B3 Group Design Project

Cultured Beef Production

Mikhail Agureev, Eunsoo Chang, Will Hough, Tracey Saber

University of Oxford

Department of Engineering Science

supervised by J. Kwan, N. Hankins, B. Nie, P. Mouthuy

Trinity Term 2024

Contents

1 Abstract **4**

2 Cell Selection **5**

2.1 Introduction to Cells 5

2.2 Stem Cells 5

3 Control systems **7**

3.1 Introduction 7

4 Medium **12**

4.1 Introduction 12

4.2 Medium composition 12

4.3 Moving away from FBS 13

4.4 Consideration 13

4.5 Summary 14

5 Proliferation seed train **15**

5.1 Growth medium selection 15

6 Control systems **16**

6.1 Introduction 16

6.2 Temperature control 16

6.2.1 Introduction 16

6.2.2 Methods 17

6.2.3 Results 18

6.2.4 Discussion 19

6.3 Oxygen control 20

6.4 Acidity control 21

7 Purification methods **22**

7.1 Lactic acid purification 22

7.2 Ammonia purification 23

8 Final product formulation **24**

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 Abstract

Introduction

It's becoming clear that the livestock farming sector is one of the main contributors to the world's water depletion, land use, biodiversity loss and greenhouse gas emissions. Over a third of the world's crop calories are used as animal feed, and only a third of those feed calories end up contributing to the human diet. [https://iopscience.iop.org/article/10.1088/1748-9326/8/3/034015] With trends in global consumption and production of meat growing, the impacts of livestock farming are set to grow. Alternative protein developments aim to substantially reduce the impact of feeding the human and domesticated animal population of the world by substituting the billions of animals grown and slaughtered every year with an alternative. There are significant developments in the plant-based, microbial, and fermented protein research, but we focus on evaluating the viability of directly substituting reared and slaughtered cattle with commercially cultured beef.

Summary

Whilst shifting crop use away from animal feed could feed an additional 4 billion people and reduce global Green House Gas emissions by about a tenth, our venture proposal wouldn't be profitable in today's political and economic climate. [https://iopscience.iop.org/article/10.1088/1748-9326/8/3/034015] [https://www.sciencedirect.com/science/article/pii/S0377840111001933 MAKE SURE TO ACCOUNT FOR INCREASE IN CROP CONSUMPTION] Unless commissioned by a special interested party – the outgoings and capital cost, along with risk associated with such a new, volatile and saturated market – mean that the proposal is unlikely to work better than other protein alternatives. If policies were introduced that reduced the subsidies on traditionally farmed meat and moved them to cultured meat, then our proposal might gain more traction to feed demand as our prices ease. [https://www.oxfordmartin.ox.ac.uk/blog/meat-and-dairy-gobble-up-farming-subsidies/]

2 Cell Selection

2.1 Introduction to Cells

Our final product is a combination of muscle cells and oleogel-based fat substitute [https://www.nature.com/articles
023-38593-4]. Of the three muscle cell types (myocytes) found in vertebrates, beef is composed of skeletal muscle cells [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5167519/]. These cells can be derived from myoblasts through a process known as myogenesis, explained further in [SECTION DIFFERENTIATION].

[https://www.researchgate.net/figure/Myoblast-differentiation-Activated-satellite-cells-are-called-myoblasts-They-express$_{fig}25_{2}31588877]ORUSETRACEY'SFIGURE4$

The definition of a myoblast is “an undifferentiated cell capable of giving rise to muscle cells” [https://www.merriam-webster.com/dictionary/myoblast], which includes many different types of stem cells. When designing our cultured meat process, we need ensure our primary cells are suitable for both the process and final product.

Myosatellite cells are the mode cell type used in industry, 23.1% of 40 companies. [GFI-APAC-cell-line-survey-report-19-June-2023]

2.2 Stem Cells

We considered the following stem cell types for seeding our bioreactors:

Skeletal Muscle Stem Cells (Myosatellite)

Under resting condition myosatellite cells are quiescent [dormant] and reside under the basal lamina [https://en.wikipedia.org/wiki/Basal$_{lamina}]ofthemyofiber[https://www.sciencedirect.com/science/article/pii/$

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are stromal cells that can self-renew. They are also capable of multilineage differentiation. MSCs can be isolated from a variety of tissues, such as umbilical cord, endometrial polyps, menses blood, bone marrow, adipose tissue, etc. [https://pubmed.ncbi.nlm.nih.gov/21396235/]. One of the major challenges is to elucidate the mechanisms of differentiation, mobilization, and homing of MSCs, which are highly complex.

