82011. DOI: 10.1371/journal.pone.0082011.

[5] Pauline M. Doran. "Chapter 9 - Heat Transfer". In: *Bioprocess Engineering Principles*. 2nd ed. Elsevier, 2013, pp. 333–377. DOI: 10.1016/B978-0-12-220851-5.00009-5.

[6] P.J. Fellows. "Heat processing". In: *Food Processing Technology: Principles and Practice*. 3rd ed. Woodhead Publishing, 2009, pp. 339–366. DOI: 10.1533/9781845696344.3.339.

[7] Mai Furuhashi et al. "Formation of contractile 3D bovine muscle tissue for construction of millimetre-thick cultured steak". In: *npj Science of Food* 5.6 (2021).

[8] J. B. Gaughan and T. L. Mader. "Body temperature and respiratory dynamics in un-shaded beef cattle". In: *International Journal of Biometeorology* 58 (2014), pp. 1443–1450.

[9] X. Guan et al. "Bioprocessing technology of muscle stem cells: implications for cultured meat". In: *Trends in Biotechnology* 40.6 (2022), pp. 721–734. DOI: https://doi.org/10.1016/j.tibtech.2021.11.004.

[10] Arthur C. Guyton and John E. Hall. *Textbook of Medical Physiology*. 11th ed. Elsevier Inc., 2006, p. 3. ISBN: 0-7216-0240-1.

[11] H. Hemeda, B. Giebel, and W. Wagner. "Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells". In: *Cytotherapy* 16.2 (2014), pp. 170–80. DOI: 10.1016/j.jcyt.2013.11.004.

[12] C. E. Jochems et al. "The use of fetal bovine serum: ethical or scientific problem?" In: *Altern Lab Anim* 30.2 (2002), pp. 219–27. DOI: 10.1177/026119290203000208.

[13] Mosa Meat. *Growth Medium without Fetal Bovine Serum (FBS)*. [Online; last accessed <29-03-2024>]. 17-01-2023.

[14] James Shen, Kim Ly, and Yvonne Hoang. *Human Stem Cell Manual*. Elsevier, 2012. Chap. 5, pp. 53–69. DOI: 10.1016/B978-0-12-385473-5.00005-9.

[15] K Subbiahanadar Chelladurai et al. “Alternative to FBS in animal cell culture - An overview and future perspective”. In: *Heliyon* 7.8 (July 2021), e07686. DOI: 10.1016/j.heliyon.2021.e07686.

[16] The Good Food Institute. *Cultivated meat cell culture media: Deep Dive*. Online; Last accessed: 28 March 2024. 2021.

[17] Sakshi Tyagi and Shalini Mani. “Media and Buffer Preparation for Cell Culture”. In: *Animal Cell Culture: Principles and Practice*. Springer International Publishing, 2023, pp. 77–88. ISBN: 9783031194856. DOI: 10.1007/978-3-031-19485-6_5.