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Cultured Meat Production: A Food Supply-Chain for the Future (EEM)

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Abstract

With the global population projected to reach 9.7 billion by 2050, traditional meat production faces increasing scrutiny due to its unsustainable environmental and ethical impacts. Cultured meat offers a promising alternative by producing real animal meat from cell cultures without the need for animal slaughter. This project addresses the challenge of designing a facility capable of producing one tonne of cultured chicken meat per year, while also evaluating its techno-economic feasibility. A novel high-density and entirely animal component free cellular process was devised to produce cultured chicken mince. It explores the integration of advanced bioengineering techniques such as serum-free media, suspension adaptation, variable perfusion, and medium recycling. The engineering solution was complemented by detailed environmental, safety, and cost assessments. In parallel, a comprehensive entrepreneurship and management strategy was developed. Market analysis, stakeholder identification, and consumer research informed the selection of an optimal launch location and marketing approach. Financial forecasting and operational planning culminated in a complete business model and funding strategy under the company name "Oxfarm." This integrated engineering and business approach demonstrates a potentially viable pathway for the commercialisation of cultured meat that could contribute to a more ethical and sustainable future for the intensive animal farming industry.

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1 Introduction - Zac Smith

1.1 Project Background

Food is an undisputed necessity of life, and with an ever-increasing population expected to reach 9.7 billion people by 2050 [1] it will become harder to satisfy food demands in ecological, ethical and sustainable ways. Agriculture plays a significant role in the current environmental situation of the Earth due to its massive land demands (half of the world's habitable land), large fresh water requirements (70% of global freshwater withdrawals), intense energy demands, and enormous greenhouse gas (GHG) emissions (26% of global emissions) [2]. More specifically, livestock farms require more than three quarters of global agricultural land [3], contribute to 80% of agriculture's GHG emissions [4], and use 70–80% of the world's antibiotics [5].

It is evident that these growing concerns must be met with innovative and sustainable solutions. Approximately 90% of the world's population eats meat [6], which highlights why effective alternatives to the environment-damaging meat industry must be found to meet consumer demands in cleaner and more ethical ways. Cultured meat is emerging as one of the most promising technological avenues to address this monumental challenge [7].

1.2 Problem Statement

Cultured meat (also known as cultivated, in vitro, lab-grown, or synthetic meat [8]) refers to meat grown from real animal cells in vitro (outside a living organism). This avoids animal slaughter by using advanced tissue engineering techniques. It has the potential to ease the burden on intensive animal farming practices and reduce their negative ethical and environmental impacts [7]. With this in mind, the engineering problem statement was simple:

Design a facility which uses cell cultures to supply 1 tonne per year of chicken meat products.

In addition to this, an engineering, entrepreneurship, and management (EEM) strategy was produced. This detailed the financial and commercial challenges and opportunities of this engineering project and used the following brief:

Develop a business plan that includes financial forecasts, market analysis, and operational strategies to assess and ensure the project's viability.

1.3 Project Objectives

- Identify, design, and integrate the individual engineering processes of the facility.
- Ensure the process is entirely animal component free, excluding the one-off purchase of the selected cell line.
- Explore the current technological landscape to make informed engineering decisions for higher efficiency.
- Consider future strategies that could improve the sustainability of the process and reduce total costs.
- Analyse the alternative protein market, industry stakeholders, competitors, and regulations.
- Identify the optimal starting location and develop an overall business strategy.

- Explore commercialisation strategies and produce a financial plan for the most promising business model.
- Reach a conclusion about the long-term viability of the business.

1.4 Problem Solution

In order to address both the engineering and EEM problem statements, the engineering objectives were first targeted. Once the process had been designed, analysed, and optimised (as laid out in Section 2), the focus was on the EEM objectives. The business design work for a hypothetical company, "OxFarm", is introduced in Section 18. This dual approach is essential in enabling a thorough and balanced investigation of a rapidly evolving concept and market. Combining the technical engineering design work with a flexible but rigorous business plan will allow an assessment of the techno-economic feasibility of producing cultured meat.

2 Engineering Introduction - Zac Smith

This paragraph details how individual research was combined to design a unique high-density cellular process to produce pure chicken mince meat. Sections 3 and 5 cover initial decisions including cell line, bioreactor, and cell culturing types, while Section 4 details the internally developed cell culture medium. These sections informed the subsequent engineering process design, outlined in Figure 1.

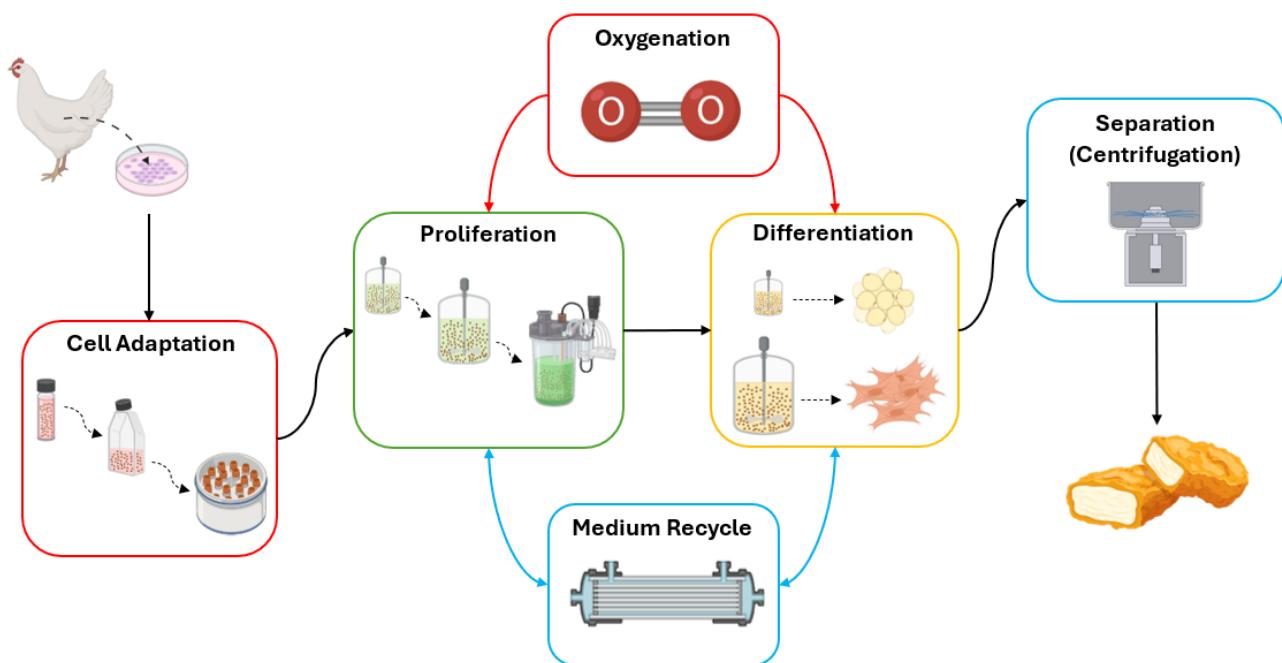


Figure 1: Overall Project Process Flow Diagram (Made in Microsoft Word with BioRender)

The process design was split into individual key sections: cell line adaptation, proliferation, differentiation (myogenesis and adipogenesis), supporting sub-systems (oxygenation, control systems and medium recycling), and cell harvest. Sections 6-14 explain this in detail, with subsequent costing, safety and risk, and environmental impact analyses conducted in Sections 15, 16, and 17.

3 Initial Considerations - Zaheer Sidik

3.1 Cell Types

When selecting a cell line the primary concerns are the proliferation limits and differentiation pathways. It is also desirable to have cells that can surpass Hayflick's limit, which states that cells are only able to proliferate 50 times before they become senescent - due to the shortening of telomeres [9]. Furthermore, the taste and texture also need to be considered as they dictate the quality of the product.

3.1.1 Fibroblasts/ MsC

Fibroblasts are a desirable choice due to a number of factors. They produce an ECM (Extracellular Matrix) of proteins such as collagen [10], which dictate the texture of meat based products. Also they are able to undergo a number of transdifferentiation pathways, such as myogenesis and adipogenesis [11], this would mean having to only use a single cell line for both the muscle cells and fat cells. Isolating fibroblasts is also a simple procedure [12], however this is not a deciding factor as we have chosen to buy a commercially available cell line rather than isolate the cells in house. They have a differentiation time period of approximately 4 days (from fibroblasts to muscle cells) [11].

3.1.2 Spontaneously Immortalised Cell Lines

SI (Spontaneously Immortalised) Cell Lines refer to those with the capacity to proliferate indefinitely (this is not a specific cell type but rather a desirable characteristic of cell lines), this is achievable with a fibroblast cell line, and is already commercially available at [13]. However, the cell line has to be monitored closely to ensure that there are no genetic changes that lead to tumour formation [14], this is not specific to fibroblast SI lines, rather it applies to all SI cell lines.

3.1.3 Embryonic Stem Cells

ESC (Embryonic Stem Cells) are pluripotent, meaning that they can undergo many differentiation pathways this allows for the generation of complex tissue structures mimicking that of traditional meat [15] , they also have a very high proliferation rate [16]; with the ability to become spontaneously immortalised. However, they require highly specialised media, which is expensive [17]. Furthermore, they have to be harvested from embryos which raises ethical concerns and there is limited availability of high-quality ESC cell lines [18]. According to [19], they typically take longer than other forms of stem cells to differentiate as they are at an earlier stage of development.

3.1.4 Differentiated Muscle Cells

Differentiated cells have an obvious benefit of not having to undergo the differentiation process, also they closely resemble the muscle tissue found in conventional meat [20] and they require less complex media, which could potentially reduce production costs [21]. However, they have a limited proliferation capacity, which would lead to the requirement of a constant source of new cells [21].

3.1.5 Conclusion

The chosen criteria to compare the choice of cell lines are:

- Proliferation Capacity - how many times the cells can proliferate before becoming senescent.
- Differentiation Complexity - how complex and costly is the differentiation process.
- Differentiation Time Period - how long does it take the cells to differentiate into muscle cells (only concerned with muscle cells here as they will make up majority of the product).
- Availability - how commercially available is the desired cell line.

A multi-criteria analysis can be performed to decide on which cell type is optimal choice, proliferation capacity has been given double the weighting due to a high proliferation capacity allowing for reduced complexity and in the case of SI cell lines negates the need for continuous sourcing of cell lines. All the other categories have been deemed to be equally important. The scores shown in the table below are given from 1-5 with a higher number indicating a better score/characteristic.

Table 1: Cell Line Multi-Criteria Analysis

Cell Line	Proliferation Capacity (W=0.4)	Differentiation Complexity (W=0.2)	Time Period Differentiation (W=0.2)	Availability (W=0.2)	Total
SI Fibroblasts	5	3	3	4	4
SI ESC	5	2	2	2	3.2
Differentiated	1	5	5	4	3.2

As shown in Table 1, the optimal cell line choice is that of SI Fibroblasts which are commercially available for £949 [13].

3.2 Bioreactor Choice

To ensure successful culturing meat at a large scale the efficiency and scalability of bioreactors must be considered. Different designs promote different shear stresses, mixing efficiencies, oxygen transfer and formation of nutrient/metabolite gradients [22]. Once a selection has been made it is also important to consider whether it is more cost-effective to have multiple smaller bioreactors or a single larger bioreactor this is carried out in Section 9.3.

3.2.1 Packed Bed

Packed bed reactors allow for very high cell densities [27] and limit the shear stress felt by cells, due to them being immobilised [28], however the lack of agitation can result in nutrient gradients forming and accumulation of metabolites which can lead to cell necrosis [29], furthermore when scaling up it becomes difficult to maintain a uniform flow distribution and the fluid is susceptible to channelling [30] (when the fluid takes certain paths over the packed bed rather than flowing uniformly). A simple schematic of a packed bed reactor can be seen in Figure 2a.

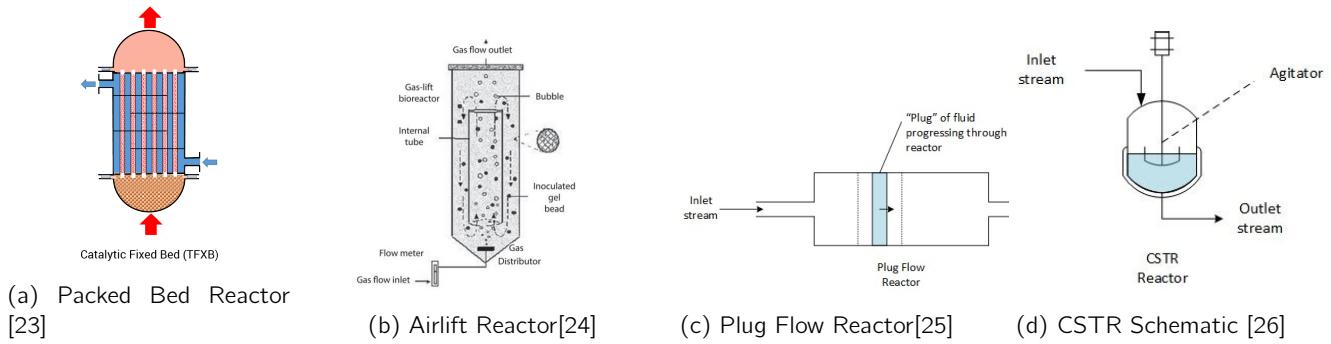


Figure 2: Bioreactor Comparison

3.2.2 Airlift Column

Airlift columns have a simple design with relatively few moving parts, which reduces the shear stress experienced by the cells [31], also they allow for sufficient gas exchange [31]. However, due to insufficient mixing there will be a non-uniform nutrient distribution resulting in some cells not receiving sufficient nutrients [32]. A simple schematic of an airlift reactor can be seen in Figure 2b.

3.2.3 Plug Flow

Plug flow reactors allow for a continuous product stream and nature of the reactor allows for predictable concentration gradients, also they generally have a higher volumetric efficiency when compared to CSTRs. However, the plug flow reactor requires turbulent flow which typically requires long pipes and baffles. Fouling typically takes place on the baffles, which is detrimental to cell cultures as it will lead to cell necrosis and hence contamination issues [33]. Furthermore, they require complex control and heating systems to ensure that the differential heat gradient along the length of the reactor is maintained. A simple schematic of a plug flow reactor can be seen in Figure 2c.

3.2.4 CSTR - Continuous Stirred Tank Reactor

CSTRs usually have a high volumetric efficiency making them suited to large scale processes [34], resulting in them being popular in industry. They also support the utilisation of perfusion, allowing for continuous removal of inhibitors and addition of growth factors [35], this limits cell necrosis and allows for a higher cell density. However, to ensure the environment inside the CSTR is uniform complex control systems are required [35]. A simple schematic of a CSTR can be seen in Figure 2d.

3.2.5 Conclusion

To decide which reactor type is best suited for our process, a multi-criteria analysis was performed with the key metrics being:

- Scalability
- Potential for High Density Culturing
- Complexity/Cost

- Nutrient Gradient Formation

Nutrient gradient formation was given a greater weighting than the other criteria as it dictates how much of the product is viable, whereas the bioreactor cost/complexity has been given a lower weight due to it being a one-off cost (single-use bioreactors have not been considered as they would contrast with our sustainability objectives).

The results can be seen in Table 2. Based on 2, the clear choice of bioreactor is the CSTR.

Table 2: Reactor Type Multi-Criteria Analysis

Reactor Type	Scalability (W=0.25)	High Density Cultures (W=0.25)	Nutrient Gradient Formation (W=0.4)	Complexity/Cost (W=0.1)	Total
CSTR	5	4	4	2	4.05
Plug Flow	3	3	3	1	2.8
Air Lift	2	2	2	4	2.2
Packed Bed	2	5	2	2	2.75

4 Cell Medium - Zac Smith

4.1 Introduction

Cell culture media is one of the most important factors in cell culture technology; it supports cell survival by providing essential nutrients, a consistent pH level, and protection from shear stresses [36]. It is also one of the most expensive, often contributing 55%–95% of the total cost of the product [37]. It is often composed of a cell-type-specific basal medium that includes the minimum necessary components such as glucose, amino acids, and vitamins. Certain growth factors or hormones are added to initiate certain cell processes (proliferation or differentiation). For decades, most media have used animal serums, commonly foetal bovine serum (FBS), as a growth promoting supplement, but these are being phased out of the cell culture industry [38] [36].

4.1.1 Serum Free vs Animal Component Free

The use of FBS raises significant ethical concerns as it is collected by syringe from the beating heart of bovine foetuses [39] after their pregnant mothers have been slaughtered [40]. FBS also suffers from batch-to-batch variation, high and unstable costs, and contamination risks, so it is necessary to develop a serum-free medium [41].

In addition to removing FBS, it was decided that a completely animal component free (ACF) medium would be used to meet the goal of creating an ethical and sustainable process that reduces animal suffering. Furthermore, this promotes a safer laboratory environment [42], eliminates dependence on animal products, and is essential for the future sustainability and regulatory approval of cultured meat production [43] [44].

4.2 Medium Composition

4.2.1 Commercially Available Media

The first step in choosing the cell medium was to research the existing commercially available media and their suitability to avian cell production. Table 3 shows the most relevant ACF media and their estimated costs per litre.

These were useful starting points, but the high costs prompted research into the internal development of media.

Table 3: Commercially Available Animal Component Free Media

Medium	Avian Cell Use	Additions Required	Cost (\$/L)
VP-SFM (AGT)	Chicken Embryo Fibroblast [45]	4mM Glutamine	38.72 [46]
Ex-Cell 320 ACF	Chicken Embryo Related [42]	4mM Glutamine, 0.1% Pluronic F68	51.61 [47]
OptiPro SFM	Immortalised Duck cells [48]	4mM Glutamine, Pluronic F68	160.02 [49]
ClearX9 Stem	Chicken Embryo Fibroblasts [50]	4mM Glutamine	147.47 [51]

4.2.2 Internally Developed Base Media

The standout choice for a serum-free basal media is equal parts Dulbecco's Modified Eagle Medium (high concentration of essential nutrients) and Ham's F-12 (wide range of components), known as DMEM/F-12 [36] [52]. It is chemically defined, ACF, and has been used as a base for several avian perfusion experiments [53] [54]. Alone, DMEM/F-12 cannot support cell growth [55], so a serum substitute is necessary. It was decided that a plant hydrolysate would be used as they can be sourced from various agro-industrial wastes, ranging from hempseed [56] to rice grains [57]. They are frequently used as sustainable and cost-effective animal protein substitutes [58]. Soybean hydrolysate (SH) was chosen for its low cost of production (raw materials, operating costs, equipment costs) and its effectiveness at low concentrations [59].

This medium composition requires additional supplementation of the following minor components: L-glutamine to adjust DMEM/F-12's concentration to the optimal 0.58 g/L (Δ) [60] [61]; Sodium Bicarbonate (NaHCO_3) to regulate the pH level between 7.1 and 7.4 [62]; Pluronic F-68, a non-ionic surfactant that protects cells from shear stresses [63], which is crucial when no serum is used [61]. Table 4 summarises this internally assembled base medium, which can then be fine-tuned and supplemented for specific cell processes to allow medium consistency throughout the cycle. Note that some costs were obtained from a bulk purchase quote from Thermo Fisher (*).

Table 4: Composition and total costs of internally developed medium

Component	Source	Amount Required	Cost per Unit (\$)	Cost per Litre (\$/L)
DMEM/F-12 Powder	Thermo Fisher	12.00 g/L *	0.30 *	3.54
Sterilised Water	ReAgent	1.00 L	0.58 [64]	0.58
Soybean Hydrolysate	Research Study	0.01 g/L [59]	0.18 [59]	0.002
L-Glutamine Powder	Thermo Fisher	0.22 g/L Δ	0.46 *	0.10
NaCHO_3 Powder	Amazon	2.43 g/L [62]	0.002 [65]	0.01
Pluronic F-68 Powder	MP Biomedical	0.50 g/L [48]	0.45 [66]	0.23
Total		-	-	4.23

4.2.3 Proliferation-Specific Medium

It is imperative to maintain high cell doubling rates throughout the proliferation stage [67], and using SH as the only source of growth factors decreased the growth rate by 40%. To compensate for this, it was decided that only 1% animal-free FBS replacement would be added with 10 $\mu\text{g}/\text{L}$ SH to match the performance of conventional media, as in experiments [59]. This is justified considering that ACF media can increase proliferation rates [68].

Several different serum alternatives were considered (KnockOut [69], FetalGro EX [70], FastGro Synthetic

[71]), but the clear choice was TNCBio's entirely ACF XerumFree [72]. It matches the growth characteristics of FBS at equivalent concentrations, does not require freezing and is compatible with DMEM/F-12, fibroblasts, and suspension culture. A recent quote from Thermo Fisher (*) prices it at \$721.22 per litre, so it raises the price of the proliferation medium to **\$11.62/L**. Despite this, it is a 96.9% cost reduction from the industry benchmark: Essential 8 Medium™[73].

4.3 Optimisation Strategies

Using an optimised and chemically defined medium minimises media waste and costs [67]. There are several traditional optimisation methods to use, including "design of experiments" or "spent media analysis" [74], to perfectly balance the medium. Manufacturing the medium in-house eliminates the need to use expensive products (DMEM/F-12 and XerumFree), drives the price down, and creates intellectual property [43]. The cost is expected to reduce to \$0.5/L based on theoretical research estimates (\$0.24/L [73]) and reported avian ACF media costs (\$0.63/L [53]), all lower than the estimated cost to achieve commercial viability of \$1/L [75].

5 Cell Culture Methods - Zac Smith

Having selected fibroblast cells as the optimal cell type as the first step of the bioprocess design (Section 3.1), it was essential to consider the most efficient cell culture environments for this cell line at the specified scale [67].

5.1 Cell Culture Environment

Mammalian cells, including avian fibroblasts [76], are inherently anchorage dependent (adherent), which means that in order to survive and proliferate, they require a surface to attach to [77]. Historically, this has been achieved by providing a fixed two-dimensional monolayer system such as a flask wall or scaffold structure [78], but large-scale cell production is unachievable under these growing conditions [79]. To solve this issue and greatly reduce the cost, volume, and carbon footprint of the plant [80], anchorage independence can be achieved by two methods that facilitate cell suspension [77]:

- **Suspension Adaptation:** a time-consuming process of identifying which cells can grow as single cells [81] or small clusters (aggregates) in suspension [82].
- **Microcarriers:** suspending organic or synthetic spherical microcarriers (100-200 μm diameter [79]) in the medium for the cells to adhere to [83] [31].

5.2 Decision

When evaluating the two different methods, suspension adaptation of the cell line was the best option for several reasons. Firstly, the final cell density of microcarriers is limited to just 8×10^6 cells/ml [83], whereas a maximum cell density of 108×10^6 was achieved using SI avian fibroblasts in single cell suspension. This translates to a 17-fold smaller processing plant compared to microcarrier culturing [81]. Microcarriers also expose cells to higher

shear stresses [84] and require costly, complex separation processes [77]. Even edible structures, which restrict the final product to only 40% meat [85], introduce more regulatory issues and operating costs [86]. Therefore, the ability to produce 100% meat products was an influential factor in the decision to use single-cell suspension.