Embryonic Stem Cells

Embryonic stem cells (ESC) are obtained from the inner cell mass of the blastocyst and are associated with tumorigenesis [https://pubmed.ncbi.nlm.nih.gov/21396235/]. Embryonic stem cells are pluripotent meaning they can differentiate into all derivatives of the three primary germ layers. ESCs are capable of self-renewal in conditions that prevent differentiation and clumping. [https://www.science.org/doi/10 [https://www.cell.com/cell/fulltext/S0092-8674%2803%2900847-X]. ESCs have a shortened G1 phase and thus divide very frequently, allowing the cells to multiply quickly. ESCs have ethical considerations as harvesting embryonic stem cells usually necessitates destroying the embryo from which those cells are obtained.

Induced Pluripotent Stem Cell (iPSC)

iPSCs can be obtained by reprogramming differentiated somatic cells. The genetic engineering required is high-cost due to its sensitivity, however base cell biopsy samples are easy to gain. Performance characteristics are similar to ESCs. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347549/]

Other Cell Types

The following cell types may be useful:

Fibroblast

Fibroblast cells are used in the production of the extracellular matrix that can be used to provide texture to the meat. They can replicate indefinitely in vitro and are found in many parts of the body including skin. [https://www.ncbi.nlm.nih.gov/books/NBK26889/]

Adipocyte

Adipocytes are the energy storage mechanism of the body, also known as fat cells. They are derived from Mesenchymal Stem Cells via adipogenesis. [https://www.ncbi.nlm.nih.gov/books/NBK555602/]

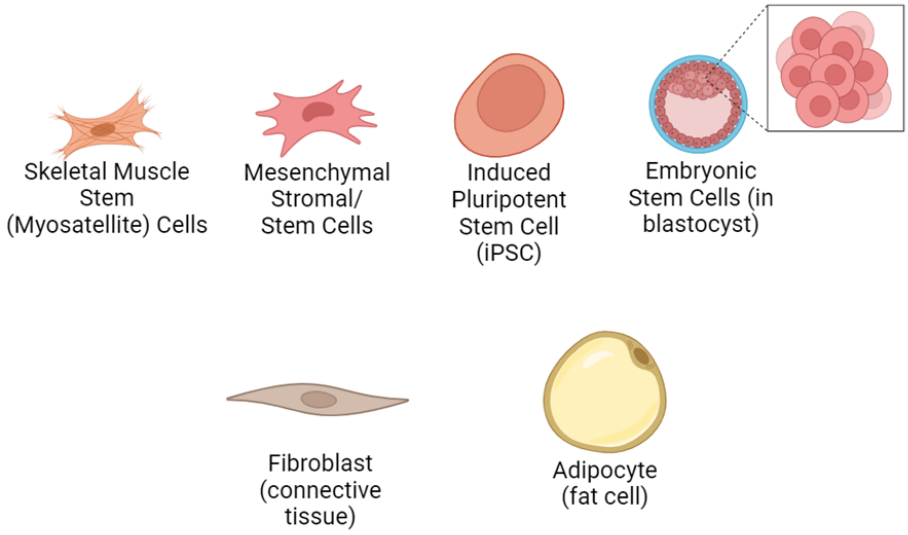

Figure 1: Cell types discussed, made in Biorender

3 Control systems

3.1 Introduction

Scaffold Selection Skeletal muscle cells are themselves adherent – they're named after the fact they adhere to bones. In vivo, cells exist within an extracellular matrix of proteins and proteoglycans. Complex interactions known as mechanotransduction occurs between the stiffness of the matrix and the cells within it. These interactions are governed by integrins and lead to downstream signaling that can affect cell polarity, migration, and differentiation. During the proliferation phase, we grow anchorage-dependent stem cells in large scale bioreactors. The non-native environment can lead to anoikis if cells are not adapted to suspension growth, tricked into via molecules such as Rho Kinase inhibitors to be anchorage independent or grown as spheroids. We can bypass activation of anoikis in suspension by using a scaffold that enables cellular adherence. Another factor to consider would be the materials used in our scaffolds. We need to ensure they meet our primary directives and the goals of the group.

[https://gfi.org/wp-content/uploads/2023/01/Figure-4-Scaffold-Types$_v$1.pdf]$Microcarriers Microcarriers are$
$//gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-scaffolding][https:$
$//www.mdpi.com/2304-8158/10/12/3116]$

Figure 1"C:- Nexus36533Workand Documents31016 Ivy Farm Industry Talk Oxford - Share.pdf" [https://www.sciencedirect.com/science/article/pii/S0268005X22001527] Porous Scaffolds Porous scaffolds try to mimic the extracellular matrix of a tissue. Generally, porous scaffolds allow adhesion,

mass transport, migration and cell distribution. To accommodate this, they need to be biocompatible to avoid an immune response and they can enhance tissue growth by being bioactive. Typical scaffold structures can be foams or lattices. If foamed, they need a manufacturing process that ensures the interconnectivity of pores and pore size, to prevent hypoxic conditions due to stagnation. Manufacturing processes like this are complex and don't guarantee performance. [https://pubs.acs.org/doi/10.1021/acsabm.2c00
Hydrogels Hydrogels are made up of three-dimensional networks of crosslinked hydrophilic polymers. They can absorb large amounts of fluids, up to several thousand %, and readily swell without dissolving. When swollen they resemble living tissues in texture – rubbery and soft. [https://www.ncbi.nlm.nih.gov/pmc/ar
Chitosan and alginate-based hydrogels meet the biocompatible requirements needed to prevent an immoresponse from our cultured cells. [https://onlinelibrary.wiley.com/doi/abs/10.1002/app.10137]

Figure 2https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3963751 Hydrogels can be obtained by various methods – most commonly physical crosslinking, chemical crosslinking, free radical polymerisation and irradiation crosslinking [see fig below]

Figure 3 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3963751 [https://link.springer.com/article/10.1007/s1
019-6318-7 and "C:- Nexus36533WorkCollaboration42242-021-00165-0.pdf"] Additional techniques 3D Printing and other additive techniques 3D printing can be used to print either the product, allowing greater control over the composition and texture, or the ECM-like porous scaffold used in cell proliferation, allowing us to print a fluid-modelled growth-structure that we're certain allows proper perfusion. Additive techniques, however, decrease cell viability after micro extrusion by around 40-86% due to extrusion pressure and shear stress. The process requires specialist, precision, equipment – increasing complexity, maintenance, and capital costs.

Figure 4 "C:- Nexus36533WorkCollaboration10856-019-6318-7.pdf" Fiber Scaffolds Much like porous scaffolds, we can make cell supporting structures using microfibres. Cellulose fibres meet our criteria for biocompatibility, mechanical strength and reactive surfaces for protein binding, however very few studies have looked into the application of cellulose microfibers as a scaffold in cell culturing due to the absence of an intrinsic 3-D structure. Use of gelatin may provide the needed 3-D architecture – gelatin is collagen derived and is nonimmunogenic, inexpensive and biodegradable. Research shows that cellulose microfiber/gelatin composites containing up to 75% cellulose fibres are more capable of withstanding mechanical loads than gelatin alone. [https://www.sciencedirect.com/science/article/pii/S17
Gelatin is produced through partial hydrolysis of collagen, which is typically from bovine or porcine sources. This means that whilst cellulose itself is plant based, cellulose fiber scaffolds don't meet our requirements for reducing reliance on existing animal agriculture and slaughter. [https://www.sciencedirect.com/to

Additional factors Typically, scaffolds are used in the proliferation process, after which the cells are harvested using trypsin, accutase and/or sonication. [https://pubmed.ncbi.nlm.nih.gov/30504375/][https://www.nature.com/articles/s41598-022-09605-y] However, harvesting puts the cells at risk of death – reducing yield – and adds further operations to an already complex process. If we consider the texture and composition of the final product, we know that since techniques for growing fibrous muscle or spatially patterned 3D substrates haven't developed yet, the structure of our final product will be a homogenised microtissue structure – as seen in ground beef. To add texture to the homogenised microtissues we need a binding/aggregating agent, which is often added to the cells after they are harvested from their proliferation scaffold. If we can find a proliferation scaffold that can stay in the final product and increase the aggregate size, we remove the need for harvesting and secondary scaffold addition. This will be a significant improvement on the product and thus should be weighted as such in our multi-criteria analysis. If we do manage to find something suitable, we note we've removed the option of recycling our proliferation scaffold, which means we need to ensure we have a well optimised scaffold manufacturing process.