6 Suspension Adaptation - Viraj Nerkar

6.1 Introduction

From Sections 3.1 and 5, the cell line of choice is spontaneously immortalised chicken embryonic fibroblasts that will be sourced from the American Type Culture Collection [13]. The cell line is already immortalised but is adherent and, therefore, to make it suitable for cell culture, it must undergo a suspension adaptation process.

6.2 Process

Based on the findings of this paper [81], the method of making spontaneously immortalised fibroblasts anchorage independent is as follows :

- Grow DF-1 cells in DMEM with 10 % Soybean Hydrolysate (FBS alternative) (Section 4.2.2) in a culture flask.
- Maintain at 37°C with 5% CO_2 and passage regularly when reaching 80% confluence.
- Transfer to low-attachment plates and reduce serum concentration gradually.
- Provide gentle agitation to prevent attachment.
- After 2 weeks, collect floating cells and transfer to new low-adherence plates.
- Centrifuge at $100 \times g$ for 5 minutes and re-suspend in fresh media.
- Repeat process every few days.
- Validate anchorage independence using the test for soft agar colony formation.

The suspension adaptation process will take 2-3 months initially. The final cell density is estimated to be 10^8 cells/ml [81]. Due to the planned cell bank, where a small portion of cells at the end of each proliferation cycle will be removed and then act as the initial cell line for the next cycle, (Section 7.4.2), this is a one-time process and therefore the total cost is \$2000 (from the initial purchase of the cell line [13]) and other process costs.

7 Proliferation - Zac Smith

7.1 Introduction

7.1.1 Proliferation Overview

Cell proliferation is the process of growing cells on a large scale, often at high cell densities [87], with the goal of producing enough biomass for the downstream differentiation stage [88]. The seed train process describes the progressive growth of a single vial of cells (from the working cell bank) up to the desired cell number for inoculation of the largest production reactor (N^{th} Reactor) [89]. This involves placing several bioreactors in series, successively increasing the volume by a factor of roughly five to ten [90].

Proliferation is a critical stage in the production of cultured meat as it serves as a platform for the entire manufacturing process [91]. It dictates the quantity, viability, and availability of cells, which impacts the efficiency of subsequent downstream systems [92].

7.1.2 Design Brief

The first step in designing the proliferation stage was manipulating the engineering problem to determine its requirements. The desired meat output of 1 tonne (1000 kg) per year was converted into a daily output number (N_{day}) of 9.04×10^{11} cells/day using Equation 1, assuming a value of 3.0×10^{11} mammalian muscle cells per kilogram of wet meat (C) averaged across several studies [67] [85] [93] [94].

$$N_{\text{day}} = \frac{1000 \text{ kg/year}}{365 \text{ days/year}} \times C \times SF \quad (1)$$

An arbitrary safety factor (SF) of 10% was applied to the daily quantity of cells to guarantee production of the desired biomass. This accounts for any losses incurred when transferring between bioreactors, separating the biomass from its medium, or a slight loss of cell viability throughout the various processes.

This daily cell number was used to design the seed train and production process, but close collaboration with the differentiation design was crucial to ensure reliable feeding of the downstream process.

7.2 Process Flow Diagram

The Process Flow Diagram below (Figure 3) outlines the entire proliferation stage, including the two seed train reactors ($N-1$, $N-2$), the main production bioreactor (N^{th}), and the medium recycle system (Section 13). The system takes suspension-adapted fibroblasts and fresh cell medium (Sections 6 and 4.2.3) plus other nutrients to meet the proliferation design brief previously explained.

7.3 Bioreactor Design

The proliferation stage design process was influenced by the downstream requirements and the capabilities of the supporting infrastructure. The design was cyclically revised as the models were developed and refined.

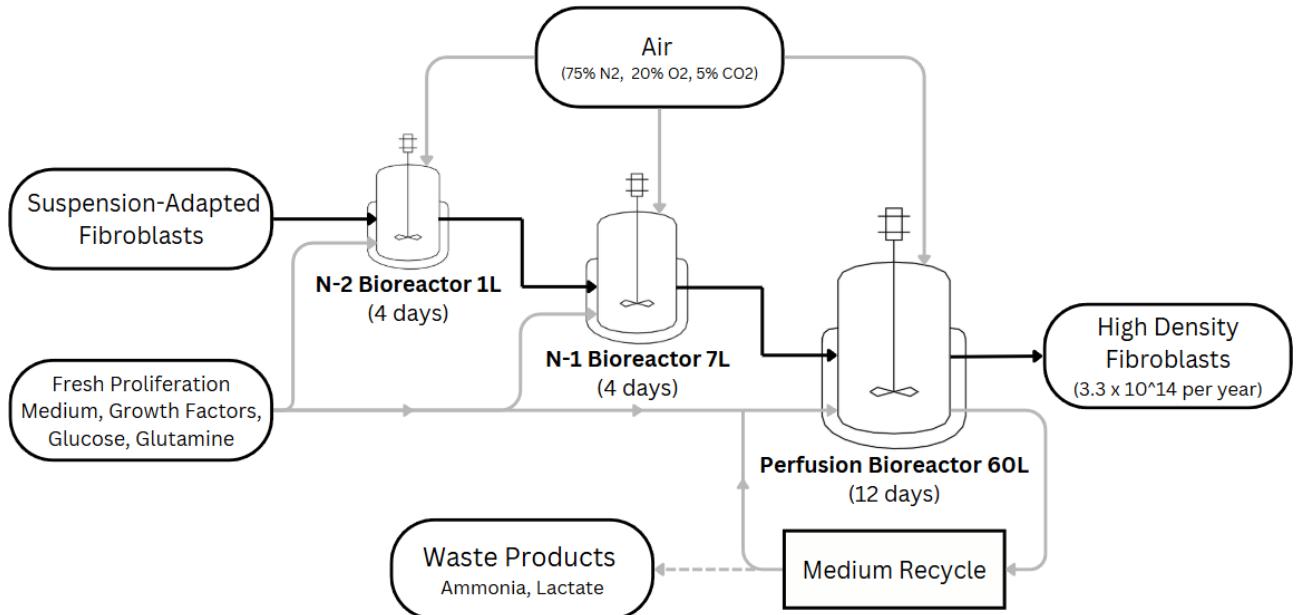


Figure 3: Proliferation Stage Process Flow Diagram (Made in Canva)

It was first decided, using Section 3.2.5, that STRs would be the best reactor type for the proliferation stage. Despite the increase in popularity of single-use STRs due to potential lower operating costs [67], it was decided that reusable stainless steel or glass bioreactors would be used to reduce plastic waste and improve sustainability. It was decided that perfusion would be used for the N^{th} reactor as it enables suspension cells to reach densities that are 40 times higher than adherent cultures to increase process yields [95]. For simplicity, a fed batch process would be used for the seed train bioreactors [31].

7.3.1 Bioreactor Sizing

It is common practice to estimate the size of proliferation bioreactors using the maximum achievable final cell density (X_f) in cells/ml [67]. The total working volume (V) required for each reactor was calculated using Equation 2, where N_f is the desired cell number output.

$$V = \frac{1 \text{ litre}}{1000 \text{ millilitres}} \times \frac{N_f}{X_f} \quad (2)$$

For the N^{th} production stage, N_f was estimated by multiplying the cell requirement (N_{day}) by the number of days per cell cycle (T_{cycle}): batch time (T_B) plus downtime for cleaning and biomass transfer. Then, using a conservative viscosity-limited maximum cell density (Section 8.1), the total

Table 5: Proliferation Bioreactors Sizing Values

Reactor	Volume (L)	Qty	Batch / Cycle Time (Days)	Final Density (cells/ml)
N^{th}	52.56	3	12 / 13.5	7.74×10^7
N-1	6.57	1	4 / 4.5	4.00×10^6
N-2	0.82	1	4 / 4.5	4.00×10^6

working volume required was divided between three parallel bioreactors for scheduling reasons (Section 7.4.2).

Equation 2 was also used to size the seed train reactors by calculating the number of cells needed to inoculate

the next reactor (Equation 6) and using a typical final cell density for fed-batch processes [96] [78]. All reactors use the optimal inoculation density (X_0) of 5×10^5 cells/ml [90] [97].

7.3.2 Impeller Design

The selection of impeller type and its rotational speed influences the homogenous distribution of cell culture components (cells, nutrients, gases), the size of suspended aggregates, and the mechanical stresses on the cells [98]. The impeller design was kept constant throughout the bioreactors, choosing a marine impeller for its low shear stress [98], [48]. Other considerations and values mentioned in Section 8.2 were assumed in the calculations. Equation 14 was rearranged to find the impeller power (P) [99], based on the researched optimal impeller speeds (n) shown in Table 6 and assuming the impeller diameters are half the diameter of the reactor tanks [100] [101].

Table 6: Proliferation Bioreactors Energy Balance per Cycle

Reactor	Impeller Speed (s^{-1})	Impeller Power (W)	Heat Of Reaction (W)	Heat Lost (W)	Heat Required (W)
N th	5.42 [81][102]	26.60	29.85	718.02	688.17
N-1	1.08 [103] [100]	2.0×10^{-3}	0.36	144.95	144.59
N-2	1.08 [103] [100]	1.8×10^{-4}	0.05	50.19	50.14

7.3.3 Heating Requirements

Heating the bioreactors to 39°C, the optimal temperature for avian fibroblasts [81], accounts for a significant proportion of the operating cost. The heating power required per bioreactor (Q_{req}) was calculated using Equation 3 with the heat lost to the surroundings (Q_{lost}) and the heat produced by the cell metabolic reactions (Q_{rct}).

$$Q_{req} = Q_{lost} - Q_{rct} \quad (3)$$

Q_{lost} was calculated using estimated or quoted reactor tank dimensions and a heat transfer coefficient of 0.8 $Wm^{-1}K^{-1}$ [104] for the glass seed train reactors alongside the values and methods explained in Section 8.3.

Q_{rct} was more complex due to the time-varying number of cells which Aspen PLUS cannot model. The simplified cellular metabolic reaction (Equation 22) and the final flow rate of the Nth perfusion cycle (Section 7.4.4) generated molar flow rates which were entered into an Aspen RSTOIC model (explained in Section 8.4). This produced an enthalpy of reaction, which was then normalised by the final cell count (N_f) and scaled for the average cell number of each reactor found by solving Equation 4 where $N(\tau)$ is the number of cells at any time (Section 7.4.1).

$$N_{avg} = \frac{1}{T_B} \int_0^{T_B} N(\tau) d\tau \quad (4)$$

This produced the average heat of reaction for the duration of each bioreactor cycle (Table 6) and therefore the average heating power required for each proliferation bioreactor was calculated.

7.4 Process Modelling

Mathematically modelling cell cultures is useful for estimating time frames, nutrient consumption, and optimisation strategies [105]. Capturing the time-varying nature of the proliferation process (which never reaches steady state because of constant cell growth) required simplifications. In order to predict crucial concepts like nutrient supplementation and medium replenish / recycling rates, a discretised model was built in Excel. This model has its limitations and assumptions that are evaluated throughout this section.

7.4.1 Cell Growth Models

The growth rate of mammalian cells can vary for different cell lines and environments [106], so careful assumptions were crucial to create realistic models to design other systems around. One such assumption is that the use of a serum-free medium will not reduce the growth rate, as justified in Section 4.2.3.

The growth equation below was used to model the number of cells at a given time ($N(t)$) of any proliferation process. Avian cells were conservatively assumed to experience a 24 hour latent phase (T_{lag}) for each new bioreactor [76] [107] as they acclimate to their new environment. The subsequent growth stage is determined by reactor-specific values: population doublings per day (PDD) and the initial number of cells (N_0).

$$N(t) = \begin{cases} N_0 & t < T_{lag} \\ N_0 \times 2^{PDD \times (t - T_{lag})} & t \geq T_{lag} \end{cases} \quad (5)$$

$$N_0 = V \times X_0 \quad (6)$$

A constant PDD of $1\ d^{-1}$ was used for the fed-batch seed train processes, averaged from multiple avian fibroblast experiments [81] [108] [106]. Due to the more intense nature of a perfusion reactor, a more conservative PDD value of $0.66\ d^{-1}$ was extrapolated from a chicken fibroblast perfusion experiment [53]. Equations 5 and 6 were used to calculate reactor batch times (T_B) in Table 5, which iteratively influenced the sizing of the N^{th} reactor until an equilibrium was achieved between batch time and working volume. Equation 5 was also used to generate time-varying cellular models of each bioreactor (in Excel) for subsequent modelling calculations.

7.4.2 Reactor Timelines

In order to maximise the efficiency of the plant, careful consideration of the bioreactor chain was necessary to reduce downtime between processes. The Gantt chart below shows the alignment of individual reactors, the transfer of biomass (arrows), and the time scales of each reactor (including scheduled cleaning periods represented by grey blocks). For speed and ease, biomass transfer throughout the proliferation stage can be achieved by transferring the cells to the subsequent stage suspended in the medium and adding fresh medium up to the working volume.

A bottleneck was first observed between the N^{th} perfusion stage and the myogenesis stage due to its shorter batch time of just 4 days (Section 9.1). It was decided that three N^{th} perfusion reactors would run in parallel, staggered to ensure constant supply of the predominant differentiation reactor as shown in Figure 4. This required

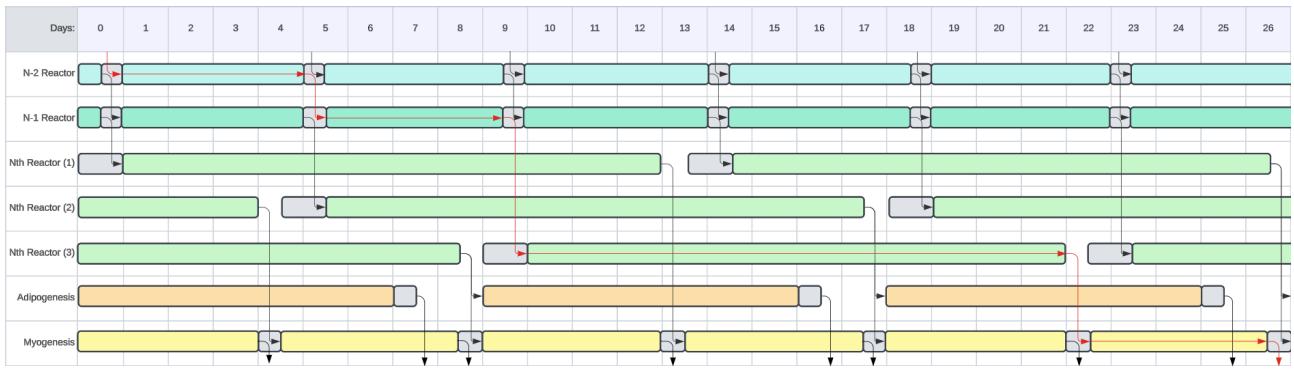


Figure 4: Timeline of all bioreactors with a full cycle shown in red (Made in Lucidspark)

a half-day rest period for the N^{th} reactor on top of the cleaning time (T_{cycle}) to bring the total cycle time (T_{cycle}) to 13.5 days. This also kept reactor volumes lower, making the cell culture environment more manageable [101] [58].

Having modelled the growth of the seed train reactors (N-2 and N-1), it was apparent that scheduling a half-day for cleaning would allow just one N-2 and N-1 reactor to sustain the entire process. This reduces the total number of reactors needed, lowering the complexity, space, and costs. Due to the longer batch time and lower cell requirement of the adipogenesis reactor (Section 10.1), it has been designed to run after alternate N^{th} reactor cycles. Its 1.5 day downtime is not as costly as only 3% of cells are sent to this reactor, and excess cells from the non-adipogenesis outputs can be bled off and used to restore the cell bank at high cell densities (Section 6.2).

7.4.3 Metabolite Concentration Models

To ensure constant growth, cell culture conditions must be kept at optimal levels by removing waste products of cell metabolism (ammonia and lactate) while maintaining a steady supply of nutrients (glucose, glutamine) [109]. The concentration of these four crucial components was modelled by adapting Equation 7 to form two discretised equations for the Excel tabulated model.

$$C_i(t) = C_{i,0} + \frac{q_i}{V} \int_0^t N(\tau) d\tau \quad (7)$$

$$C_{i,wst}(k) = C_{i,wst}(k-1) + \frac{q_{i,wst}}{V} N(k-1) \times T - C_{i,wst}(k-1) \times VVD \times T \quad (8)$$

$$C_{i,nut}(k) = C_{i,nut}(k-1) + \frac{q_{i,nut} MW_{i,nut}}{V} N(k-1) \times T + \frac{m_{i,nut}(k)}{V} \quad (9)$$

These assume the cell number (N) of step $k-1$ remains constant for a time period T to calculate the production or consumption of substrate i using its specific rate, q_i . Equation 8 models the waste product concentration (millimolars as standard), calculating the moles removed by medium replenishments (perfusion (VVD) or one-off replacement ($VVD = 1$)). Equation 9 models glucose and glutamine levels (grams per litre for costing) by calculating the mass $m_{i,nut}$ of nutrients added in that time step (by fresh media or supplementation).

The initial concentrations ($C_{i,0}$) of glucose and glutamine (3.15 and 0.58 g/L respectively) are determined by the proliferation medium (Section 4.2.2). Initial waste product levels depend on the final concentration of the previous reactor after dilution with fresh medium. These models were used to determine the necessary interventions

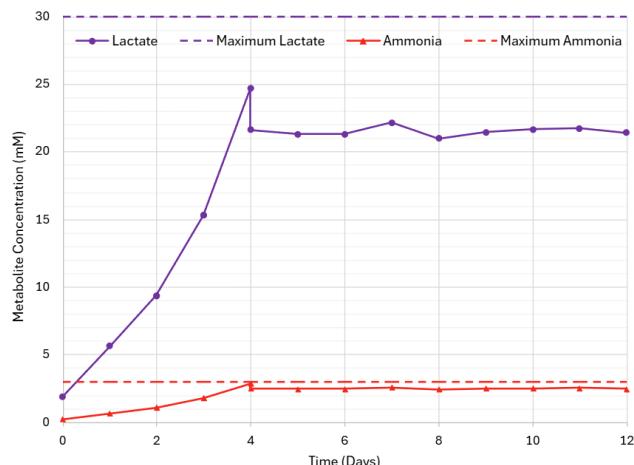
that a control system (Section 12.2) would implement to avoid inhibitory concentrations of lactate and ammonia (3 mM and 30 mM respectively [110] [109]) and maintain optimal initial nutrient levels.

The specific rates in Table 7 (negative signifies consumption) are for baby hamster kidney fibroblasts [109], so similar values for avian fibroblasts may vary slightly. However, processes have been designed to a higher performance level than necessary as these values appear to be overestimates when consulting other sources [82] [53].

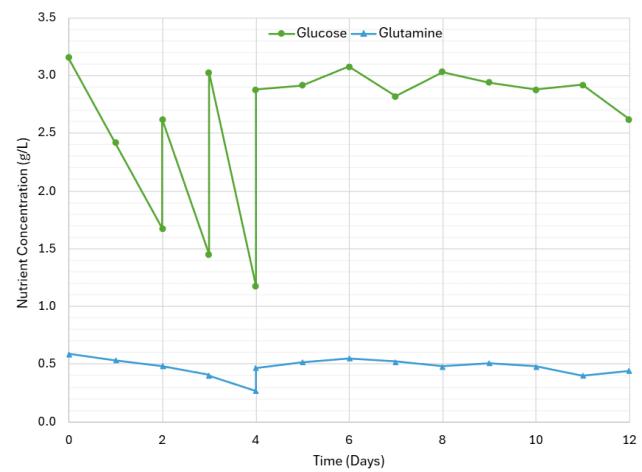
For the N^{th} reactor, the complications of the perfusion process were simplified by modelling at a time period (T) of one day. This is valid because the slower growth rate of cells means that they have not doubled in this time period. Figure 5a shows how the toxin concentration level is affected by the start of the perfusion process after four days. The perfusion rate was calculated daily (Section 7.4.4), assuming 100% of the waste products are removed from the medium that passes through the recycle system. The nutrient concentrations are also maintained by the perfusion process (Figure 5b), which adds fresh and recycled medium supplemented with additional nutrients. Daily additions of glucose are necessary until the perfusion process starts.

Table 7: Production and Consumption Rates

Metabolite	Specific Rate, q_i (10^{-10} mmol/day/cell)
Lactate	74.9
Ammonia	8.6
Glucose	-82.1
Glutamine	-7.2
Oxygen	-60.5



(a) Lactate and Ammonia variations over time



(b) Glucose and Glutamine variations over time

Figure 5: Essential Substrate Concentrations after Daily Supplements (day 2+) and Perfusion (day 4+) in N^{th} Reactor (Made using Excel)

The seed train reactors have identical cellular growth profiles as only the working volume changes, so the concentrations were modelled identically. A shorter time period was used to account for the faster doubling rate (Figure 6). Again, the ammonia concentration is the limiting value and would reach its threshold before the batch process has finished so an entire medium replenishment is necessary at the end of the third day, as in Figure 6a. Daily glucose additions are necessary from day two (common for fed batch cell cultures [81]), despite the restoration of glucose and glutamine concentrations from the scheduled medium replacement.

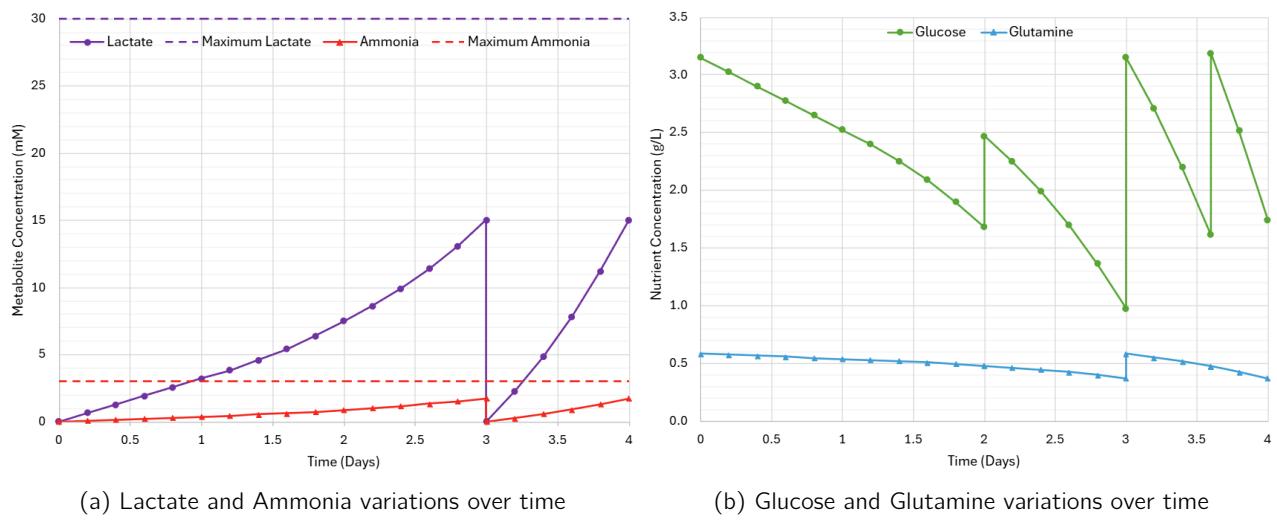


Figure 6: Essential Substrate Concentrations after Daily Supplements (day 2+) and Medium Replacement (day 3) in Seed Train Reactors (Made using Excel)

7.4.4 Perfusion Rate

The perfusion rate for the Nth bioreactor was determined using the concentration model of the limiting substrate: ammonia. To ensure sufficient removal of ammonia (and consequently other waste products), the necessary volume of medium replaced per working volume per day (*VVD*) was estimated using Equation 41, assuming that 100% of the metabolites are removed from the recycled medium. The model built by Equation 8 adjusts this perfusion rate to maintain a maximum concentration of 2.5 mM as a safety buffer.

Figure 8 shows how the calculated perfusion rates increase throughout the cycle, starting at just 0.2 d^{-1} at the end of day 4 and reaching 16.9 reactor volumes replaced throughout day 12. This extreme final value is necessary to achieve such high final cell densities. Typically, *VVD* is normalised to current cell density (X ; 10^6 cells/ml), giving the cell specific perfusion rate (*CSPR*) in picolitres per cell per day [111]:

$$\text{CSPR} = \frac{1000 \text{ picolitres}}{1 \text{ nanolitres}} \times \frac{\text{VVD}}{X} \quad (10)$$

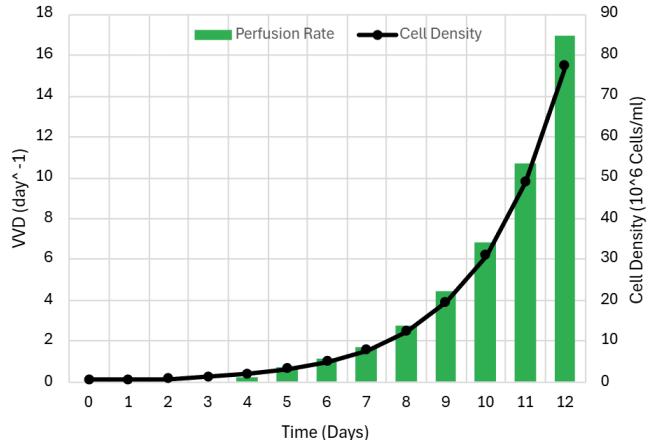


Table 8: Perfusion Rate and Cell Density of Nth Reactor

Once the perfusion process has stabilised (day 5), the system undergoes a *CSPR* of $220 \pm 2.7 \text{ pL cell}^{-1} \text{ day}^{-1}$. The use of a constant value ensures optimum conditions for cells [96] and is 8.3% lower than the target used in the successful perfusion of immortalised avian fibroblasts [53], resulting in less medium used.

7.4.5 Media Requirements

The proliferation bioreactors will use the ACF proliferation medium detailed in Section 4.2.3 and supplementations of glucose and glutamine delivered in high concentration doses. Table 9 details the key usages for each proliferation

cycle, where the medium used is calculated by summing the number of reactor volumes (RV) replaced throughout the cycle plus the initial volume of fresh medium required to adjust the transferred biomass medium to the required working volume.

Table 9: Total / Fresh Medium and Supplementation Usage in each Proliferation Bioreactor Cycle

Reactor	RV Replaced	Medium Used (L)	Fresh Medium (L)	Glucose (g)	Glutamine (g)
N th	45.23	2423.49	521.49	8792.20	448.62
N-1	1	12.32	12.32	15.52	0.00
N-2	1	1.64	1.64	1.94	0.00
Total	-	2437.45	535.45	8809.67	448.62

Due to the high medium usage of Nth perfusion reactors, it was decided that a medium recycle system (detailed in Section 13) would be implemented to reduce the amount of fresh medium required [54]. To preserve medium quality, it cannot be recycled infinitely and must be fully refreshed at least every 6 days [43]. In a serum-free perfusion experiment, a cycle of replenishing 20% of the medium after 3 days and then using 100% fresh medium after 6 days ensured constant cell viability and expansion [54]. The low perfusion rate of this experiment ($VVD = 0.95$) led to an adaptation of their method to a higher perfusion process by applying their cycle per RV replaced rather than per day. This results in 20% of the medium used in the perfusion process being replaced by fresh medium, as used in Table 9.

The glucose and glutamine required per reactor is determined by summing the supplements added throughout the processes. These were calculated for each discrete time step to maintain nutrient concentrations to the levels shown in Figures 6b and 5b, mimicking the function of a control system.

7.5 Costing

7.5.1 Operating Costs

The operating costs were calculated over one full proliferation cycle: a full cycle of the N-2, N-1, and Nth reactors. The use of three Nth Reactors allows one proliferation cycle to finish every 4.5 days. The supplemented glucose is bought in bulk from Sigma Aldrich [112], other medium costs are from Section 4.2, and energy costs are assumed to be \$0.11 / kWh [113] for the chosen starting location - Washington State, USA (Section 22).

Table 10: Proliferation Operating Costs per 4.5 day Cycle

Component	Quantity per Cycle	Cost per Unit (\$/g, L, kWh)	Cost per Cycle (\$)
Glucose Additions (g)	8,809.67	0.02 [112]	175.83
Glutamine Additions (g)	448.62	0.46 (4.2.2)	207.64
Fresh Medium (L)	535.45	4.45 (4.2.2)	2,380.98
FBS Alternative (L)	5.35	721.22 (4.2.3)	3,861.83
Impeller Energy (kWh)	7.66	0.11 [113]	0.84
Heating Energy (kWh)	221.02	0.11 [113]	24.20
Total Cost per Cycle	-	-	6,651.31

7.5.2 Capital Costs

The major capital costs of the proliferation stage are the purchase of bioreactors. Rough quotations were obtained for a reusable perfusion reactor and two fed-batch HyPerforma™ Glass STRs at the appropriate volumes from Thermo Fisher (Table 11). These were verified using the equation in Figure 16a and have been further developed in Section 15.3 to account for delivery and installation.

Table 11: Proliferation Capital Costs

Reactor	Volume (L)	Bare Cost (\$)
N th	60	264,600
N-1	7	176,400
N-2	1	138,600

7.6 Optimisation Strategies

This paragraph briefly outlines a few strategies that were not implemented in this design, but should be considered in the future to limit waste, increase efficiency, and reduce costs.

Thermal Manipulation: Research indicates that exposing avian cells to higher temperatures (39.5 to 41°C) for just six hours during embryogenesis can increase proliferation rates by up to 20% [114] [115]. This could be applied relatively cheaply during suspension adaptation to achieve faster growth cycles [116] that could lower required reactor volumes.

Continuous Harvesting: Proposed as a solution for high-volume cell production in the biopharmaceutical industry, harvesting cells continuously enables high densities to be maintained and farmed for up to three months [77]. This increases process yields and reduces production costs, growth lag time, and production downtime [53]. This method could be applied to our perfusion reactors if several differentiation reactors were staggered to accept continuous input. This is more feasible in a larger-scale plant.

Bioreactors: As bioreactor technology improves, the ability to use larger volumes without limiting cell density may become more cost efficient. It is hoped that airlift or wave bioreactors will be able to achieve this in the future [117] [78]. Seed train intensification through high density cell banks is another promising solution [118] to gain economies of scale by scaling up.

Others: Several other possible optimisations have been mentioned elsewhere that would increase the efficiency of this section. These include the use of co-culturing or adapted cell lines to improve the medium recycle system or the cost reduction of the proliferation medium (Sections 13.5 and 4.3).

7.7 Summary

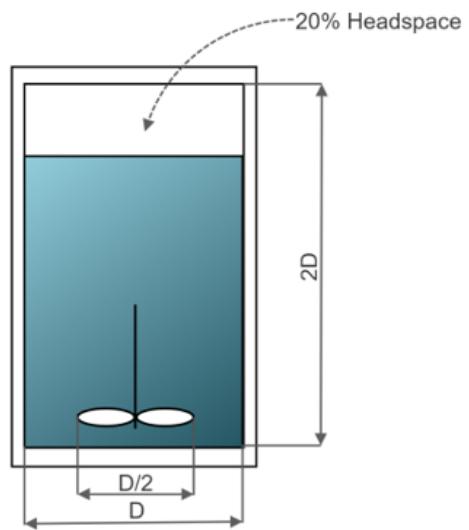
A complete process for the high-density expansion of suspension-adapted avian fibroblasts has been developed to ensure sufficient biomass supply for the downstream differentiation stage. The design of a three-stage bioreactor system was made possible by developing cell growth and metabolite concentration models, constructing a bioreactor timeline, and integrating a medium recycle system to support the intensive perfusion design. The energy usage, media requirements, and total costs were calculated and finally, a series of further optimisations were suggested.

8 Differentiation Bioreactor Design - Zaheer

As determined in Table 2, a CSTR will be used for the differentiation processes, to effectively design the bioreactor it is vital to consider key factors such as cell density, viscosity and oxygen requirement. This section explores the interaction between these factors and attempts to optimise the bioreactor parameters for the myogenesis and adipogenesis processes.



(a) Typical Industrial CSTR [taken from [119]]



(b) Simplified Depiction of Differentiation Bioreactor

In Figure 7a a few typical CSTRs can be seen, the adipogenesis bioreactor is to be modelled similar to the smaller glass bioreactor whilst the myogenesis bioreactor will resemble the larger steel bioreactors. A schematic depicting the salient features of the bioreactors can also be seen in Figure 7b.

8.1 Cell Density Limitations

As stated in [58] the maximum allowable cell density occurs at a volume fraction of approximately 0.25. For 3000 pg cells with a $17.7 \mu m$ diameter this results in a maximum attainable cell density of 86×10^6 cells/ml. As stated in [58] the maximum mass density is fixed for cells of different sizes and masses. Applying this to our cells with a mass given from 1 tonne being approximately 3.0×10^{14} mammalian cells averaged across several studies [67] [85] [93] [94]. The mass of a single cell is approximately 3.3 ng. Using the equation:

$$\rho_{process,max} = \rho_{study} \times \frac{m_{study}}{m_{process}} \quad (11)$$

where:

- ρ refers to the cell densities for the proposed process and the study respectively (cells/ml)
- m refers to the mass of a single cell (grams)

We find that due to viscosity considerations the maximum possible cell density is 7.47×10^7 cells/ml.

8.2 Impeller

To ensure that there is no formation of nutrient gradients and that the cells are not subject to levels of shear stress that result in cell death, the design parameters that must be optimised for the impeller are: blade geometry, diameter and rotational speed. To ensure that significant cell death does not occur, the Kolmogorov length scale should be equal to or larger than the cell diameter [58], this is to ensure the size of the smallest turbulent eddies that form are no smaller than the cell diameter. For cell culture applications a marine blade impeller (which can be seen in Figure 8) is typically used as it provides low levels of shear stress whilst allowing for sufficient mixing [101], the Impeller Power Number (N_p) is taken as being 0.6, an average value from [120], [121]. Furthermore, the impeller diameter is taken as being half the tank diameter as this is typical of application in cell culturing [122]. Having fixed these parameters, Equations 12,13,14,15,16 can then be combined to determine the impeller speed and power required.



$$L = \left(\frac{\nu^3}{\epsilon} \right)^{\frac{1}{4}} \quad (12)$$

$$P = \bar{\epsilon} \times \rho \times V \quad (13)$$

$$n = \left(\frac{P}{N_p \cdot \rho \cdot D^5} \right)^{\frac{1}{3}} \quad (14)$$

$$v = n \times \pi \times D \quad (15)$$

$$v_f = k_v \times v \quad (16)$$

Figure 8: Marine Blade Impeller

Table 12: Impeller Equation Constants and Units

Symbol	Unit	Explanation
L	m	Cell diameter, set as $7 \mu\text{m}$.
ν	$\frac{\text{m}^2}{\text{s}}$	Kinematic viscosity, set as 1.07×10^{-6} from [123].
ϵ	$\frac{\text{m}^2}{\text{s}^3}$	Maximum Turbulent Energy Dissipation Rate.
$\bar{\epsilon}$	$\frac{\text{m}^2}{\text{s}^3}$	Mean Turbulent Energy Dissipation Rate, taken as 10 times less than ϵ according to [58].
ρ	$\frac{\text{kg}}{\text{m}^3}$	Density of the medium, taken as 1015 from [123].
V	m^3	Working volume of the bioreactor.
n	s^{-1}	Rotational speed of the impeller.
v	m/s	Velocity of the impeller.
P	W	Power required by the impeller.
D	m	Diameter of the impeller, taken as half the tank diameter.
N_p	-	Impeller Power Number (dimensionless), taken as 0.6.
v_f	m/s	Fluid velocity.
k_v	-	Proportionality constant, taken as 0.6 from [124].

8.3 Heating

Assuming that the bioreactor has walls of thickness 3mm and an insulating jacket of thickness 9mm, the heat loss through the walls of the bioreactor can be considered using the Sieder-Tate equation for laminar flow in stirred tanks [125]:

$$Nu = 1.86 \left(\frac{Re \cdot Pr \cdot D}{L} \right)^{1/3} \left(\frac{\mu}{\mu_0} \right)^{0.14}, \quad (17)$$

$$Re = \frac{\rho U D}{\mu}, \quad Pr = \frac{\nu}{\alpha}, \quad \alpha = \frac{k}{\rho C_p}, \quad Nu = \frac{hD}{k}. \quad (18)$$

Table 13: Heat Transfer Equation Constants and Units

Symbol	Unit	Explanation
Nu	-	Nusselt Number (dimensionless).
Re	-	Reynolds Number (dimensionless).
Pr	-	Prandtl Number (dimensionless).
D	m	Diameter of the reactor.
L	m	Length of the reactor, assuming an aspect ratio (Length/Diameter) of 2 [126].
μ	Pa·s	Dynamic viscosity.
μ_0	Pa·s	Reference viscosity, assumed to be the same as that of water, 8.9×10^{-4} [127].
ρ	$\frac{\text{kg}}{\text{m}^3}$	Density of the medium, taken to be 1015 [123].
U	m/s	Fluid velocity, determined using the impeller correlations.
α	$\frac{\text{m}^2}{\text{s}}$	Thermal diffusivity.
C_p	$\frac{\text{J}}{\text{kg}\cdot\text{K}}$	Specific heat capacity of the medium, taken to be the same as water, 4182 [127].
k	$\frac{\text{W}}{\text{m}\cdot\text{K}}$	Thermal conductivity of the medium, taken to be the same as water, 0.628 [127].
h	$\frac{\text{W}}{\text{m}^2\cdot\text{K}}$	Convective heat transfer coefficient.
h_r	$\frac{\text{W}}{\text{m}^2\cdot\text{K}}$	Heat transfer coefficient of the reactor interior, derived from 17.
A_i	m^2	Internal curved area of the bioreactor.
k_w	$\frac{\text{W}}{\text{m}\cdot\text{K}}$	Thermal conductivity of the wall
L	m	Height of the bioreactor, given from the bioreactor aspect ratio.
r_o	m	External radius of the bioreactor, as shown in 10
r_i	m	Internal radius of the bioreactor, as shown in 10
h_{H2O}	$\frac{\text{W}}{\text{m}^2\cdot\text{K}}$	Heat transfer coefficient of water inside the insulated jacket, taken as 0.628
A_j	m^2	Surface area of the jacket.
k_j	$\frac{\text{W}}{\text{m}\cdot\text{K}}$	Thermal conductivity of the jacket
h_o	$\frac{\text{W}}{\text{m}^2\cdot\text{K}}$	External heat transfer coefficient, taken as 55 [128]
A_o	m^2	External surface area.
q	W	Heat transfer.
U	$\frac{\text{W}}{\text{m}^2\cdot\text{K}}$	Heat transfer coefficient, taken as $(1/H)$, H is the thermal resistance from 19, 20.
ΔT	K	Difference in temperatures between the reactor interior and exterior.

Applying these correlations in conjunction with those for the impeller (see Section 8.2), yields a value for the heat transfer coefficient of the reactor interior. To account for the heat transfer through the base, top and sides of the reactor both the conductive and convective heat transfer must be considered, as shown in Equations 19, 20, 21. The temperature profile of the bioreactor wall can be seen in Figure 9.

$$H_{curved} = \left(\frac{1}{h_r A_i} \right) + \left(\frac{1}{2\pi k_w L} \right) \ln \left(\frac{r_o}{r_i} \right) + \left(\frac{1}{h_{H2O} A_j} \right) + \left(\frac{1}{2\pi k_j L} \right) \ln \left(\frac{r_o}{r_i} \right) + \left(\frac{1}{h_o A_o} \right) \quad (19)$$

$$H_{\text{top}} = \frac{1}{h_r \pi r_i^2} + \left(\frac{1}{\pi k_w r_i^2} \right) \ln \left(\frac{L+t}{L} \right) + \frac{1}{h_o \pi r_o^2} \quad (20)$$

$$q = U \cdot \Delta T \quad (21)$$

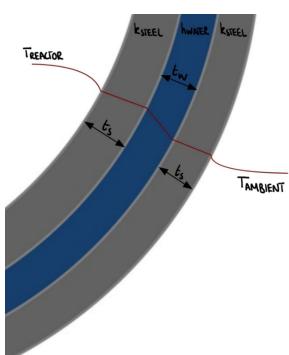


Figure 9: Reactor Temperature Profile

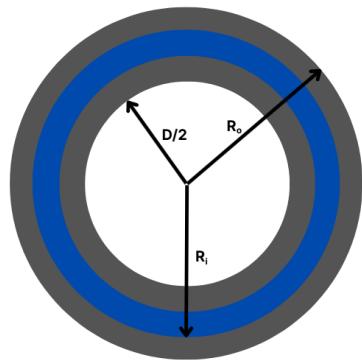
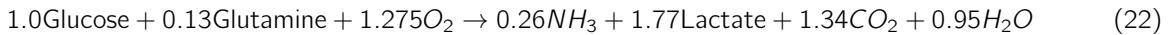


Figure 10: Reactor Cross-Section

8.4 ASPEN Plus Simulation

To model the heat generated within the bioreactors the simplified reaction, Equation 22, taken from [58] was considered to take place within an RSTOIC reactor as seen in Figure 12. Equation 22 represents a simplified model of the metabolic activities taking place within the cells.



Simulation Parameters	Values
Temperature (°C)	39
Pressure (bar)	1

Figure 11: Simulation Parameters

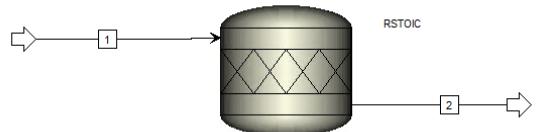


Figure 12: Aspen PLUS RSTOIC Reactor

9 Myogenesis - Zaheer

9.1 Overview

Muscle cells are responsible for the texture and structure of the product [130]. To achieve a final product that closely resembles traditional meat, a target of 97% muscle cells and 3% fat cells has been chosen; as reported in [131].

To achieve this product composition, performing the myogenesis and adipogenesis in series was considered as demonstrated in [11]. This involves putting all the cells through the myogenesis process initially. Then all the cells would be transferred to an adipogenesis bioreactor, in which only a fraction of the cells would undergo adipogenesis.

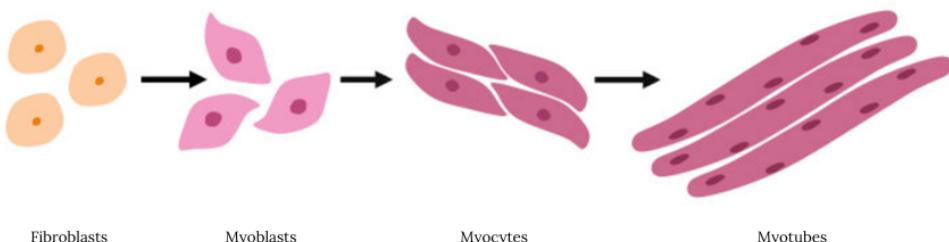


Figure 13: Myogenesis Process (adapted from [129])

Though this yields a final product with a level of triglycerides comparable to traditional chicken breast/thigh; it is inefficient. When compared to a parallel process in which cells undergo only a single differentiation process, the series process (in which all cells go through both differentiation reactors) results in:

- An increased time period
- Increased energy requirements
- Increased media quantity and growth factors requirements
- Larger bioreactor for the adipogenesis process

This led us to the conclusion that the parallel process is more cost efficient whilst only being slightly more complex.

The proposed myogenesis process requires:

- A time period of 4 days [11] to allow for full maturation.
- Addition of DOX (Sigma, #D3000000) at a concentration of 50 ng / 10^6 cells [11].
- A temperature of 39 degrees celsius [11].
- A 5% CO₂ atmosphere to maintain the optimal pH level [11].
- A pH of approximately 7.4 [132].

9.2 Process Requirements

As stated in Section 1, a total mass of 1 tonne of chicken meat must be produced, which is equivalent to 3.0×10^{14} mammalian muscle cells averaged across several studies [67] [85] [93] [94]. Given that the product will be 97 % muscle cells this amounts to approximately 2.91×10^{14} cells per year. As each myogenesis cycle takes 4 days and there is a half a day cleaning period, using Equation 23 with:

- T, time period (days) taken to be 4.5 days (4 days process + 0.5 days cleaning)
- α , proportion of cells, taken to be 97%

$$N_{cycle} = N_{yearly} \times \alpha \times \frac{365 \text{ days/year}}{T_{myogenesis}} \quad (23)$$

Approximately 3.6×10^{12} cells or 12kg of muscle cells must be produced per cycle.

9.3 Bioreactor Requirements and Comparison

9.3.1 Bioreactor Sizing

As stated in Section 9.2, the process will be required to produce approximately 12kg every cycle, assuming the maximum possible cell density of 7.47×10^7 cells/ml, a working volume of 48.3 litres is required. The headspace is taken to be 20% as mentioned in [133], therefore for a single bioreactor system the total bioreactor volume would be 60 litres. The total working volume of 48.3 litres can either be distributed across multiple smaller bioreactors or consolidated in a single larger bioreactor, the options being considered are shown in Table 14.

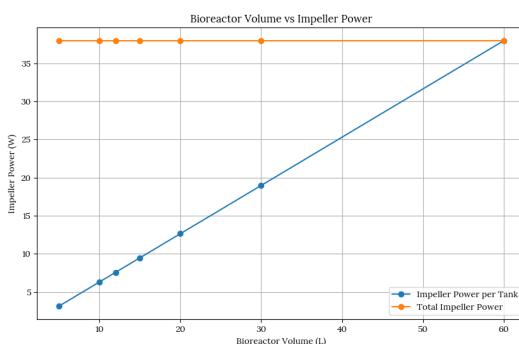
Table 14: Different Bioreactor Configurations

Number of Bioreactors	Bioreactor Size
1	60
2	30
3	20
4	15
5	12
6	10
12	5

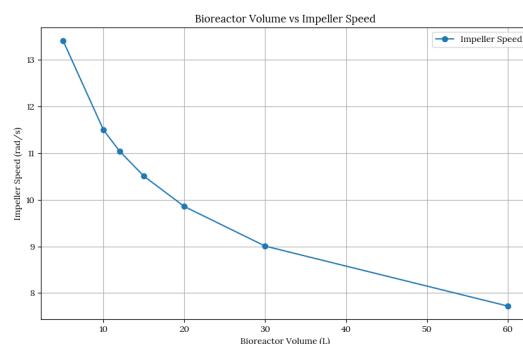
To perform the analysis the length:diameter ratio of the bioreactors is assumed to be 2:1 [126] and the ambient temperature is assumed to be 25°C, that of a typical laboratory.