Figure 5 Structured Final Beef Burgers https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-023-38593-4/MediaObjects/41467$_{2}023_{3}8593_{M}OESM6_{E}SM.mp4$

Microcarrier Materials and Manufacturing [https://www.sciencedirect.com/science/article/pii/S0268005X2200 Materials Chitosan (Av. MW 890,000 Da, degree of deacetylation: 93%) was purchased from Glentham Life Sciences (Wiltshire, UK). Sodium tripolyphosphate (TPP) was purchased from Alfa Aesar (Ward Hill, MA, USA). Bovine Achilles tendon collagen and porcine gastric pepsin were purchased from Sigma Aldrich (Rehovot, Israel). Epigallocatechin gallate (EGCG) was supplied by Healthy origins (Pittsburgh, PA, USA). Substitute tendon collagen for biomimetic plant based collagen. [https://go.gale.com/ps/i.do?id=GALE%7CA776940777sid=sitemapv=2.1it=rp=HRCAsw=wuserGroupName=anc web-entry] Substitute pepsin with animal free pepsin. [https://theeverycompany.com/news/pepsin] Manufacturing of cell microcarriers A 2% chitosan (CS) solution in 0.2M acetic acid was electrosprayed through a 27G needle into a 2% TPP crosslinking solution. Alternatively, a solution of 1% collagen (COL) in 0.5M acetic acid was prepared using 0.1% pepsin and electrosprayed into a 1% EGCG crosslinking solution. In the case of composite microcarriers, chitosan and collagen solutions were mixed at a mass ratio of 90:10, respectively, to achieve a final polymer concentration of 2%. The spray distance, applied voltage, flow rate, and polymer concentration were optimized according to Tables S1 and S2. The obtained MCs were sterilized using 70% ethanol and then washed 2 times for 60 min in double distilled water, once in phosphate buffer saline (PBS), and once in a growth

medium before seeding the cells. For the seeding, microcarriers were incubated with the cells at 37 ℃ and 5% CO2 overnight in a ratio of 5000 cells/cm2. The seeding area was determined as S = 4 × × R2 × N, where S is the seeding area, R is the radius of MCs, and N is the number of MCs. Optimal size being spherical microparticles with a smooth surface and narrow size distribution of 571 ± 66 m diameter.

Bacterial collagen substitute [https://www.frontiersin.org/articles/10.3389/fchem.2014.00040/full]

Plant collagen [https://www.sciencedirect.com/science/article/pii/S0268005X22001527bib45]

Muscle cell @ 3.5e-13kg [Mike ref]

collagen/chitosan scaffold aerogels @ 0.0468g/cm3 = 46.8kg/m3 [https://www.sciencedirect.com/science/arti
For the seeding, microcarriers were incubated with the cells at 37 ℃ and 5% CO2 overnight in a ratio of 5000 cells/cm2. The seeding area was determined as S = 4 × × R2 × N,

The addition of a low EGCG concentration (0.02%) resulted in spherical microparticles with a smooth surface and narrow size distribution of 571 ± 66 m diameter. This means each microcarrier has a volume of 97.48e-12m3, and thus a mass of V*rho = 4.562e-9kg. A surface area of 4*pi*r^2 = $pi * d^2 = 1.0243e-6m2$

Our starting density is 50e6 cells per m2 of microcarrier surface area. This is 51.2 cells per microcarrier. [https://www.sciencedirect.com/science/article/pii/S0268005X22001527appsec1: :text=2.5.%20Cell-,seeding,-and%20cultivation%20on] However, if we go with the later studied seeding density of 9000 cells per cm2 or 90e6 cells per m2 we get a seeding of 92.187 cells per microcarrier. [https://www.nature.com/artic
023-38593-4MOESM4: :text=Cell%20seeding-,Edible,-cell%20microcarriers%20were]