9.3.2 Impeller

Using the Equations 13, 14, 15 and the constants stated in Table 12, the impeller power required for the different configurations stated in Table 14 can be compared. The different impeller powers and the speeds can be seen in Figure 14.



(a) Plot of Impeller Power against Bioreactor Size



(b) Plot of Impeller Speeds against Bioreactor Size

Figure 14: Effect of changing tank sizes on the impeller power and speed

9.3.3 Heating

Using Equations 19, 21, 20, and the constants in Table 13 the heat loss for each of the configurations can be calculated, these are shown in Figure 15. It is assumed that the bioreactors are made of steel and hence k_w is

taken as $13.1 \frac{W}{m \cdot K}$ [134] Accounting for the heat generated in the reaction, (Equation 22) and a recycle flowrate of 57.42 L/h derived in Section 13, an Aspen PLUS RSTOIC simulation using the method outlined in Section 8.4 with the parameters in Table 15 yielded a value of the heat generated by the reaction which can be seen in Table 15.

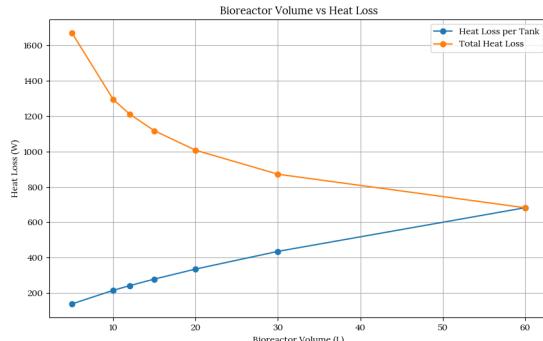


Table 15: Aspen PLUS Myogenesis Input Stream

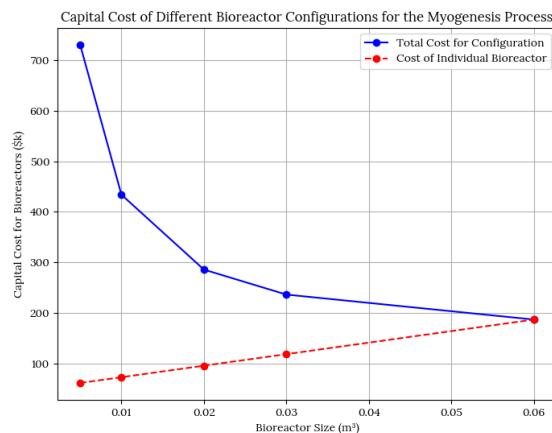
Component	Myogenesis Flowrate (kg/hr)
Glucose	0.302
Glutamine	0.0302
Oxygen	26.522
Water	57.456
Heat Generated (W)	306.519

Figure 15: Bioreactor Heat Loss against Size

9.3.4 Capital Cost

When comparing the different bioreactor configurations the cost of both the bioreactor and installation must be considered. Based on the correlation given in [135], which is shown in Figure 16a, the cost of the bioreactor and installation can be determined.

$$\text{Cost}(\$/k) = \begin{cases} 30.7 \times V + 800 & V \geq 0.33\text{m}^3 \\ 2285 \times V + 49.5 & V < 0.33\text{m}^3 \end{cases}$$



(a) Bioreactor costing correlation from [135]

(b) Costing for different configurations based on [135]

Figure 16: Bioreactor Capital Costing

9.3.5 Conclusion

Based on the heating, impeller energy requirements and the capital cost for the bioreactors shown in Figure 14, 15 and 16 respectively, the clear choice is **a single 60L bioreactor for the myogenesis process** as it has both the lowest energy requirements and the lowest capital cost.

9.4 Culturing Environment Requirements

9.4.1 Process Flow Diagram

A process flow diagram of the myogenesis process can be seen in Figure 17, due to the high cell density culture, a recycle loop has been incorporated to prevent an accumulation of waste products which will ensure that the cells are in an optimal culturing environment.

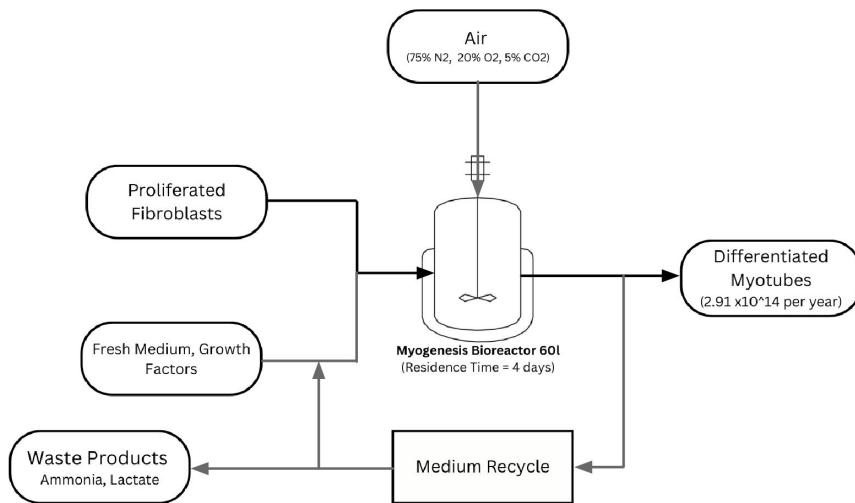


Figure 17: Myogenesis Process Flow Diagram (created in Canva)

For fibroblasts to differentiate successfully into myotubes a base medium is required with the supplementation of growth factors, soybean hydrolysate has been chosen instead of using FBS to ensure that our product is independent of animal sources [59] as explored in Section 4.2.

9.4.2 Gas Requirements

The air mixture being sparged into the bioreactor will be 5% CO₂, 75% N₂ and 20% O₂ to ensure that the pH environment within the bioreactor is optimal for cells to differentiate [11]. The amount of oxygen required per cell is 6.05×10^{-9} mmol/day/cell [109], the flowrate of oxygen is derived in Section 11.5.5. The total gas flowrate requirements can be seen in Table 16.

Quantity	Flowrate (kg/hr)
Oxygen	0.032
Nitrogen	0.120
Carbon Dioxide	0.008

Table 16: Myogenesis Bioreactor Gas Requirements

9.4.3 Media Requirements

The myogenesis bioreactor will utilise a recycle loop to ensure the concentrations of lactate and ammonia don't exceed 2.5 mM and 25 mM respectively, with the limiting concentration being taken as 3 mM and 30 mM [136]. The flowrate of the medium, is derived in Section 13 based on the production of lactate and ammonia by the cells and consumption of glucose and glutamine which can be seen in Table 7. The flowrate has been determined to be 57.42 L/hr. Supplementation is required to ensure the cells are able to differentiate successfully this is shown in Table 17. The base medium composition has been outlined in Section 4.2.

As mentioned in [54], 20% of the medium should be replaced after three days and then the entirety of the medium should be replaced after another 3 days. This has been adapted for this process to consider the flow of volume out of the reactor, hence instead of 3 days, medium will be replaced after 3 reactor volumes have flown out of the bioreactor. This is reflected in the values in Table 18. The pH of the medium will be maintained at approximately 7.4 [132].

Component	Supplementation (g/hr)
Glutamine	230
Glucose	16.4

Table 17: Myogenesis Bioreactor Supplementation Requirements

9.4.4 Differentiator Requirements

To ensure differentiation into myotubes the medium will require the addition of DOX at a concentration of 50 ng / 10^6 cells [11]. This equates to a requirement of 0.18g for the entire bioreactor working volume.

9.5 Costing

9.5.1 Operating Cost

The flowrate of 57.42 L/h derived in Section 13, the replacement of reactor volumes mentioned in Section 9.4.3 and the cell requirements [11] have all been considered to produce the values shown in Table 18. The total glucose supplementation consists of both the initial supplementation, which is due to the medium produced in Section 4.2 having a glucose concentration of 3.15 g/L, and the optimal value for cells being 4.5 g/L [137] and the intermediary supplementation due to the consumption of glucose by the cells.

Myogenesis	Amount Required per Cycle	Cost per Unit (\$/g or L)	Total Cost per Cycle (\$)
Glucose (g)	22,810.80	0.02[?]	456.22
Glutamine (g)	1,518.54	0.46(4.2.2)	702.86
DOX (g)	4.27	3,044.41[138]	12,989.49
Medium (L)	1,150.76	4.23 (4)	4,863.13
Total Cost per Cycle	-	-	19,011.70

Table 18: Annual Material Myogenesis Requirements

The energy requirements can be seen in Table 19, this includes the energy required for both the impeller and heating.

Parameter	Value
Bioreactor Volume (L)	60
Heat Generated (W)	307.5
Heat Loss (W)	682.22
Heat Required (W)	374.72
Impeller Power Required (W)	37.96
Impeller Speed (rad/s)	7.72
Total Energy Required (W)	412.68

Table 19: Myogenesis Bioreactor Energy Balance

9.5.2 Capital Cost

As discussed in Section 9.3 the most economically attractive bioreactor configuration is a **single 60L bioreactor which costs \$186,600** which is based on Figure 16a and can be found in [58].

10 Adipogenesis - Zaheer Sidik

10.1 Overview

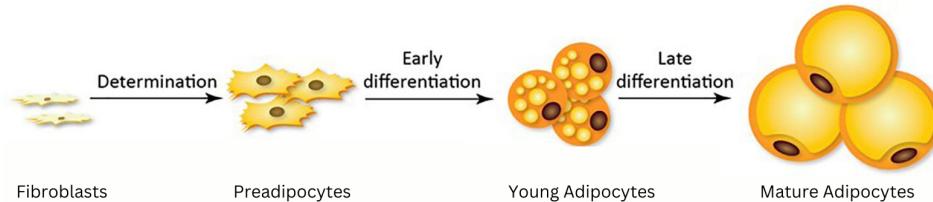


Figure 18: Adipogenesis Process (adapted from [139])

Fat cells are vital to the product, even though they only make up a small portion (3%) of the final product, they are responsible for the flavour and smell of the chicken, this is observed when cooking chicken and lipid-oxidation occurs [140]. The adipogenesis process is modelled as requiring:

- A time period of 7 days [81].
- Operating conditions of 39°C and 5% CO₂ [81].
- Oleic acid at a concentration of 200 μM for a cell density of 2×10^5 cells per mL [81].
- The presence of a differentiator - Rosiglitazone, Pristanic Acid or Phosphatidylcholine [81].
- A pH of approximately 7.4 [132].

10.2 Choosing a Differentiator

As stated in [81] there are 3 possible differentiators that can be used in the presence of oleic acid to cause adipogenic differentiation. Their efficiencies, costs and required amounts are stated in Table 20. The adjusted cost stated in the table accounts for the amount of the component required per ml (the different differentiators require different amounts per ml of medium) and the efficiency of the differentiator. The table does not account for the extra medium costs that arise due to the differentiation process not being 100% effective, as the medium costs are trivial when compared with the cost of the differentiator components.

Component	Efficiency	Required (g/ml)	Cost (\$/g)	Adjusted Cost (\$/ml of medium)
Phosphatidylcholine	0.84	0.00464	\$1,985.70	\$10.97
Rosiglitazone	0.84	0.00138	\$8,215.20	\$17.71
Pristanic Acid	0.93	0.00636	\$69,804.00	\$477.37
Oleic Acid	–	0.022	\$7.19	–

Table 20: Cost Analysis of Adipogenesis Differentiators

The proposed adipogenesis differentiator is Phosphatidylcholine.

10.3 Bioreactor Requirements

10.3.1 Bioreactor Sizing

As derived in Section 8.1 the chosen cell density is 7.47×10^7 cells/ml, given that the process requirements dictate a yearly output of 9×10^{12} fat cells per year, this equates to a total yearly working volume of 120.5 litres. Accounting for the efficiency of phosphatidylcholine being 84% [81], a yearly working volume of 143.45 litres will be required. Taking into consideration a cycle time of 7 days and then 2 days offline (so that there are 2 myogenesis cycle for each adipogenesis cycle) as shown in Figure 4. There will be 40 cycles per year, hence the working volume per cycle is 3.64 litres. Accounting for the constraint of a minimum of 20% headspace [133] a total bioreactor volume of 5L will be required.

10.3.2 Impeller

From Equations 13, 14, 15 the impeller power and speed can be derived based on a bioreactor length:diameter ratio of 2:1 and the constants stated in Table 12, the results of this can be seen in Table 22.

10.3.3 Heating

From Equations 19, 21, 20 the heat loss for the bioreactor can be found, which is displayed in Table 22. The heat generated was found using the Aspen PLUS method mentioned in Section 8.4 and the parameters shown in Table 21. The results can be seen in Table 22.

Component	Flowrate (kg/hr)
Glucose	0.02277
Glutamine	0.002277
Oxygen	1.99874
Water	4.33
Total	6.353787

Table 21: Adipogenesis Aspen PLUS Parameters

Parameter	Value
Bioreactor Volume (L)	5
Heat Generated (W)	23.1
Heat Loss (W)	109.4
Heat Required (W)	86.2645
Impeller Power Required (W)	3.16
Impeller Speed (rad/s)	13.4
Total Energy Required (W)	89.4245

Table 22: Adipogenesis Bioreactor Energy Balance

10.4 Culturing Environment

10.4.1 Process Flow Diagram

A process flow diagram for the adipogenesis process can be seen in Figure 19.

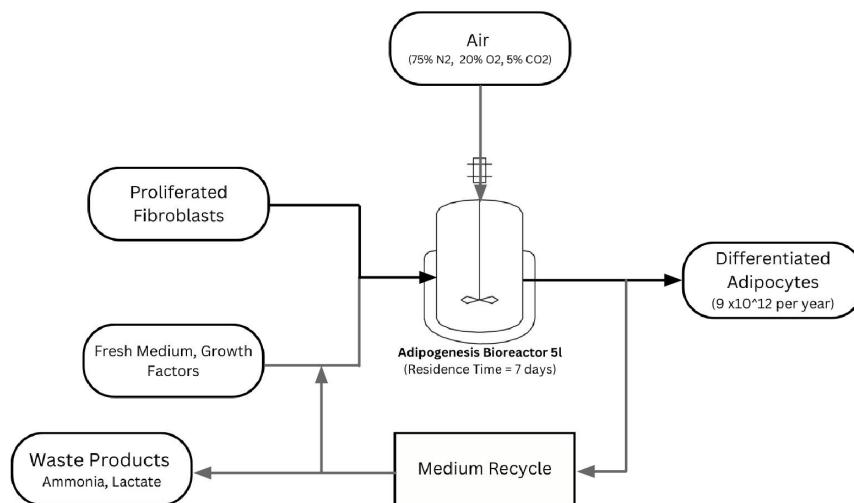


Figure 19: Adipogenesis Process Flow Diagram

10.4.2 Gas Requirements

The air mixture being sparged into the bioreactor will be 5% CO₂, 75% N₂ and 20% O₂ to ensure that the pH environment within the bioreactor is optimal for cells to differentiate [11]. The amount of oxygen required per cell is 6.05×10^{-9} mmol/day/cell [109] and the flowrate of oxygen is derived in Section 11.5.4. The total gas flowrate requirements can be seen in Table 23.

10.4.3 Media Requirements

The adipogenesis bioreactor will utilise a recycle loop to ensure the concentrations of lactate and ammonia don't exceed 2.5 mM and 25 mM respectively, with the limiting concentration being 3 mM and 30 mM [136]. The

Quantity	Flowrate (kg/hr)
Oxygen	0.0024
Nitrogen	0.009
Carbon Dioxide	0.0006

Table 23: Adipogenesis Bioreactor Gas Requirements

flowrate of the recycle loop is 4.33 L/h as derived in Section 13. The glucose and glutamine consumption rates are taken as the same as the myogenesis process, hence supplementation will be required; which can be seen in Table 24. The base medium composition will be that derived in Section 4.2, this will be supplemented with Oleic Acid and Phosphatidylcholine as shown in Table 24

As mentioned in the myogenesis media requirements Section 9.4.3, 20% of the medium will be replaced after the flow of 3 RVs and then after a subsequent 3 RVs it will be replaced entirely. The pH of the medium will be maintained at approximately 7.4 [132].

10.5 Costing

10.5.1 Operating Cost

The flowrate of 4.33 L/h derived in Section 13, the replacement of reactor volumes mentioned in Section 9.4.3 and the cell requirements [11] have all been considered to derive the values in Table 24. The total glucose consumption supplementation consists of both the initial supplementation which is due to the medium produced in Section 4.2 having a glucose concentration of 3.15 g/L and the optimal value for cells being 4.5 g/L [137] and the intermediary supplementation due to the consumption of glucose by the cells.

Table 24: Annual Adipogenesis Material Requirements

Adipogenesis	Amount Required per Cycle	Cost per Unit (\$/g or L)	Total Cost per Cycle (\$)
Phosphatidylcholine (g)	674.644 [141]	442.26	298,368.06
Glucose (g)	3,008.375	0.02[?]	60.17
Glutamine (g)	200.275	0.46(4.2.2)	92.70
Medium (L)	149.0375	4.23 (4)	629.83
Oleic Acid (g)	3,184.2	6.12 [142]	19,472.69
Total Cost per Cycle	-	-	318,623.45

As seen in Table 24, the majority of the cost associated with the adipogenesis process is the cost of Phosphatidylcholine (Differentiator), hence the next step in development would be to explore manufacturing this component in house to reduce costs.

10.5.2 Capital Cost

The cost for the **5 litre bioreactor is \$60,925**. This is based on the correlation found in [58] which can be seen in Figure 16a.

11 Oxygenation - Viraj Nerkar

11.1 Introduction

For the continued and optimal growth and multiplication of cells, a system capable of reliable supply of oxygen is crucial. Lack of oxygen can inhibit cell growth while, conversely, excess oxygen can saturate the cell medium. Therefore, it is critical to maintain DO (dissolved oxygen) levels within an acceptable range [143].

11.2 Methods of Oxygen Supply

There are multiple methods to deliver oxygen to cells in the CSTR bioreactor with each method having its own unique advantages and disadvantages. Three methods were investigated in further detail to assess which method is optimal for usage in this application: Direct Sparging, Membrane Diffusion, and Surface Aeration.

11.2.1 Direct Sparging

A commonly used method of delivering oxygen is to feed it directly into the culture medium [58].

- Oxygen is introduced into the bioreactor through a sparger located at the bottom of the reactor.
- The gas forms bubbles, which rise through the liquid medium.
- As the bubbles rise, oxygen dissolves into the liquid phase, supplying cells.
- The impeller is used to break up bubbles and distribute them uniformly to improve mixing and gas-liquid mass transfer.

Table 25: Analysis of Direct Sparging

Advantages	Disadvantages
<ul style="list-style-type: none"> • High oxygen transfer efficiency • Can dynamically adjust oxygen levels • Optimal for high-cell-density cultures 	<ul style="list-style-type: none"> • Bubble-induced shear stress can cause cell damage • Risk of foaming and contamination

11.2.2 Membrane Diffusion

An alternative method of delivering oxygen to the bioreactor is without direct gas-liquid contact. This is particularly useful in applications where shear stress and bubble related damage need to be minimized [144].

- Oxygen is supplied on one side of a gas-permeable membrane, which acts as a barrier between the gas phase and the liquid culture.
- Due to the partial pressure gradient, oxygen molecules diffuse across the membrane into the liquid medium without forming bubbles.

- The dissolved oxygen spreads through the liquid phase, supplying cells with the oxygen needed for metabolism.

Table 26: Analysis of Membrane Diffusion

Advantages	Disadvantages
<ul style="list-style-type: none"> Low shear stress (lack of bubble formation) Low contamination risk 	<ul style="list-style-type: none"> Low oxygen transfer efficiency Membrane fouling over time

11.2.3 Surface Aeration

Surface Aeration promotes gas exchange at the gas-liquid interface. This technique is also advantageous for low-shear applications[145].

- The oxygen in the air diffuses into the liquid at the surface of the bioreactor.
- The impeller increases turbulence at the surface, enhancing oxygen transfer.
- The dissolved oxygen disperses into the bulk liquid phase, supplying cells with oxygen for metabolism.
- As cells consume oxygen, the concentration gradient drives continuous oxygen absorption from the air.

Table 27: Analysis of Surface Aeration

Advantages	Disadvantages
<ul style="list-style-type: none"> Simple design and low contamination/fouling risk Minimal shear stress 	<ul style="list-style-type: none"> Very low oxygen transfer rate Limited scalability for high-density cultures

11.2.4 Final Choice

To decide on a final oxygenation system, a multi-criteria analysis with the following metrics was used:

- Transfer efficiency - effectiveness of oxygen transfer from gas to liquid phase
- Ease of control - how easy is it to adjust the rate and duration of oxygen transfer
- Risk of cell damage - likelihood of damaging cells and reducing yield
- Design complexity - difficulty of designing the system

All metrics were given equal weight. The results of the analysis can be seen in Table 28. Based on this, the system of choice is direct sparging.

Table 28: Oxygenation System Multi-Criteria Analysis

System	Transfer Efficiency	Ease of control	Risk of cell damage	Design Complexity	Total
Direct Sparging	5	5	1	4	15
Membrane Diffusion	3	2	4	2	11
Surface Aeration	2	1	5	4	12

11.3 Continous Flow

A continuos flow method works by ensuring that the oxygen transfer rate - OTR (rate at which oxygen is transferred by the oxygenator to the culture) is set equal to the oxygen uptake rate - OUR (rate at which oxygen is consumed by the cells)[146]. This ensures that the system continually meets the oxygen demand. The following equations are referenced from this study [147].

$$OUR = Q_{O_2} \times V \quad (24)$$

Eq.24 shows that the OUR is the product of the molar oxygen uptake rate Q_{O_2} ($mmol/hr^{-1}L^{-1}$) and the working volume of the bioreactor V (L).

$$OUR = kLa \times (C^* - C_L) \times V \quad (25)$$

where kLa (s^{-1}) is the oxygen transfer coefficient, C^* (mgL^{-1}) is the saturated dissolved oxygen concentration (a constant for a given medium density, temperature and pressure) and C_L (mgL^{-1}) is the current concentration of dissolved oxygen in the medium.

$$C_L = DO \times C^* \quad (26)$$

where DO (%) is the dissolved oxygen levels in the medium.

$$kLa = C \times \left(\frac{P}{V} \right)^\alpha \times v_g^\beta \quad (27)$$

where P (W) is the power generated by the CSTR impeller, v_g (ms^{-1}) is the superficial gas velocity and α, β and C are empirical constants.

$$v_g = \frac{Q}{S} \quad (28)$$

where Q (m^3s^{-1}) is the volume flow rate of oxygen and S (m^2) is the surface area.

Method :

- Calculate the OUR as a function of time
- Use equations 24, 25, 26 and 27 to arrive at v_g as a function of time

Assumptions and Modeling Approximations:

- The medium can be approximated as water at 39 °C and 1 atm
- The empirical constants can be assumed to be the same as in [147].
- On any given day, the OUR can be approximated as constant throughout the day.

The data in the following table refer to the Nth reactor in the proliferation stage.

Table 29: Nth Reactor Specification

Parameter	Value
V (L)	52.56
C^* (mgL^{-1})	6.7
DO (%)	40
α (const)	0.4
β (const)	0.5
C (const)	0.03
P (W)	26.6

Based on an initial OUR of $0.13 \text{ mmol/hr}^{-1}L^{-1}$ (218.6 mg hr^{-1}), the required v_g would be $1.85 \times 10^{-8} \text{ ms}^{-1}$, which is much too low to accurately sparge. A possible reason for this is that the paper from which the constants are referenced [147] and the paper from which they were experimentally derived [148], both used much larger bioreactors by volume (approximately 40x and, therefore it is unclear whether these values are appropriate at a much smaller scale). Nevertheless, because of the lack of available literature on this, these are the most appropriate values to be used.

11.4 Pulse Train

An alternative approach is to sparge oxygen at regular intervals to raise DO levels back to 40% once they have fallen to 30% [48]. If a graph of DO levels vs time was plotted based on this method, it would look like a periodic function.

$$\frac{dC}{dt} = kLa \times (C^* - C) \quad (29)$$

where $\frac{dC}{dt}$ is the rate of change of oxygen concentration. Solving the differential equation :

$$\ln\left(\frac{C^* - C}{C^* - C_o}\right) = -kLa \times t \quad (30)$$

where C_o is the initial DO concentration and C is the current DO concentration at time t.

$$t = \Delta C \times \frac{V}{OUR} \quad (31)$$

where ΔC is the change in oxygen concentration.

Method:

- Choose an appropriate value for v_g
- Using eq. 27 calculate a fixed value for kLa
- Using eq. 30 calculate the time taken to replenish the oxygen (how long to sparge oxygen)
- Using eq. 31 calculate the time for oxygen levels to fall below threshold (30%) from target level (40%)
- Repeat for each day (assumption: OUR is constant on any given day hence the above steps only need to be calculated once per day)

11.5 Calculations

There are 3 reactors in the proliferation stage ($N^{th}, N - 1, N - 2$) and 2 reactors in the differentiation stage (Adipogenesis and Myogenesis) for a total of 5 reactors.

11.5.1 N^{th} Reactor

Based on the method listed in Section 11.4 and Table 29, the sparge intervals for each day are calculated in Table 30. The value of v_g was chosen as a balance [149] between increasing oxygen transfer efficiency and ensuring that cell damage from the resulting shear stress is minimized.

Table 30: Nth Reactor Calculations

Day	OUR ($mmol/hr^{-1}L^{-1}$)	Time (s) for DO level to drop from 40% to 30%
1	0.13	579.8
2	0.13	579.8
3	0.2	376.9
4	0.32	235.5
5	0.5	150.8
6	0.79	95.4
7	1.29	58.4
8	1.97	38.3
9	3.12	24.2
10	4.97	15.2
11	7.8	9.7
12	12.33	6.1
13	19.5	3.9

Table 31: Nth Reactor Specification

Parameter	Value
V (L)	52.56
P (W)	26.6
v_g ($m s^{-1}$)	0.005
Q ($m^3 s^{-1}$)	0.0006
kLa (s^{-1})	0.022
Sparge time (s)	6.95

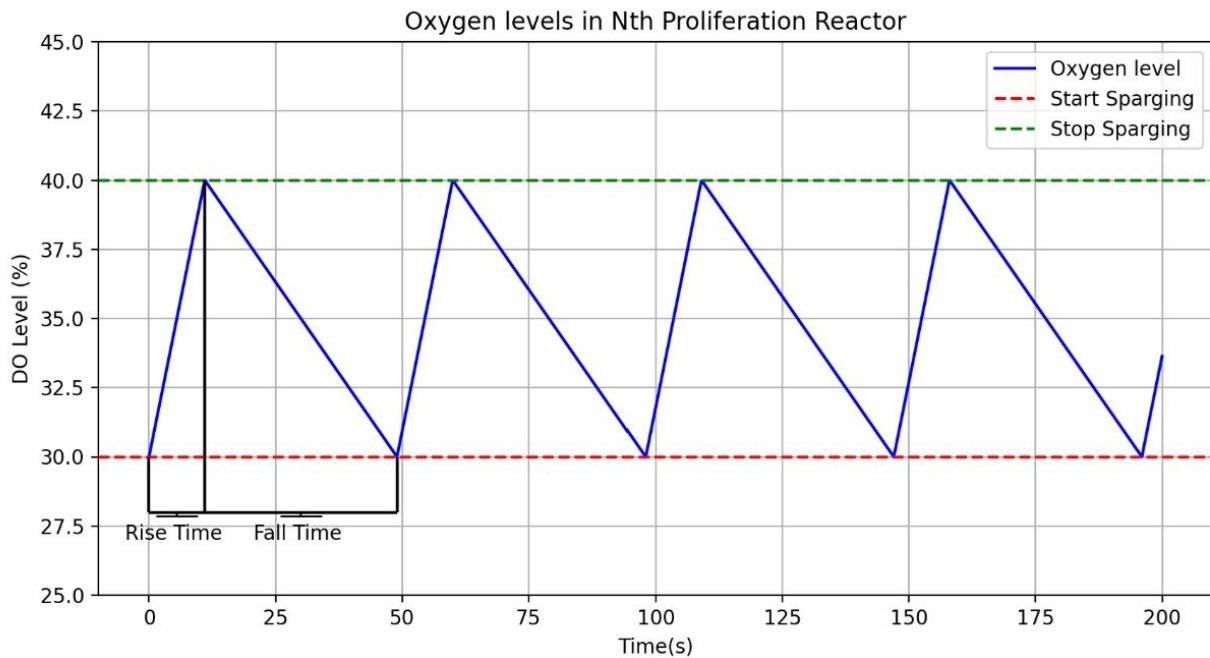


Figure 20: Oxygen levels on Day 8

11.5.2 $N - 1$ Reactor

The same method is repeated for the $N - 1$ reactor. The specification is in Table 33.

Table 32: N-1 and N-2 Reactor Calculations

Day	OUR ($\text{mmol hr}^{-1} L^{-1}$)	Time (s) for DO level to drop from 40% to 30%
1	0.13	579.8
2	0.13	579.8
3	0.25	301.5
4	0.5	150.8
5	1.01	74.6

Table 33: N-1 Reactor Specification

Parameter	Value
V (L)	6.51
P (W)	2.0×10^{-3}
v_g (ms^{-1})	0.005
Q ($\text{m}^3 \text{s}^{-1}$)	9.19×10^{-5}
k_{La} (s^{-1})	0.0011
Sparging time (s)	134.2

11.5.3 $N - 2$ Reactor

The same method is repeated for the $N - 2$ reactor. The calculations are the same for both reactors as OUR is unchanged. The specification is in Table 34.

Table 34: N-2 Reactor Specification

Parameter	Value
V (L)	0.82
P (W)	1.8×10^{-4}
v_g ($m s^{-1}$)	0.005
Q ($m^3 s^{-1}$)	3.47×10^{-5}
kLa (s^{-1})	9.97×10^{-4}
Sparging time (s)	154.6

11.5.4 Adipogenesis

The same method as above is used for the differentiation reactors, although made simpler by the fact that the OUR is constant throughout the cycle. The specification is in Table 35.

Table 35: Adipogenesis Reactor Specification

Parameter	Value
OUR ($m g h r^{-1}$)	984
V (L)	3.64
P (W)	1.9
v_g ($m s^{-1}$)	0.01
Q ($m^3 s^{-1}$)	1.26×10^{-3}
kLa (s^{-1})	0.032
Sparging time (s)	4.85
Interval time (s)	8.92

11.5.5 Myogenesis

Same method as the adipogenesis reactor. The specification is in Table 36.

Table 36: Myogenesis Reactor Specification

Parameter	Value
OUR ($m g h r^{-1}$)	31840
V (L)	48
P (W)	22.8
v_g ($m s^{-1}$)	0.01
Q ($m^3 s^{-1}$)	1.26×10^{-3}
kLa (s^{-1})	0.0306
Sparging time (s)	5.04
Interval time (s)	3.64

11.6 Costing

The CSTR bioreactors chosen for proliferation and differentiation processes have built-in oxygen-sparging systems. Therefore, no additional fixed capital is required for the oxygen system. The only variable cost is the oxygen supply itself. This cost is outlined in the material requirement section (15.1).

12 Control System - Viraj Nerkar

12.1 Controllers

Inside the continuous stirred tank bioreactors, it is essential to maintain and control conditions to achieve optimal growth. The CSTR displays complex non-linear dynamics due to the continuous inflow and outflow of media within the system. To combat this, a proportional-integral-derivative (PID) controller can be designed. It has many benefits, such as ease of tuning and operation. The proportional component applies a gain to the difference between the current and target values, known as the error. The integral component accumulates the error over time, increasing until the error reaches zero. The derivative component reduces the output if the process variable changes rapidly, providing damping to prevent excessive approach speed. These parameters are tuned to ensure the controller reaches the target efficiently while minimizing overshoot and transient behaviour.

12.2 Applications

Temperature

For chicken cells, the optimal temperature is 39 °C. A substantial difference between the CSTR temperature and the optimal temperature can affect cell viability, cause damage, and slow cell metabolism. To ensure that the CSTR temperature is maintained at the target value, a temperature sensor can measure the current temperature and act as input to a controller which can send a signal to the heating jacket [150]. This will ensure that the optimal temperature is reached.

pH

For chicken cells, the optimal pH value is 7.4. Cell consumption of O₂ and production of CO₂ caused by metabolic processes that convert glucose into lactate will make the medium more acidic. A PID controller can be designed that takes the current value of the pH probe and adds a basic solution such as NaOH to control the pH value and steer it back to the target of 7.4 [151].

Nutrient and Waste

A PID controller can dynamically control nutrient levels and remove metabolic byproducts by managing the media inflow and outflow rates. A similar approach was implemented in [152] with respect to the wastewater management system to improve stability and efficiency.

12.3 Design for Temperature

Heat inflow and outflow in the myogenesis bioreactor is caused by the flow of the medium, the cell metabolic reaction and heating from the heating jacket. An experiment carried out in literature [150] to model the temperature control of the bioreactor developed the following equation:

$$V\rho C_p \frac{dT}{dt} = F\rho C_p(T_f - T) - \Delta H V k_o e^{\frac{E}{RT}} C_A - UA(T - T_j) \quad (32)$$

where C_A is the nutrient concentration in the tank, ΔH is the enthalpy of reaction, R is the ideal gas constant, E is the activation energy, K_0 is the rate of reaction constant, F is the fluid flow rate, V is the CSTR volume, ρ is the medium density, C_p is the specific heat capacity, T , T_f and T_j are the CSTR, fluid inlet and heating jacket temperatures respectively, U is the heat transfer coefficient and A is the heating jacket area. This model can be further simplified by assuming that the heat input from the medium and heat uptake from the cell differentiation process is constant. Let P be the value for heat input from the fluid,

$$P = F\rho C_p(T_f - T) \quad (33)$$

and Q be the heat uptake rate of the cells,

$$Q = \Delta H V k_o e^{\frac{E}{RT}} C_A \quad (34)$$

Substituting this back into eq. 32

$$V\rho C_p \frac{dT}{dt} = P - Q - UA(T - T_j) \quad (35)$$

Let $a = V\rho C_p$, $b = P - Q$ and $c = UA$, and dividing through by a

$$\frac{dT}{dt} = \frac{b}{a} + \frac{c}{a}(T_j - T) \quad (36)$$

Taking the Laplace transform of both sides,

$$sT(s) - T_0 = \frac{b}{as} + \frac{c}{a}(T_j(s) - T(s)) \quad (37)$$

where T_0 is the initial temperature of the CSTR. Rearranging for $T(s)$,

$$T(s) = \frac{1}{s} \frac{aT_0 s + b}{as + c} + \frac{cT_j}{as + c} \quad (38)$$

Now that the system dynamics have been determined, a PID controller can be designed.

12.4 Model Parameters

From Table 36, V is $48L$ and from Table 19 P is $307.5W$ and Q is $682.22W$. ρ is 1015kgm^{-3} , C_p is $4180\text{Jkg}^{-1}\text{K}^{-1}$ and A is 0.725m^2 and U is $38.89\text{Wm}^{-2}\text{K}^{-1}$. These values come from the modelling done in Section 9.3.3. T_0 can be assumed to be at room temperature 25°C .

Substituting the values of a , b , and c in Eq 38,

$$T(s) = \frac{1}{s} \frac{5.15 \times 10^6 s - 374.47}{2.06 \times 10^5 s + 28.2} + \frac{28.2T_j}{2.06 \times 10^5 s + 28.2} \quad (39)$$

Table 37: Controller Model Parameters

Parameter	Value
T_0 ($^{\circ}\text{C}$)	25
ρ (kgm^{-3})	1015
C_p ($\text{Jkg}^{-1}\text{K}^{-1}$)	4180
V (m^3)	0.048
Q (W)	682.22
P (W)	307.75
a	2.06×10^5
b	-374.47
c	28.20

12.5 Simulink

Eq 39 can be implemented in Simulink to design a PID controller. Figure 21 shows the closed loop system.

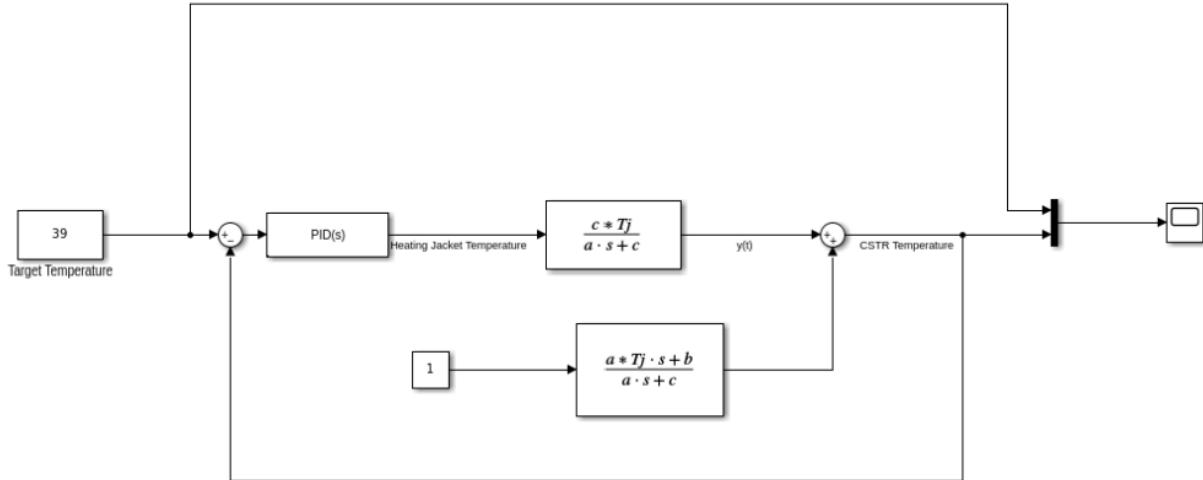


Figure 21: PID Controller Simulink Model

Using Simulink's auto-tuning feature, the PID controller's proportional, integral, and derivative gains can be calculated. The resulting temporal response graph is shown in Figure 22. This shows a rise time of 4.2 hours and an overshoot of 0.5 $^{\circ}\text{C}$ which is within the range for cell viability [153].

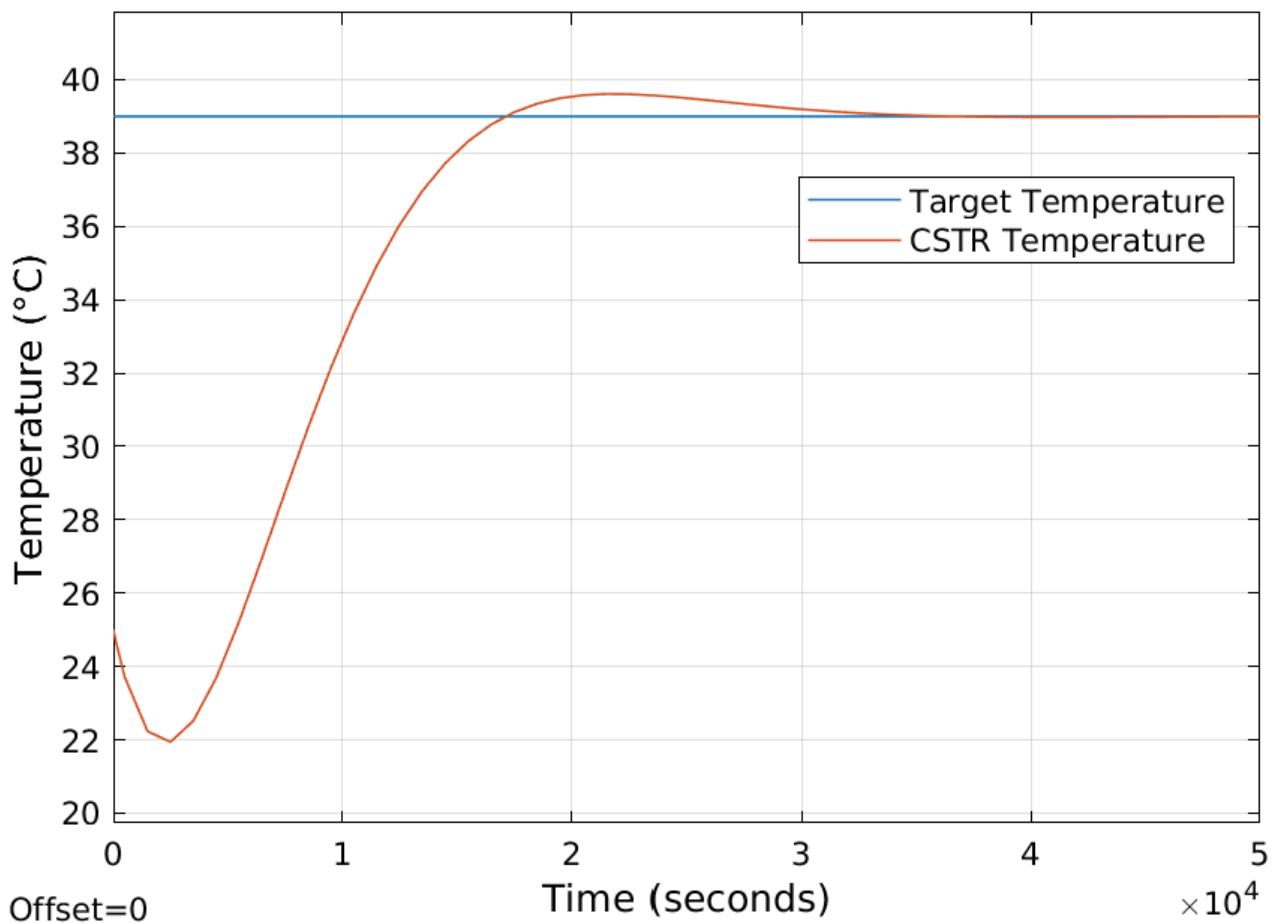
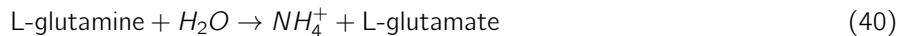


Figure 22: CSTR Temperature response

13 Medium Recycle - Luke Nijkamp

During cell proliferation and differentiation, medium components are consumed, while waste products (most notably ammonia and lactate) are generated, with inhibitory effects on cell growth. Only these two metabolites were considered in the scope of this report, however others such as aconitic acid, methylsuccinic acid, and trigonelline have similarly been reported to suppress cell growth [154].

Ammonia is produced through the spontaneous decomposition and degradation of L-glutamine, described by the following equation [37]:



Ammonia directly impacts cellular metabolism and energy transfer by inhibiting glutamate dehydrogenase, which in turn disrupts adenosine triphosphate (ATP) production of the cell in the tricarboxylic acid (TCA) cycle [37]. ATP is often termed the 'energy currency' of the cell, and reduced synthesis of this molecule therefore imposes a significant limitation on cellular proliferation [155].

Lactate in cell culture is mainly produced through glycolysis, where glucose is rapidly consumed and converted to lactate to regenerate NAD+, especially at high glucose concentrations. Additionally, amino acid catabolism,

particularly following glutamine metabolism, contributes to lactate production via the conversion of alanine by lactate dehydrogenase [37]. Lactate production, like ammonia, has been shown to limit cell growth and alter cell processes as well as reducing medium pH through the release of H⁺ ions [156].

13.1 Process Overview

For this plant, metabolite removal by adsorption was concluded to be most suitable, given current costs and technology readiness levels (TRLs).

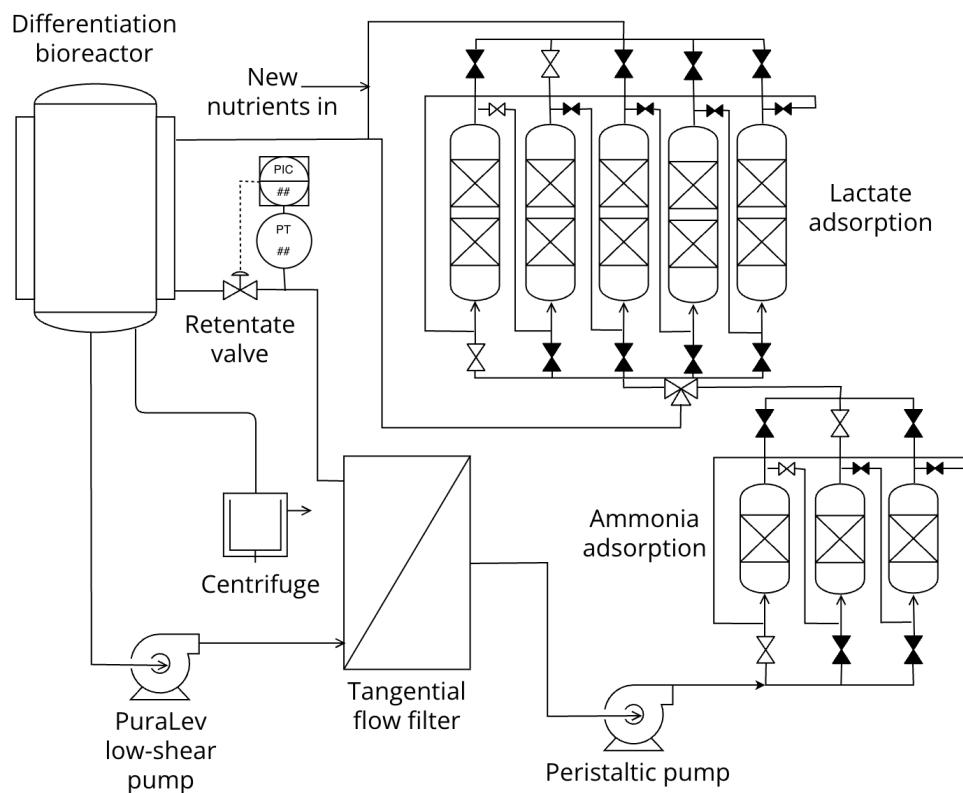


Figure 23: Medium Recycle System

Two, largely identical medium recycle systems based on adsorption were designed for lactate and ammonia removal (shown in figure 23); one for final three proliferation reactors, and one for the differentiation systems. The systems differ only in filter and column dimensions.

However, the cost of the proposed process remains too high to be viable and relies on the assumption that available resins and adsorbents will become cheaper, and more effective in future.

Owing to this, several other strategies with lower TRLs, such as co-culturing and cell line adaptation have additionally been considered for potential future adoption.