Our final amount of meat each cycle is about 100kg At 20%m/m of fat this leaves 80kg of cells + microcarriers. Optimistic cell densities achieved in production STRs are around 2e6cells/mL = 2e12cells/m3. [https://www.frontiersin.org/articles/10.3389/fsufs.2019.00044/full] With random packing of equal spheres we get approx. 63.5% of space taken up. This means that in a 1m3 space we can pack 1/(97.48e-12m3/63.5%) = 6.514e9 microcarriers/m3 This gives a microcarrier to cell ratio of 1:307 or roughly 1:300. This gives a microcarrier to cell mass ratio 1:0.2355 or 4.246:1 Thus, of our 80kg, we have 15.25kg of raw cells and 64.75kg of microcarriers.

Further attempts to reduce the collagen concentration in the MC led to a decreased cell viability: (Fig. S3), hence the 90:10 CS/COL-MCs were used in our following studies.

Figure S3. Viability of C2C12 cells cultured on CS/COL-MCs produced using different ratios of chitosan to collagen. https://www.sciencedirect.com/science/article/pii/S0268005X22001527

4 Medium

4.1 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).

4.2 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.

4.3 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]).

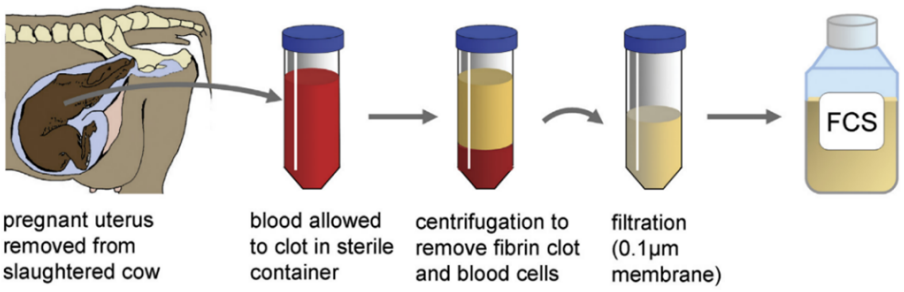

Figure 2: Process of FBS, also known as foetal calf serum FCS, ([11])

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.

4.4 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.

4.5 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.

5 Proliferation seed train

5.1 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.

6 Control systems

6.1 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.

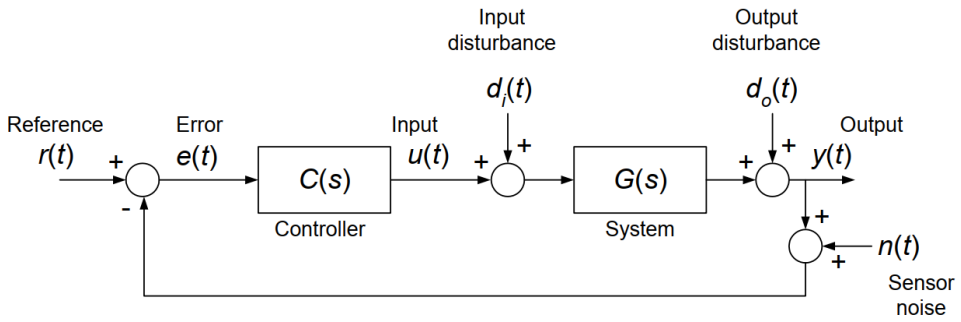

Figure 3: A schematic of a negative feedback control system Cannon [2]

6.2 Temperature control

6.2.1 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.

6.2.2 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.

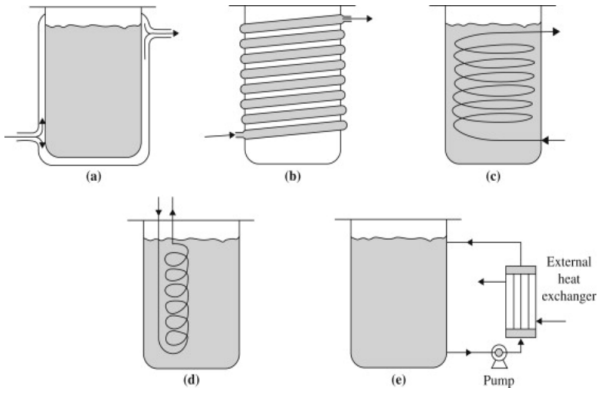

Figure 4: Heat exchanger configurations for a bioreactor [5]

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.