13.2 Adsorption and Design

13.2.1 Literature Review

Current metabolite removal strategies include those that limit production (e.g. gene engineering, medium optimisation) as well as methods that actively remove or break down metabolites such as biocatalysis, electrochemistry

and adsorption.

Initially it was hypothesised that inhibition by lactate could be offset via neutralisation of the media with a buffer or alkali. However, the resultant increase in solute concentration causes osmotic effects that could lead to cell damage [37].

Gene engineering can produce cells with lower waste metabolite production levels, but poor public perception, and risks around genetically modified food ruled this out as an option [37].

Electrochemical oxidation and electrodialysis are commonly used in fermentation and wastewater treatment for the removal of lactic acid and ammonia nitrogen respectively. However limited research exists applying these methods in live cell culture, and systems can be complex and hard to regulate. Hence electrochemical methods were ruled out for our application [37].

Adsorption has strong potential as a viable method of metabolite removal in cell culture. Adsorption is widely used in wastewater treatment to remove contaminants such as heavy metals, organic pollutants, and ammonia, and there continues to be significant research in this area [157]. Moreover, ion-exchange membranes, such as AmberLite™ resins, have proven to be effective methods for lactate removal [158].

Despite this, the application of these strategies in cell culture imposes new constraints such as cytotoxicity considerations and working pH and temperature ranges [37].

Finally, with developments in cell culturing, various novel methods have emerged for the removal of waste metabolites. These include using biocatalysts such as lactate dehydrogenase to convert lactate into new carbon sources for the cells [159], as well as co-culturing strategies with lactate-consuming bacteria or micro-algae for ammonia removal [160][161]. Recent research has also demonstrated the potential for the adaptation of cells to a lactate-supplemented medium to significantly reduce lactate production [110]. These have been considered in the context of future process developments, but were not implemented into our preliminary design.

13.2.2 Parameters

Cell-specific lactate and ammonia production rates were assumed to be 3.12E-13 and 3.60E-14 mol/h/cell respectively [109]. The study from which these figures were derived refers to baby hamster kidney fibroblasts, so values are likely to deviate from those of chicken fibroblasts. However, a comparison with the perfusion rates in [?] revealed that this is, if anything, a conservative estimate. Considering the maximum cell numbers in the proliferation and differentiation reactors provided a basis to determine the maximum lactate and ammonia production rates:

Table 38: Maximum ammonia and lactate production levels

Reactor	Max Cell Number	Ammonia (mol/h)	Lactate (mol/hr)
Differentiation	3.74×10^{12}	0.135	1.166
Proliferation	4.78×10^{12}	0.172	1.493

Furthermore, limiting lactate and ammonia concentrations of 30mM and 3mM, respectively, were obtained from [81], which also uses chicken fibroblasts. This project's waste removal system has been designed to keep concentrations below 25mM and 2.5mM respectively. Assuming 100 percent waste removal in the recycle stream,

the required flow rates were calculated as

$$\text{recycle flow rate} = \frac{\text{waste metabolite production rate}}{\text{equilibrium metabolite concentration}} \quad (41)$$

and are given in the table below:

Table 39: Required perfusion rates for lactate and ammonia removal

Reactor	Ammonia (L/h)	Lactate (L/h)
Differentiation	53.83	46.66
Proliferation	68.90	59.71

13.2.3 Adsorption Theory

All adsorbents and ion-exchange resins studied in this project follow the Langmuir Isotherm adsorption model, described by the equation below

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e} \quad (42)$$

where q_e and q_{\max} (mol/g adsorbent) are the equilibrium and maximum capacities of the adsorbents respectively and K_L (L/mol) is the Langmuir constant. The constants q_{\max} and K_L can be derived from experimental data: By adding different amounts of adsorbent to a solution containing the component X to be removed, different equilibrium points will be reached. By plotting the data for each equilibrium point on a plot of $\frac{C_e}{q_e}$ against C_e we find that:

$$\text{slope} = \frac{1}{q_{\max}}, \text{ intercept} = \frac{1}{K_L q_{\max}}$$

13.2.4 Lactate Removal

Three adsorbents were evaluated for lactate removal: Mg-Al LDH, Amberlite IRA-67, and activated carbon. Mg-Al LDH was selected for its low cytotoxicity and high adsorption capacity [162], Amberlite IRA-67 for its selectivity and resistance to organic fouling [163], and activated carbon for its low cost and availability. This comparison revealed trade-offs between cost and effectiveness of lactate removal.

Adsorbent properties were derived from published data. Mg-Al LDH data came from an aqueous L-lactate solution at 37°C [162] while Amberlite IRA-67 and activated carbon were tested in a model fermentation broth at 25°C [164]. The effectiveness of Amberlite IRA-67 is largely temperature-independent [165], though the study medium has a significantly lower pH than the assumed reactor conditions of this project, which should be considered.

Table 40: Lactate adsorption parameters for different adsorbents

Adsorbent	K_L (L/mol)	q_m (mmol/g)	q_e at 25mM (mmol/g)	Source
Mg-Al LDH	82.6	1.212	0.862	[162]
Amberlite IRA-67	4.32	3.700	0.425	[164]
Activated Carbon	2.34	2.846	0.186	[164]

Using this, together with current costs from online data, the total cost per molar capacity of lactate was

calculated for each adsorbent:

Table 41: Adsorbent costs for lactate removal

Adsorbent	q_e at 25mM (mol/kg)	Cost (\$/kg)	Cost/capacity (\$/mol)	Source
Mg-Al LDH	0.862	201.60	247.25	[166]
Amberlite IRA-67	0.425	65.02	180.27	[167]
Activated carbon	0.186	64.37	409.23	[168]

Whilst the capacity is lower for Amberlite IRA-67 than Mg-Al LDH, its cost is significantly lower, making it cheaper overall. Activated carbon is eliminated at this point due to its high total cost and mass required. Ease of regeneration is another important metric for adsorption. It was found that both Mg-Al LDH and Amberlite IRA-67 can be easily regenerated by flushing the adsorbent with NaOH[169][170]. Although the latter requires a more concentrated solution of NaOH [169], weak base resins like IRA-67 are more easily regenerated than strong ion-exchange resins like IRA-400 [169]. Moreover the Amberlite IRA-67 shows no indication of a reduction in efficiency, whilst Mg-Al LDH capacity has been shown to drop to below 30 percent after 10 cycles with 0.1M NaOH [170].

13.2.5 Ammonia Removal

Two adsorbent materials were considered for ammonia removal. Zeolites are commonly used for this purpose in aquaculture [171] and significant research exists on the use of zeolites in wastewater treatment [172]. Recently however, zirconium phosphate has emerged one of the most effective adsorbents for the removal of low concentrations of ammonia [37].

A similar analysis to that for lactate removal above was conducted for the removal of ammonia using L-type zeolite and zirconium phosphate:

Table 42: Ammonia adsorption parameters for different adsorbents

Adsorbent	K_L (L/mol)	q_m (mmol/g)	q_e at 25mM (mmol/g)	Source
L-type zeolite	47.63	2.66	0.283	[173]
α -Zirconium phosphate	-	-	6.500	[174]

Table 43: Adsorbent costs for Ammonia Removal

Adsorbent	q_e at 25mM (mol/kg)	Cost (\$/kg)	Cost/capacity (\$/mol)	Source
L-type zeolite	0.283	201.60	180.91	[175]
α -Zirconium phosphate	6.500	534.24	82.19	[176]

Note that, due to lack of pricing data for L-type zeolite, costs were based on Zeolite 4A. Since this would likely yield an underestimate, zirconium phosphate still represents the more cost-effective solution.

13.2.6 Column Design

From this data, the total mass of adsorbent could be calculated for a certain column online time (the time it takes for all the adsorbent in an adsorption column to become saturated), via the following equation:

$$m_a = \frac{r_m \times t_o}{q_e} \quad (43)$$

where m_a is the mass of adsorbent (kg), r_m is the rate of metabolite production (mol/hr), t_o is the online time (hours) and q_e is the equilibrium (or in our case limiting) capacity of the adsorbent (mol/g).

The results of this, and total adsorbent costs are summarised in Table 44 below. For the ammonia removal process the column online time was set at 48 hours, however, for the lactate removal process the infrastructure footprint and adsorbent requirement would be too large. Instead an online time of five hours was chosen with an array of five columns, to still ensure sufficient time for regeneration once each column is saturated.

Table 44: Total adsorbent requirement and costs

Process	Online Time (h)	m_a per column (kg)	Total mass (kg)	Total cost (\$)
Lactate - Differentiation	5	16.17	80.85	5256.75
Lactate - Proliferation	5	20.70	103.50	6727.81
Ammonia - Differentiation	48	0.99	2.98	1592.85
Ammonia - Proliferation	48	1.27	3.82	2038.60

The superficial velocity (described as liquid flow rate/ column area) is an important parameter that influences the residence time for the adsorption process. For liquid adsorption processes this is typically between 0.001-0.004 m/sec [177]. This, combined with the flow rate sets the column area.

The column length (in metres) was calculated as:

$$L = \frac{m_a}{\rho_b \cdot A \cdot (1 - \epsilon)} \quad (44)$$

where m_a is the mass of adsorbent per column (kg), ρ_b is the bulk density of the adsorbent (kg/m²), A is the cross-sectional area of the column (m²) and ϵ is the void fraction of the packed bed.

To size the pumps in Section 13.4 some calculations were done to explore both the frictional and gravitational head losses in the columns. The frictional head losses can be modelled by the Ergun Equation:

$$\frac{\Delta P}{L} = \frac{150(1 - \epsilon)^2 \mu v}{\epsilon^3 d_p^2} + \frac{1.75(1 - \epsilon)\rho v^2}{\epsilon^3 d_p} \quad (45)$$

where ΔP is the pressure drop (Pa), L is the bed length (m), μ is the dynamic viscosity of the fluid (Pa·s), ρ is the fluid density (kg/m³), v is the superficial velocity (m/s) based on the empty column cross-section, ϵ is the void fraction of the packed bed (dimensionless), and d_p is the diameter of the packing particles (m).

The medium was assumed to have the same properties as water at standard conditions ($\rho = 1000$ kg/m³, $\mu = 0.001$ Pa·s) and the diameter of the adsorbent molecules were taken at 1mm. The superficial velocities were set

at 0.001 and 0.003 m/s for the ammonia and lactate removal columns respectively, to ensure practical dimensions.

The assumed adsorbent physical properties along with bed dimensions and pressure drops are summarised in Table 45. Note that the pressure drop applies to two columns due to the system's configuration.

Table 45: Adsorption Column Parameters for Ammonia and Lactate

	Ammonia		Lactate	
	Differentiation	Proliferation	Differentiation	Proliferation
Adsorbent Properties				
Void fraction (ϵ)		0.4		0.45
Adsorbent bulk density (kg/m ³)		3300		1060
Column Parameters				
Bed Length (m)	0.10	0.10	2.14	2.14
Column diameter (cm)	7.97	9.01	12.85	14.53
Frictional pressure drop (Bar)	0.00540	0.00540	0.0218	0.0218

Typically, adsorption systems use three columns, where the solution flows through a lead column, followed by a guard column to capture any breakthrough (adsorbate that has not been adsorbed). This allows the lead column to be operated to full saturation. The final column is the column being regenerated, sometimes referred to as the standby column [177]. This is illustrated by the valve settings in Figure 23.

As shown in Figure 23 an extra two columns were added in parallel for lactate adsorption for the reasons mentioned earlier in this section.

13.2.7 Column Dynamics

To understand the effects of residence time on the effectiveness of the column, the column dynamics were modelled in Python. The adsorbent-adsorbate binding process was modelled as a chemical reaction



where A is the adsorbate, B is the adsorbent and C is the adsorbent-adsorbate complex. Second order reaction kinetics were assumed where the rate of the reaction is first order with respect to both A and B.

To model the concentration of B, the adsorbent, a 'mole' of B was considered as the amount of adsorbent needed to absorb one mole of A at the equilibrium capacity.

The resulting ODE describing the dynamics is:

$$\frac{dC_A}{dt} = D \frac{d^2C_A}{dz^2} - \frac{Q}{A_c} \frac{dC_A}{dz} - k C_A C_B \quad (47)$$

$$\frac{dC_B}{dt} = -k C_A C_B \quad (48)$$

Where: C_A is the concentration of species A (mol m⁻³), C_B is the concentration of species B (mol m⁻³), D is the diffusion coefficient (m² s⁻¹), z is the spatial coordinate along the reactor length (m), Q is the volumetric flow

rate ($\text{m}^3 \text{s}^{-1}$), A_c is the cross-sectional area column (m^2), k is the second order rate constant ($\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$), t is the time (s).

This was solved using numerical methods in Python, with $k = 0.005 \frac{\text{m}^3}{\text{mol}\cdot\text{s}}$ and $D = 0.00025 \frac{\text{m}^2}{\text{s}}$. The spatial concentration profiles as well as the variation of lactate concentration with time are shown below:

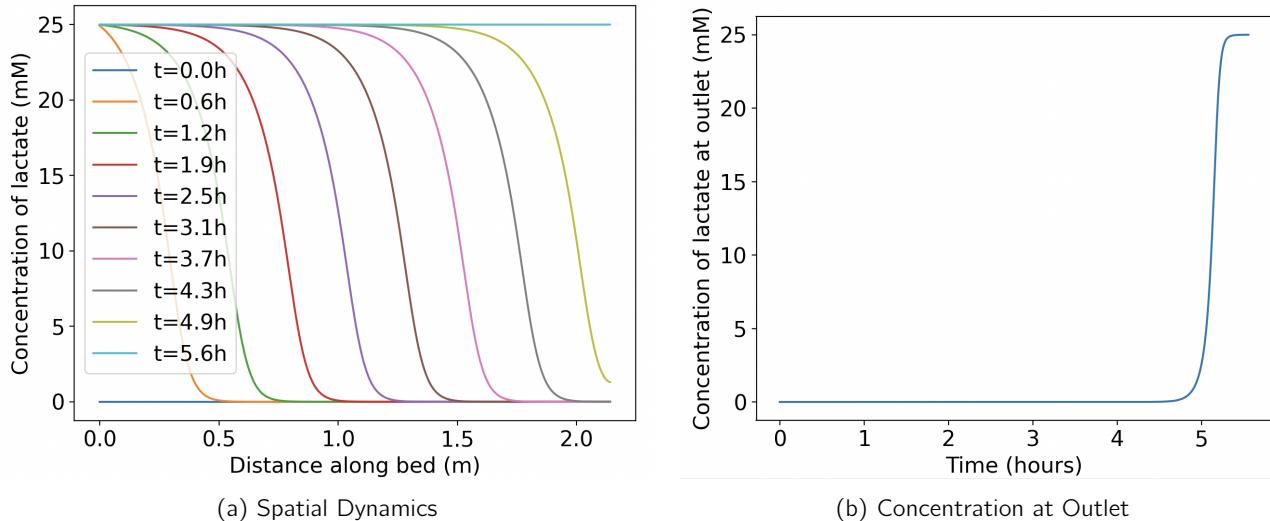


Figure 24: Lactate Adsorption Dynamics

13.2.8 Adsorbent Regeneration

Both adsorbents can be regenerated relatively easily. Lactate saturated Amberlite IRA-67 resin can be regenerated with NaOH at room temperature [169], whilst spent zirconium phosphate can be regenerated using sodium and hydrogen ion solutions (e.g., sodium chloride, sodium acetate, acetic acid) to displace adsorbed cations [37].

13.3 Cell Retention

13.3.1 Literature Review

Current cell retention mechanisms include continuous centrifugation, gravitational settlers, acoustic settlers and filtration systems. According to a 2023 industry survey, 12 of 23 respondents reported having medium recycling in their process and 13 said they used tangential flow filtration (TFF) or alternating tangential flow filtration (ATF) systems [90]. Whilst these filtration methods are favoured, they are costly and suffer from membrane fouling, reducing their effectiveness over time [178].

Consequently, more novel methods are also being trialled, such as inertial sorting that uses microfluidics to hydrodynamically sort cells from the medium in a spiralled channel. This has the potential to significantly reduce costs and simplify the system, however retention efficiency limits cell densities to around 44 million cells/ml, which is below the assumed design criteria [178].

Acoustic separation methods use standing waves to isolate cells. They are a promising technology since they are non-invasive and gentle on cells, but retention efficiencies of commercial products remain limited to 90-99% at up to 20 million cells/ml [179]. Similarly, continuous centrifugation and gravitational settlers are also unsuitable

since they are unable to handle the cell densities aimed for in this process [178].

In TFF systems, the feed stream flows tangentially across the surface of a membrane, retaining the cells whilst some of the medium passes through as the permeate. ATF is a variation of TFF, where the direction of flow alternates periodically to reduce fouling. However, a recent study revealed that, at high cell densities, TFF is likely to be the most suitable technology. This is because TFF systems can achieve higher cross-flow velocities, effectively preventing clogging, something ATF systems would struggle with due to pressure constraints [53].

A weighted multi-criteria analysis (MCA) was applied to identify the best solution. Since the design demands large recycling rates, the greatest importance was placed on cell retention efficiency.

Table 46: MCA for cell-retention methods

Criteria	Max cell density	Retention efficiency	Scalability	Low cost	Energy use	Total score
Weight	3	4	2	3	2	-
Centrifugation	2	1	4	2	0	24
Inertial separation	2	2	2	4	3	36
TFF	4	4	4	1	2	41
ATF	3	4	4	1	2	38
Acoustic separation	1	1	2	3	3	26
Gravitational settlers	1	0	3	4	4	29

The values in the table are based on data from [178],[180],[179].

13.3.2 System Overview

Taking account of the MCA results in Table 46, a TFF cell retention device was incorporated in the perfusion loop, to ensure no cells passed through the adsorption processes.

The filter area requirement is determined by the perfusion flowrate (the maximum of the two values in Table 39), and the filtrate flux achievable by the filter at the operating point.

There are two important variables in a TFF that must be regulated in order to prevent membrane fouling. The transmembrane pressure (TMP) is the force that pushes medium across the membrane. Increasing TMP, for example by restricting the outlet of the feed channel, causes permeate flux to increase. However, above some TMP, a critical flux will be reached in which the fouling effect of accumulating cells at the membrane prevents any further increase in permeate flux.

The onset of this critical flux can be delayed by increasing the cross-flow velocity (the flow rate in the feed channel), which acts to remove cells accumulating at the membrane. Thus, the greater the cross-flow velocity for a given TMP, the greater the permeate flux as shown in Figure 25.

13.3.3 Filter Requirements

Hollow fibre cartridges are recommended for the clarification of mammalian cell cultures [181]. The permeate flux is best determined by empirical analysis. Although Pasitka et al. only manage to achieve a permeate flux of 7.7 L/h/m² (LMH) [53], a GE manual suggests 30LMH to be a good starting point for cell cultures [181]. Based on

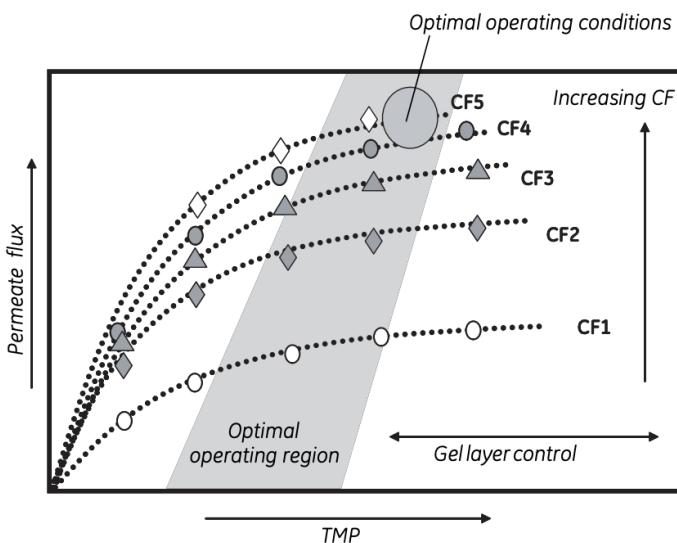


Figure 25: Permeate flux vs TMP at different cross-flow rates, from [181]

this, a flux of 20LMH was assumed. Filter area was calculated as filtrate flow rate/filtrate flux, indicated in Table 47.

Table 47: Filter parameters for different systems

System	Flow rate (L/h)	Filter flux (LMH)	Filter area (m^2)
Proliferation	68.90	20	2.69
Differentiation	53.83	20	3.44

Various products are available that satisfy these requirements, for example the K06-E65U-07-N (0.65 μ) filter by Repligen which has an area of 2.9 m^2 and a cost of 5,953 USD [182].

13.4 Pump Sizing

Two pumps are incorporated into each recycle system, one for the feed and one for the permeate (see Figure 23). Following advice from suppliers at Levitronix GmbH and Drifton, appropriate pump types and sizes were chosen.

The feed pump is a PuraLev i100MU [183], that uses a magnetically levitating impeller for low-shear operation. The maximum TMP reported by Pasitka et al. was 0.5 bar [53], hence a conservative maximum head requirement of 0.75 bar was assumed. The flow rate requirement was assumed to be 10 times the flow rate of the permeate.

Since the filter permeate contains no cells, shear effects are of smaller concern. Hence a cheaper, peristaltic pump can be used. The head requirement is the sum of the frictional heads associated with both adsorption units, plus the gravitational head between the top and bottom of the bioreactor ($h = 0.674m$). This corresponds to an overall pressure requirement of 0.16 bar. The flow rate requirement is taken from the maximum proliferation recycle flow rate, equal to 1.15 L/min. The Shenchen LabN6 Peristaltic pump was recommended by Drifton [184].

13.5 Future Strategies

The proposed medium recycling system, based on established techniques, presents several challenges. High adsorbent costs and the infrastructure footprint for lactate removal are significant concerns, particularly when scaling

up. Additionally, issues with fouling and costs related to tangential flow filtration (TFF) add further complexity. Therefore, three alternative or additional strategies are proposed below, with potential for future integration.

13.5.1 Biocatalysts

Microbial metabolic processes or enzymes can catalyse the conversion of ammonia and lactate into other substances. Lactate is metabolized by lactate dehydrogenase to pyruvate, which is further oxidized in the mitochondria, generating carbon dioxide and water while providing a vital carbon source for the cell [37]. A bi-catalyst system could employ lactate dehydrogenase to convert lactate to pyruvate and NADH, which is then oxidized by NADH oxidase to regenerate NAD+, enabling continuous lactate conversion [159].

However, the cost of NADH oxidase is substantial, priced at \$500 for 5 mg [185], with an estimated requirement of 400 mg for this process. Consequently, significant development in this area is needed to reduce costs.

13.5.2 Co-culturing

Various studies have investigated the potential of cultivating cells in the presence of cyanobacteria or micro-algae, which consume ammonia while simultaneously providing essential nutrients to the cells [161]. Additionally, research has explored the use of genetically modified bacteria for the removal of lactate [160][186].

13.5.3 Cell Adaptation

A recent study introduces Lactate Supplementation and Adaptation (LSA) technology, which reduces lactic acid and ammonium production in animal cell cultures by acclimating CHO cells to a lactate-enriched medium. This method resulted in near-complete elimination of lactic acid and a significant reduction in ammonium production, albeit for a lower density culture than the one considered in this report [110].

13.6 Summary

In conclusion, while the adsorption-based system aligns well with a minimum viable product (MVP) approach, it will require further enhancements to remain competitive in the future. As the industry evolves, the strategies outlined are expected to undergo significant development, and their implementation will be adapted as necessary to meet emerging demands.

14 Cell Harvest and Product Formulation - Luke Nijkamp

14.1 Cell Harvest

Once the cells have differentiated, they must be removed from the medium for product formulation. In the proposed system, cells are removed by a tubular bowl centrifuge, after which they are sold in their current form as a mince-type product. In future, the process also considers co-extruding the cell slurry with textured pea protein to create a hybrid product suitable for the mass market.

14.1.1 Literature Review

Like cell separation, there exist various methods for cell harvest from culture medium. In this case, cell retention efficiency was less critical, since the cells only flow through the system once, and instead more emphasis was placed on the speed, cost and scalability of the technologies.

For cell harvest, three technologies are consistently mentioned in the literature [187]. Centrifugation uses centrifugal force to separate components by density, concentrating cells at the walls while the supernatant flows out.

TFF and depth filtration separate cells by size. Unlike TFF, depth filtration directs fluid flow perpendicularly to the filter area, capturing particles throughout the filter matrix rather than solely at the membrane edge. Although this enhances the filter's capacity before fouling, such systems are primarily used for liquid purification rather than solid recovery [188].

The reasoning for centrifugation is further outlined in the multi-criteria analysis below:

Table 48: MCA for cell harvest [187]

Criteria	Ease of solids removal	Retention efficiency	Scalability	Low cost	Fouling	Total score
Weight	4	2	3	2	3	-
Centrifugation	3	2	4	1	3	39
TFF	2	4	2	2	1	29
Depth Filtration	1	4	3	3	2	33

14.1.2 Centrifuge Design

Carr Biosystems recommended their Viafuge Pilot system (Figure 26) as a good fit for our application and parameters. Disc stack centrifuges offer higher separation efficiency [189], but tubular bowl centrifuges are sufficient for this application due to lower cost and lower shear forces. Should cell densities be too high, the mixture can be pre-diluted before entering the centrifuge.

The following bowl dimensions were given by the supplier:

$$\text{Inner radius } (r_i) = 0.0485 \text{ m}; \text{Outer radius } (r_o) = 0.0762 \text{ m}; \text{Height} = 0.127\text{m}$$

From this, the maximum flow rate for a given relative centrifugal force (RCF) can be determined. First RCF is converted to RPM via:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{1.118 \times r}} \times 1000 \quad (49)$$

where RCF is the relative centrifugal force ($\times g$), r is the outer radius (cm), RPM is the revolutions per minute.

From this, the flow rate of the cell culture through the centrifuge was determined as:

$$Q = \left[\frac{d^2(\rho_S - \rho_L)g}{18\mu} \right] \left[\frac{\pi z(r_o^2 - r_i^2)\omega^2}{g \ln\left(\frac{r_o}{r_i}\right)} \right] \quad (50)$$

where Q is the volumetric flow rate (m^3/s), d is the particle diameter (m), ρ_s is the cell density (kg/m^3), ρ_L is the medium density (kg/m^3), g is the acceleration due to gravity (m/s^2), μ is the dynamic viscosity of the medium ($\text{Pa}\cdot\text{s}$), z is the bowl height (m), r_i, r_o (m) are the inner and outer radii of the bowl respectively and ω is the angular velocity (rad/s).

Recommended RCF values vary widely in the literature, particularly as some refer to use cases where the cells must remain viable for reuse. An RCF of 500g-2000g was quoted as a common range for mammalian cells, of which the lower bound was taken for a conservative estimate [191]. Assuming a particle diameter of $10 \mu\text{m}$ [53], a cell density of 1060 kg m^{-3} , and a medium density of 1000 kg m^{-3} , a maximum flow rate capacity of 3.93 L/min was estimated.

According to the centrifuge supplier, the maximum achievable packed cell volume is 90% of the bowl's total volume. Assuming a random spherical packing density of 0.65 [192], and a cell diameter of $10\mu\text{m}$, 2.25 bowl volumes are required. Hence using three batches of 24.1 L , and assuming 15 minutes to pump out the cell slurry each time, yields a total processing time of 82 minutes.

14.2 Final Product

At least initially, it is planned to produce a pure chicken mince as the final product. It was anticipated that the combination of pure biomass with a filler would fail to generate sufficient cost savings for a mass market product, and that a pure product would be more appreciated by high-end restaurants (our initial primary customer).

14.2.1 Hybrid Co-extrusion

According to [193], taste concerns often limit the success of products with over 30% plant-based content, though many European consumers are open to hybrids with a 50:50 mix for which commercial products already exist [194].

Extrusion is a high-temperature, shear-intensive process that unfolds and denatures proteins, promoting aggregation and cross-linking, which, upon cooling, results in a fibrous, meat-like texture. Research has demonstrated that co-extruding a 1:1 blend of plant and meat proteins, such as texturised pea protein (TPP) and beef, can successfully produce hybrid products with improved taste and texture [195].

Future iterations of the process may incorporate this technique once biomass costs decrease. According to the UK-based supplier Andina Ingham, textured pea protein (TPP) is available in bulk for £4–6/kg. Additionally, a suitable extrusion machine has been identified for integration into the proposed production system [196].

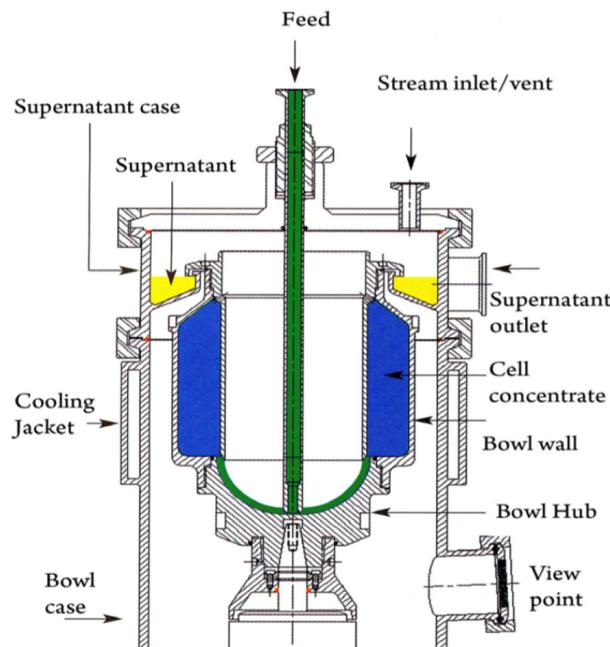


Figure 26: ViaFuge Pilot Centrifuge [190]

15 Process Requirements and Costing - Zaheer Sidik

This section explores the costs associated with construction of the plant, these can be seen in Table 51. It also looks at the operating costs due to the energy and material requirements as seen in Table 50 and Table 49.

15.1 Material Requirements

The material requirements for the overall section can be seen in Table 49. This also includes the cost of the workers which have been classed as an operating cost as they will have to be paid each year, this has been explored in Section 22.4.

Table 49: Annual Material Requirements and Cost Breakdown (including labour)

Item	Quantity (/year)	Cost (\$/g or L)	Total Cost (\$)
Fresh Medium (L)	1.426×10^5	4.45 (4)	634,591.05
Glucose (g)	2.004×10^5	0.02[?]	40,087.95
Glutamine (g)	3.806×10^4	0.46 (4.2.2)	17,506.08
Phosphatidylcholine (g)	2.699×10^4	442.26[141]	11,934,722.22
Oleic Acid (g)	1.374×10^5	0.12[142]	79,492.16
DOX (g)	345	3,044.41[138]	1,052,148.79
FBS Alternative (L)	434.31	721.22 (4.2.3)	313,121.25
Oxygen (g)	431.64	0.21[197]	90.56
Zirconium phosphate	6	534.20[176]	3,632.58
Amberlite resin	184.4	65.00[167]	11,986.00
Workers	8	59,920.00 (22.4)	479,360.00
Total			15,268,853.01

15.2 Energy Requirements

The annual energy requirements associated with each process can be seen in Table 50. The cost of 1 kWh of energy has been taken as \$ 0.11 [113]. Note that the oxygenation process requires no energy as the oxygen is held at 200 bar and the bioreactors operate at 1 bar. The lab heating cost has been determined based on the choice of location as outlined in Section 22.

Table 50: Annual Energy Requirements and Cost Breakdown

Process	Energy (kWh/year)	Cost (\$/kWh)	Total Cost (\$)
Proliferation	1.85×10^4	0.11	4,085.40
Myogenesis	3.20×10^3	0.11	352.55
Adipogenesis	6.00×10^2	0.11	66.13
Oxygenation	0	0.11	-
Cell Separation	2.49×10^3	0.11	273.72
Medium Recycle	42.44	0.11	4.67
Lab Heating	1.78×10^4	0.11	1961.08
Total	4.26×10^4		4,689.57

15.3 Capital Costs

All the equipment requirements and their costs are shown in Table 51. A DCF (Direct Cost Factor) calculated as (Installed/Bare) can be seen in Table 51, the value of 4 has been taken from [198]. The bioreactors have a

DCF value of 1 as the correlation in Figure 16a includes the cost of installation. The lab has been assumed to be 100 m² and with a cost of \$1000 per square foot, taken from [199]. The land cost has been discussed further in Section 22.3.

Table 51: Annual Capital Requirements and Cost Breakdown

Item	Cost (\$/unit)	Units Required	Bare Cost (\$)	DCF	Installed Cost (\$)
Adsorption columns	300.00[200]	16	4,800.00	4	19,200.00
Levitating Impeller pump	12,645.36[183]	2	25,290.72	4	101,162.88
Peristaltic pump	1,580.81[184]	3	4,742.43	4	18,969.70
Filters	5,953.00[182]	2	11,906.00	4	47,624.00
Centrifuge	7,000.00[201]	1	7,000.00	4	28,000.00
n-2 Bioreactor	138,600.00 (7.5.2)	1	138,600.00	1	138,600.00
n-1 Bioreactor	176,400.00 (7.5.2)	1	176,400.00	1	176,400.00
nth Bioreactor	264,600.00 (7.5.2)	3	793,800.00	1	793,800.00
Adipogenesis Bioreactor	69,925.00 [58]	1	69,925.00	1	69,925.00
Myogenesis Bioreactor	186,600.00[58]	1	186,600.00	1	186,600.00
Laboratory	1,078,000.00[199]	1	1,078,000.00	1	1,078,000.00
Land (acre)	80,400.00 (22.3)	1	80,400.00	1	80,400.00
Total					2,729,681.58

15.4 Overall Costing

The overall energy and cost associated with construction of the plant and producing a tonne of product per annum can be seen in Table 52.

Table 52: Total Cost associated with construction and first year of production

	Material (\$)	Energy(\$)	Capital(\$)	Overall(\$)
Total Cost	15,268,853.01	4,689.57	2,729,681.58	18,001,317.06

Based on a plant lifetime of 10 years as mentioned in Section 32, the cost of the cultured chicken is approximately **\$15,500/kg.**

16 Safety and Risk - Viraj Nerkar

As part of the overall design of the plant, a risk assessment was performed to ensure that all hazards were identified and categorized. Various risk measurement and analysis methods including F/N curves, HAZAN, checklists, fault trees, and event trees were considered. However, a risk matrix and HAZOP were chosen. Qualitative methods were preferred due to the lack of easily available data and the lack of means to empirically measure data for quantitative techniques.

16.1 Risk Matrix

A risk matrix is a simple method to identify and prioritize different hazards. The overall risk for a given hazard is calculated by combining the two key aspects of risk- likelihood (probability) and severity (consequences).

RISK MATRIX		LIKELIHOOD (or probability)			
		High	Medium	Low	Remote
CONSEQUENCES	Severe	High	High	Medium	Low
	Moderate	High	Medium	Medium/Low	Effectively Zero
	Insignificant	Medium/Low	Low	Low	Effectively Zero
	Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero

Figure 27: Risk Matrix used by the Department of Engineering Science, Oxford

Figure 27 shows that the overall risk is assigned to five categories of decreasing importance: high, medium, medium/low, low, and effectively zero. This matrix is applied to the operation of the CSTR bioreactor in Table 53.

Table 53: Risk Matrix

Hazard	Likelihood	Severity	Overall Risk
Contamination	Low	Severe	Medium
Chemical exposure (cleaning agents)	Low	Moderate	Medium/Low
Bioreactor rupture (overpressure)	Remote	Severe	Low
Oxygen leak (fire hazard)	Low	Moderate	Medium/Low
Human Operator injury	Medium	Negligible	Effectively Zero

16.2 HAZOP

HAZOP (Hazard and Operability Study) is a structured technique to identify operational problems in a process system by examining deviations from standard operating conditions. The two critical nodes in the CSTR bioreactor system are the tank and the oxygen supply. Table 54 and Table 55 show the HAZOPs conducted for oxygen supply and bioreactor tank respectively.

Table 54: HAZOP for oxygen system

Deviation	Cause	Consequence	Safeguards
No flow	Closed valve, blockage	DO levels drop and could cause cell death	Alarm connected to DO sensor
Low flow	Partial blockage	Inadequate oxygen supply causing cell growth to slow down	Alarm connected to DO sensor
High flow	System error	Higher shear stress causing cell damage	Pressure release valves
Other flow (e.g. air)	Operator error/ wrong cylinder connection	Cell contamination	Clear gas cylinder identification labels, gas purity sensor

Table 55: HAZOP for bioreactor tank

Deviation	Cause	Consequence	Safeguards
Overpressure	Blocked exhaust, excessive sparging	Vessel rupture, major injury, batch loss	Pressure release valves connected to sensor, manually operated valve
Underpressure	Sudden cooling, blocked inlet	Vessel collapse, loss of sterility	Vacuum breakers
High temperature	Heating malfunction	Cell death	Temperature alarms, redundant sensors
Low temperature	Heating malfunction	Reduced cell metabolism	Temperature alarms, redundant sensors
High agitation speed	System error	Higher shear stress causing cell damage	RPM sensors
Low agitation speed	Motor damage	Poor mixing, non-uniform culture	RPM sensors

17 Environmental Impact Assessment - Luke Nijkamp

17.1 LCA

17.1.1 Definition of Goal and Scope

A preliminary life cycle assessment (LCA) was conducted with the primary objective of identifying key hotspots of greenhouse gas emissions within the system. The analysis was not intended to provide a comprehensive or directly comparable evaluation against conventional farm-reared chicken. The scope of the assessment followed a cradle-to-gate approach and excluded emissions associated with construction activities and raw materials.

17.1.2 Inventory Analysis and Impact Assessment

The following tables present the calculated global warming potential (GWP) of the culture medium and overall production process (in carbon dioxide equivalent units). Due to limited data availability, multiple sources were used for impact data, which will negatively affect the validity of the analysis.

Table 56: Estimated GWP per litre of culture medium

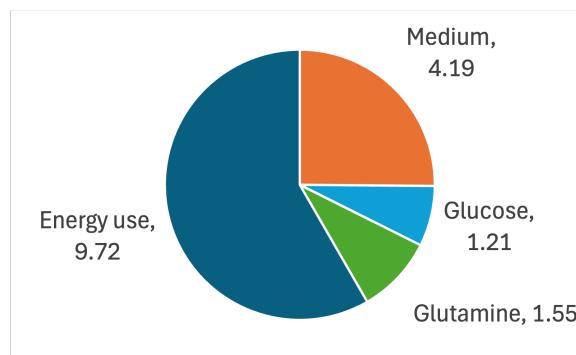
Component	Quantity/L medium	Unit GWP (CO ₂ eq)	Source	GWP contribution (kg CO ₂ eq)
Soybean Hydrolysate	0.01 g	0.743 kg/kg	[202]	7.43E-06
DMEM F12	0.99 L	0.0253 kg/L	[203]	0.0250
FBS alternative	0.01 L			
NaCHO ₃	2.43 g	0.0323 kg/kg	[204]	7.86E-06
Glutamine	0.219 g	19.14 kg/kg	[93]	4.20E-03
Total				0.0293 kg

Table 57: Estimated GWP per kg of cultured meat (CM)

Component	Quantity/kg CM	Unit GWP (CO ₂ eq)	Source	GWP Contribution (kg CO ₂ eq)
Medium	143 L	0.0293 kg/L	Table 56	4.19
Glucose	2000 g	0.603 kg/kg	[93]	1.21
Glutamine	8.09 g	19.14 kg/kg	[93]	1.55
Oxygen	0.314 g	0.7 kg/kg	[205]	2.20E-04
Energy use	42.6 kWh	0.228 kg/kWh	[206]	9.71
Total				16.67 kg

17.1.3 Interpretation

As shown by this analysis, the estimated GWP of the cultured chicken product is 16.67 kg CO₂ eq per kilogram of meat. From various studies, the GWP of conventionally produced chicken in the United States was estimated to range between 2.00 and 2.62 kg CO₂ eq per kilogram of meat (cradle-to-gate), based on an assumed muscle-to-body weight ratio of 50% [207, 208]. These values are substantially lower than those reported in many other countries [209]. Moreover, it has been observed that higher production efficiencies may in fact be inversely correlated with animal welfare [210].

Figure 28: GWP of Cultured Chicken in kg CO₂ eq (oxygen contribution negligible)

As shown in Figure 28, energy use is the largest contributor to the carbon footprint of the process, followed by the culture medium. Future work should explore opportunities to reduce electricity-related emissions, for example through the integration of on-site renewable energy sources.

Further analysis should account for indirect water and energy use, such as that associated with cleaning, to provide a more comprehensive estimate of total water consumption. This is of particular interest given that conventional chicken production required approximately 500 litres of water per kilogram in 2020 [207]. The preliminary analysis (excluding cleaning water) suggests a figure less than half of that.

17.2 Further Considerations

17.2.1 Transport and Logistics

Although product distribution was excluded from the LCA, it remains a significant contributor to overall emissions. Low-carbon distribution methods (particularly for last-mile delivery) should be prioritised and environmentally responsible packaging (such as bio-based options) selected, to minimise environmental impact.

17.2.2 Waste Streams

Ideally our process would incorporate circular economy principles to minimise material and energy waste. By-products such as lactate and ammonia have potential commercial applications in the food and agriculture sectors, whilst waste heat could be recovered and reused in other applications (e.g. heating buildings). Further work would be necessary to determine, via LCA, whether the environmental benefits outweigh the energy inputs required for recovery and purification.

18 EEM Introduction - Viraj Nerkar

In conjunction with the design of the overall cultivated chicken production system, we investigated the market for such a product and developed a business strategy accordingly. Sections 19-26 focus on understanding the cultured meat market, from analysing the broader alternative protein industry to conducting primary research and mapping the key stakeholders.

These sections refer to a traditional end-to-end business-to-consumer (B2C) or direct-to-consumer (DTC) model where we would source raw materials (chicken fibroblasts), produce biomass, and market and distribute the product to the end user. Sections 27 - 29 look at marketing, supply-chain dynamics, and use Porter's Five forces to assess the B2C model in depth. The key conclusion derived from these sections was the lack of viability of such a plan.

Therefore, a decision to shift to a business-to-business (B2B) model in the future is explored where we operate as a technology and service provider to large corporations looking to expand their product offerings to cultivated meat. The current 1 tonne-per annum (tpa) design specification will be used as a proof of concept for our process and the quality of the end product. A future 10 tpa plant will demonstrate the scale-up potential of our process. The biomass generated from these plants will be sold through high-end restaurant partners to consumers to develop consumer trust and generate cash flows before the eventual switch to a technology and service provider. Sections 30 - 32 detail the strategy and financials to reflect this.

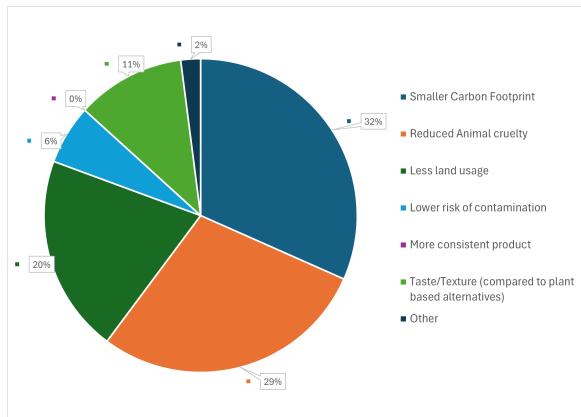
19 User Research and Market Segmentation - Zaheer Sidik

Even though we do not plan to sell to consumers directly, for cultured chicken to succeed commercially it is crucial to understand consumer preferences, behaviours, and motivations. Market segmentation and user research play a pivotal role in this. User research provides deeper insights into potential customers' perceptions, purchasing decisions, and the main barriers to adoption. Effective market segmentation allows for identification of distinct consumer groups based on demographics, lifestyle choices, dietary preferences, and ethical concerns. This section aims to highlight potential barriers to adoption and consumer preferences and values that we have to consider.

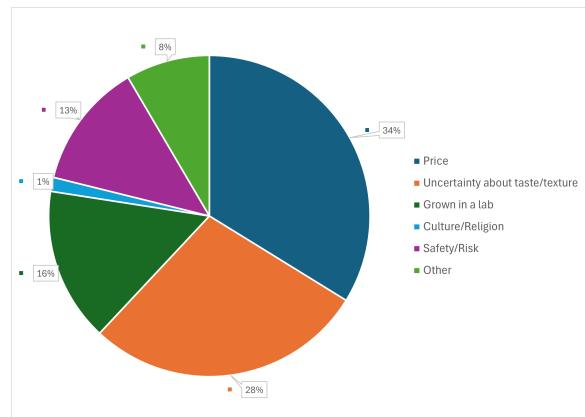
19.1 Primary Research

To explore the public perception of cultured meat and chicken, primary research was carried out through the form of an electronic survey aiming to find out consumers' perceived selling points and pain points of cultured meat. A copy of the circulated survey can be seen here. The survey was circulated within WhatsApp groups and mailing lists. The perceived selling points can be seen in Figure 29a, majority of respondents agreed that the reduced animal cruelty and smaller carbon footprint are the two main selling points of cultured meat when compared to traditional farming techniques. The perceived pain points can be seen in Figure 29b, with the primary concerns being the price and the taste of the product. The prices consumers are willing to pay can be seen in Figure 29c with the majority of respondents willing to pay up to \$10 per kg.

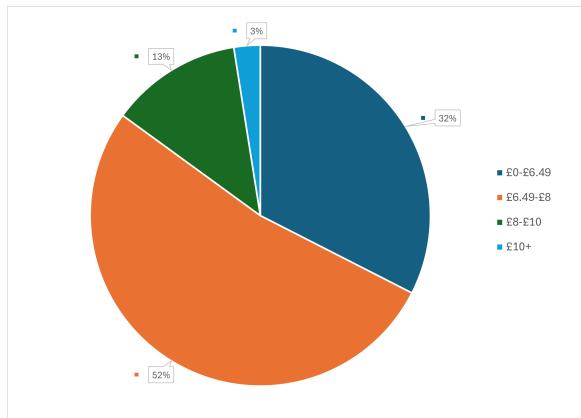
A copy of the survey results can be seen here. There are some caveats associated with the survey results, the majority of answers were from 18-25 year old, students based in the UK, furthermore there were only 40 survey results which is a relatively small sample size. This is likely due to the fact that the survey was only live for a limited time-frame and there was no incentive/reward for individuals to fill out the survey. Furthermore, our target market of Washington, USA has not been accounted for. Research done by the Good Food Institute, [211] does indicates that the UK and US move in tandem with regards to plant-based and cell-based meat trends suggesting that the findings from our survey are still relevant and meaningful however further market research in both the target location and with our target market will be required as detailed in Section 19.4.