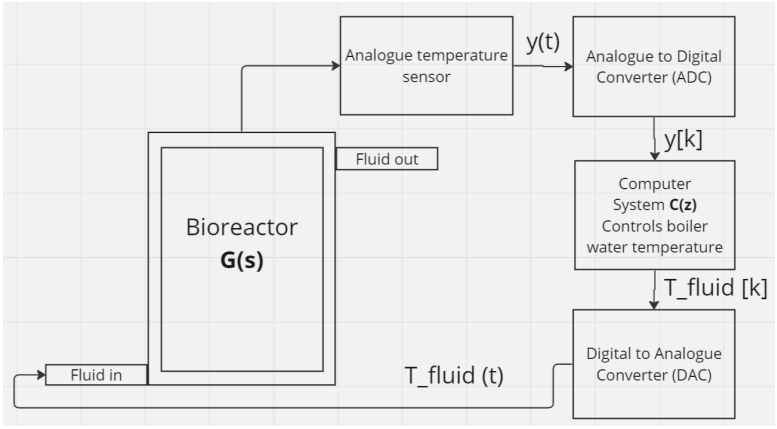

Figure 5: Practical implementation of the control system

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.

$$\rho V c_p \frac{\partial T}{\partial t} = Q_{met} V - hA(T - T_{fluid}) \tag{1}$$

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 2. 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 3. Substituting Equation 3 into Equation 2 and taking the Laplace transform leads to the desired transfer function, as shown in Equation 4.

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

$$Q_{met} V - hA(T_{ss} - T_{fluid,ss}) = 0 \tag{3}$$

$$G(s) = \frac{\Delta T(s)}{\Delta T_{fluid}(s)} = \frac{hA}{\rho V c_p s + hA} \tag{4}$$

6.2.3 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 5. 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 6.

$$c_p = \eta c_{p,cell} + (1 - \eta) c_{p,water} = 3.810\ kJkg^{-1}K^{-1} \tag{5}$$

$$G(s) = \frac{10.06}{6.872s + 10.06} \tag{6}$$

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 $C(s) = K_p + \frac{K_i}{s} + K_d s$.

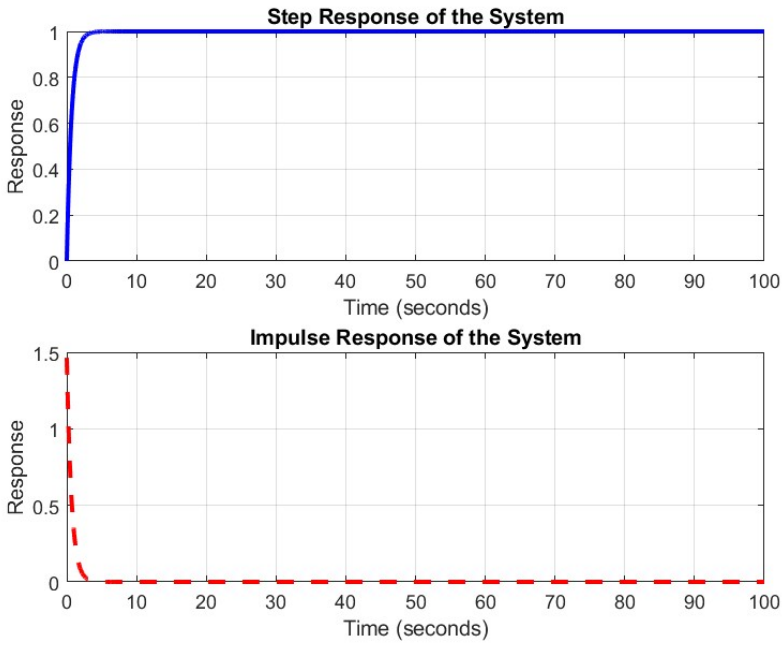

Figure 6: The step and impulse responses of the plant

6.2.4 Discussion

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

6.3 Oxygen control

6.4 Acidity control

7 Purification methods

7.1 Lactic acid purification

7.2 Ammonia purification

8 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.