(a) Survey Results - Perceived Selling Points



(b) Survey Results - Perceived Pain Points



(c) Survey Results - Perceived Value

Consider a future in which lab-grown meat alternatives are indistinguishable from animal meat in terms of taste, nutrition, and cost. In this scenario, would you prefer to eat...?

All adults (9272 US adults - May 29, 2024)



(d) Secondary Research - Cultured Meat Adoption

Figure 29: Primary and Secondary Research Findings

19.2 Secondary Research

Secondary user research plays a critical role in understanding the evolving landscape of consumer attitudes toward cultured meat, it is also less costly and typically available quicker than primary research. By analysing existing data we can gain a better understanding of public perception, key trends, and potential barriers to adoption. When used in conjunction with primary research, secondary research can be used to validate findings and fill gaps. Some results from Aleph Farms market research includes that younger consumers are generally more willing to try cultured meat, with "90% of all Gen Z respondents in the US" willing to try cultured meat [212]. Furthermore, [213] states that

"US research found that younger consumers had stronger preferences for alternative meat."

One of the key selling points of cultured meat are the environmental benefits when compared to traditional farming, in the US 71% of the 2018 consumers asked were concerned with climate change [213] and mentioned that their concerns affected food and beverage purchases and 67% were concerned with the impact of food production on climate change [213], however, it is also noted that there is generally a "surprisingly low" consumer awareness of the impact of meat production on the environment [213].

It is mentioned in [212] that the early majority are likely to be consumers with an elevated interest in environmental issues and the early adopters are likely to be novel foodies as reflected in Figure 29a.

A widespread barrier to acceptance of cultured meat is neophobia - a reluctance to eat/avoidance of novel foods [213]. This is reflected in survey data that states that 1/3 of Americans respondents were unwilling to try cultured chicken or beef in a restaurant [214]. Furthermore, Figure 29d shows that even if cultured meat was indistinguishable from traditional meat majority of respondents would still prefer to eat traditional meat. There is also a well documented societal attachment to meat consumption, meaning that traditional meat has an important social status [213]. Techniques and strategies to attempt to overcome this barrier have been outlined in Section 27.

19.3 Market Segmentation

From both the primary and secondary user research, a basic value based segmentation can be done for the consumer cultured meat market (further detail on layering the segmentation model can be found in Section 19.4):

- Environmentally Conscious
- Price Sensitive - these consumers will be difficult to target until the cost of production reduces significantly.
- Novel Foodies - these consumers typically have large amounts of disposable income and enjoy fine dining.

As mentioned in [215] "chefs are interested in the benefits and appeal of cultivated meat options - even if it means paying more for it" as well as that "Chefs and restaurants are often the first to embrace new food innovations". Hence there is also a gap in the market for a B2B venture, mentioned in Section 30, in which we provide a customised plant for restaurants/brands/supermarkets to produce cultured meat tailored to their specific requirements. We believe that our early adopters are likely to be luxury restaurants and hence the foodies that frequent these restaurants. Whilst the early majority are likely to be wealthy consumers with a concern about the environment. However as outlined in Sections 27 28 31, and it is more economically attractive to service the early majority indirectly through the B2B model.

19.4 Future Research

To accurately understand the consumer market a multi-layered segmentation model would have to be considered, accounting for the demographic and psychographic of consumers as well. This has been considered through the use of personas in Section 19.5. Initial market research would be required to find more quantitative data to supplement

that shown in Figure 29, this would be done by circulating surveys closer to our target area, with our initial target consumers which would likely be luxury restaurants/chefs, their opinions and views are likely to be heavily influenced by luxury/novel foodies. Further market research beyond this would be required to find more qualitative information about the consumers in the individual market segments, this would be done through focus groups and interviews. These methods would provide insight into the consumer taste, texture, packaging preferences and their general priorities when considering purchase. However, these methods have inherent bias. Observational methods have the benefit of allowing information that cannot be communicated to be revealed, however, they typically require more time and are more costly. The two proposed methods are naturalistic and ethnographic observation, to carry out these two methods with a particular focus on the early adopters would involve frequenting luxury/Michelin star restaurants in the targeted area of Washington and observing customer behaviour. By combining these research methods, information on the price sensitivity, environmental concerns, taste requirements and any unseen barriers to adoption would be revealed.

19.5 Personas

As discussed in Section 19.3, there are three market segments we would need to consider: environmentally friendly, price sensitive and luxury/novel foodies. Through the use of personas as shown in Table 58, we are better able to understand the consumer needs and desires that drive their purchases. To drive market adoption, this information would be required to be able to tailor marketing, packaging, product education and distribution to resonate with the consumers in each market segment.

19.6 Consumer Road Map

Given the personas in Table 58, a consumer road map depicted in Figure 30 can now be created for each individual persona, from awareness through to brand loyalty. This has been explored for a B2C model below.

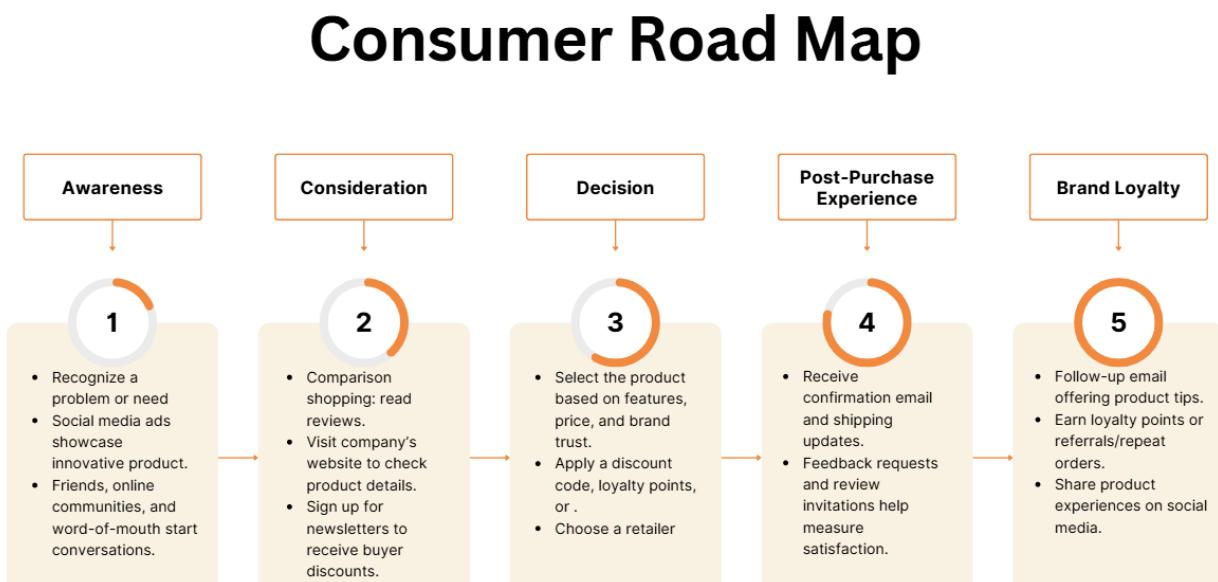


Figure 30: Consumer Road Map (created in Canva)

Table 58: Personas for Cultured Chicken

Trait	Eco-Conscious Emily	Price-Sensitive Paul	Gourmet Chef Gabriel
Age	32–40	35–50	40–55
Education	University degree	College	Culinary school graduate
Income	Upper-middle income	Moderate income	High income
Location	Urban area - Seattle	Suburban Washington	Urban area - Bellevue
Values	Sustainability, ethical consumption, reducing carbon footprint	Affordability, practicality, cost-effectiveness	Innovation, exclusivity, sustainability, premium quality
Lifestyle	Actively follows eco-friendly trends, participates in community sustainability initiatives	Budget-conscious, compares prices, seeks value for money	Passionate about culinary excellence, seeks out unique and high-end ingredients, prioritizes food ethics and traceability
Motivations	Willing to pay a premium for products that align with her environmental values	Interested in trying alternative products if they offer clear savings compared to traditional options	Excited by the opportunity to introduce cutting-edge, sustainable ingredients to fine dining menus
Media Habits	Reads environmental blogs, watches environmental documentaries and follows sustainable promoting influencers on social media	Reads product reviews, uses price comparison sites and watches budgeting influencers	Follows Michelin-starred chefs, reads food industry journals and attends high-end culinary expos
Pain Points	Skepticism about “green-washing”, desire for clear evidence of environmental benefits, limited availability of truly sustainable products	High cost as a barrier to trial, concerns that premium pricing may not deliver proportional benefits, preference for familiar products with proven value	Skepticism about taste and texture compared to free-range poultry, perceived lack of prestige, will require reliable supply chains and consistent product quality

19.6.1 Awareness

If we are pursuing a B2C model, awareness would be driven through strategic social media campaigns and influencer partnerships. For instance, eco-conscious influencers could appeal to environmentally motivated consumers like Emily, while culinary influencers and chefs could help engage individuals like Gabriel. At this stage, targeting price-sensitive consumers such as Paul would be futile, as further R&D is needed to reduce production costs and compete with traditional meat.

Additional awareness strategies could include billboards, magazine/journal articles and appearances in gourmet magazines to appeal to audiences such as Gabriel. Messaging should be tailored to align with the individuals values: for Emily, highlighting sustainability, transparency, and ethical sourcing. For Gabriel this would involve, emphasising premium quality, culinary innovation, and sustainability, since chefs are increasingly aware of the downsides of conventional meat production [215].

19.6.2 Consideration

To ensure that each consumer type is made aware of product features that align with their values as shown in Table 58: a heavy emphasis on sustainability, transparency and ethical production should be given when aiming to target consumers such as Emily, this can be done through production reports, reviews from external environmental agencies and charts comparing environmental factors such as CO₂ produced per kg of chicken. For consumers like Gabriel, the quality, innovation and exclusivity should be demonstrated, this can be done through testimonials, magazine/journal articles and exclusive interviews.

19.6.3 Decision

As this is a novel product, early market adoption would prove challenging, therefore we would offer incentives to our target customers. For consumers such as Gabriel, the incentives could take the form of tasting experiences, partnership discounts and early access to new products. For those like Emily, incentives would likely be in the form of clearly displayed eco-friendly certification.

19.6.4 Post Purchase Experience and Brand Loyalty

If we decided to utilise online fulfilment as a distribution channel for our B2C model, when consumers would order online we would include communication about the environmental benefits of the product e.g. a generated statement in the confirmation email indicating how much CO₂ was saved and how many animal's lives were saved. As well as that we would include coupons, loyalty programmes and seasonal promotions during the holidays in which meat/chicken is typically consumed. A QR code would also be attached to the packaging where customers can provide feedback and answer a short survey, this would ensure that we understand consumer's ever changing preferences and do not become obsolete.

For businesses and restaurants that we service, we would carry out personal outreach, aiming to receive feedback from chefs through the use of focus groups and interviews. We would also propose exclusive/early product launches. This commitment to improvement of our product and listening to consumer feedback can be expected to foster brand loyalty with our customers.

19.7 Conclusion

This section outlines a comprehensive approach to consumer engagement under a potential B2C model. However, such a strategy would require significant investment in consumer research, marketing, and awareness-building which is unlikely to be feasible for a new start-up. As seen in the consumer road map and proposed future research, a B2C model would require substantial investment into areas such as user research, marketing and awareness/education, hence we considered alternative commercialisation models as outlined in Section 30.

20 Industry Analysis - Viraj Nerkar

Protein is one of the fundamental building blocks of organic life. For a healthy and balanced human diet, 25-35% of the calories consumed should come from protein [216]. Proteins are biopolymeric structures composed of amino acids and serve as structural support, biochemical catalysts, hormones, enzymes, building blocks, and initiators of cellular death [217]. Although most of protein intake has traditionally come from foods such as lean meat, seafood and dairy, the alternative protein industry has gained traction over the last decade (Figure 31).

20.1 Alternative Protein Industry

The alternative protein industry is growing rapidly. Its largest driver is the aim of reducing the harmful impact from the production of traditional protein products such as meat. Meat accounts for 9% of the calories consumed globally [218] but causes 56% of greenhouse gases released by the food industry in its entirety [219].

As seen in Figure 31, the cumulative capital invested in this industry in the last decade is almost \$15 billion. The largest proportion of this has been in plant-based products although cultivated meat has gained a lot of traction in the last 3 years.

Cumulative and annual alternative protein invested capital, by pillar



Figure 31: Annual Investment into Alternative Proteins [220]

The North American region is home to the largest part of the global alternative protein sector accounting for 38% of the overall market, as seen in Figure 32, followed by Europe and the Asia-Pacific region. Likely factors

behind this distribution are that the populations in these regions are more progressive and possess higher amounts of disposable income, as alternative protein tends to be more expensive. This was one of the key factors in our decision to base Oxfarm in the United States (Washington state Section 22.5).

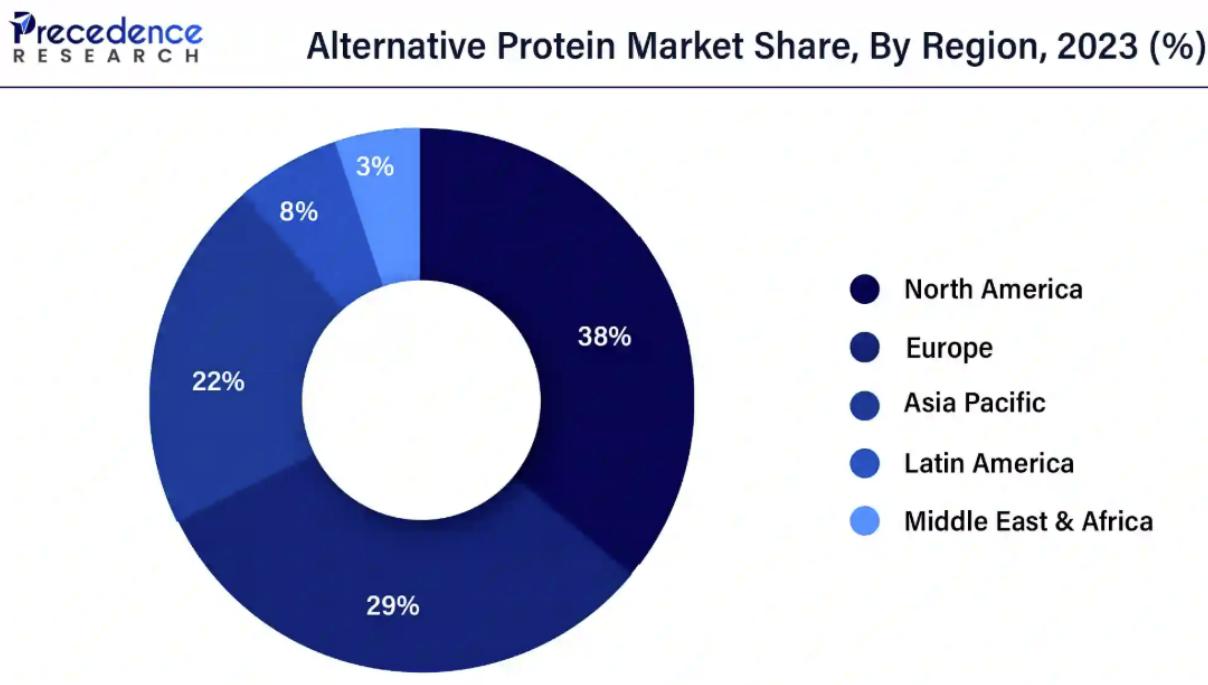


Figure 32: Global Alternative Protein Market by Region [221]

20.2 Alternative Protein Categories

The alternative protein market can broadly be divided into 3 main technologies : plant-based meat, cultivated meat, and fermented meat. It is forecasted to grow from a current size of \$16.6 billion [221] to \$77 billion [222] by 2030, although this represents an optimistic estimate for the CAGR (Current Annual Growth Rate) at 36%.

20.2.1 Plant-based Meat

Plant-based meat aims to replicate the taste, texture, and nutrient composition of conventional meat but without the use of animal products. Animal meat is made up of protein, fat, vitamins, minerals, and water. Although plants do not have muscles, they do contain protein, fat, vitamins, minerals, and water. Plant-based meat takes advantage of this biochemical similarity between plants and animals [223]. The appeal of plant-based meat is threefold: it uses significantly less water, land and energy; it allows traditional meat eaters to enjoy the taste of meat while contributing to the environmental benefits of vegan/ vegetarian lifestyle and it brings economic benefits of not relying on food products susceptible to disease outbreaks (eg, avian influenza).

Although the benefits of this technology are clear, widespread adoption has been elusive. The key factors behind this include the still significant discrepancy between taste and texture compared to animal meat, as well as the politicization of plant-based meat [224].

20.2.2 Cultivated Meat

Cultured or cultivated meat is grown from actual animal cells. Stem cells are taken from animals and cultured in bioreactors. The bioreactor and cell medium replicate the conditions inside an animal to achieve optimal growth. A key benefit of cultivated meat over alternatives is that it is actual animal meat and thus can be produced to have similar nutritional properties (fat/protein ratio) and characteristics such as appearance, taste and texture. Although energy usage from production is similar to traditional meat, a considerable amount of water is saved.

The main drawbacks include the enormous cost of production per kg, along with regulatory hurdles and consumer hesitation regarding lab-grown meat[217].

20.2.3 Fermented Products

Fermentation is the metabolic process in which microorganisms (such as yeast or bacteria) convert carbohydrates into alcohol or acids. Within the alternative protein industry, fermentation is used in three primary ways: traditional fermentation, biomass fermentation, and precision fermentation. Traditional fermentation uses live microorganisms to develop and modulate ingredients [225]. Biomass fermentation utilises the high protein content and fast growth of certain microorganisms to produce protein [225]. Precision fermentation uses microbial hosts to produce specific ingredients. The key benefit is the ability to efficiently produce a vast quantity of protein while the drawbacks include consumer reservations and differences in taste, texture, and appearance [226].

20.2.4 Comparisons

Table 59, shows a comparison of the key strengths and challenges of building a business based on each of the 3 main alternative protein categories discussed.

Table 59: Comparison of Alternative Proteins

Alternative Protein	Advantages	Disadvantages
Plant-based meat	<ul style="list-style-type: none"> Proven and commercialised technology Acceptable to a wider consumer market Low energy and water usage 	<ul style="list-style-type: none"> Inferior taste and consumer experience Can lack the nutritional profile of animal meat
Cultivated meat	<ul style="list-style-type: none"> Similar or enhanced nutritional characteristics to animal meat Reduced risk of disease from meat consumption 	<ul style="list-style-type: none"> Possible lack of consumer acceptance to lab-grown food Extremely high production costs
Fermented proteins	<ul style="list-style-type: none"> Highly energy efficient method of protein production 	<ul style="list-style-type: none"> Requires incorporation with other protein products before sale.

21 Legislation and Regulation - Zac Smith

Whenever novel products are produced, especially those intended for human consumption, they require a strict regulatory framework to ensure their quality and safety [5]. Cultured meat is a perishable good with an intricate processing path that involves the use of living organisms (cell culture) and complicated chemicals, increasing regulatory restrictions worldwide. Cultured meat's first regulatory milestone was the approval of lab-grown chicken in Singapore at the end of 2020 [227], a move which solidified their position as a global industry leader. The United States (US) followed suit in June 2023, and subsequently, other countries are reviewing their regulatory frameworks. This section aims to investigate the different international regulatory bodies and the requirements of certain influential locations, which are crucial for the following reasons:

- **Product Launch Location** - The country and region selected for our company base and initial launch was heavily influenced by its regulatory conditions and ease of approval.
- **Product Launch Timeline** - Studying the approval process of our competitors for both the time taken and the hurdles cleared is essential for the development of our business strategy.
- **Product Labelling** - Rules on the labelling of cultured meat products vary by region and must be followed.
- **Future Expansion** - Understanding the future legislative trends of potential markets will influence our company growth and diversification.

21.1 Approved Locations

21.1.1 Singapore

On 2nd of December 2020, the Singapore Food Agency (SFA) approved Eat Just's (branded GOOD Meat™) cultured "chicken bites" after a two-year safety review through their "novel food" regulatory framework [228]. The product went on sale just 17 days later in restaurants, but is now sold through delivery apps and food stalls. They have recently permitted the sale of a cultivated quail product made by the Australian company Vow™[229], amid approval for other companies to host formal cultivated meat tastings to obtain consumer feedback [230].

Singapore's government, driven by their desire to improve national food security, has maintained their progressive reputation by matching the funding raised by several local alternative protein start-ups [231]. It is also adjusting the regulations to help the industry develop, notably by granting commercial approval for a cultivated meat manufacturing platform - Esco Aster™. This allows other companies to use their food-approved facility to manufacture their own cell-based products for market launch [232].

21.1.2 United States

The US has a more unique regulatory framework, with oversight shared between the Food and Drug Administration (FDA) and the Department of Agriculture (USDA). The FDA monitors cellular processes (cell bank, proliferation,

differentiation, etc.) and the USDA is responsible for the production and labelling stages [91]. Once the FDA's pre-market consultation has been completed by receiving a "no questions" letter, the USDA inspects the processing plant frequently before final approval is granted. This process was first completed by UPSIDE Foods™ and GOOD Meat™ in June 2023, with both American companies authorised to sell "cultivated chicken" products after a four-year wait [233]. Subsequent applications are expected to be faster [230].

Despite this regulatory success, Florida and Alabama have banned its sale [230]; Arizona, Texas, South Dakota, and Nebraska are moving to ban or moderate it [229] [233]; Tennessee and Arizona attempted but failed to ban it [234]; several additional states including Iowa, West Virginia, and South Carolina require these products to be labelled "lab-grown" or "cell-cultured" [230] [234]. These state laws are all subject to litigation and will need to be constantly monitored alongside overarching federal laws to ensure full compliance.

21.1.3 Israel

The first approval of cultured bovine meat came in early 2024, when the Israeli company Aleph Farms™ received a "no questions" letter from their Ministry of Health [229]. This was a global first for non-chicken products and allowed Aleph Farms to commercialise their cultivated steaks: Aleph Cuts [235] [233]. As the third country to authorise cultured meat, Israel is a hub for alternative proteins with more than 80 startups and considerable governmental and academic support [230]. In March 2023, the chief rabbi affirmed that cultivated steak is kosher [236], but research into the possibility of gaining Halal status is far more complex [237].

21.1.4 Hong Kong

In November 2024, Hong Kong's Centre for Food Safety (CFS) granted regulatory approval to Vow™'s cultivated quail products. Their two hybrid 'foie gras' were made available in a restaurant inside the Mandarin Oriental™ hotel, an international luxury hotel chain [229].

21.2 Possible Future Locations

21.2.1 European Union

For all member states of the EU, cultured meat falls under the Novel Foods Regulation (Reg. EU, 2015/2283) which dictates that premarket approval must be obtained from the European Food Safety Authority (EFSA). This precautionary process can take a minimum of 18 months and hopeful companies must submit detailed safety assessments with nutritional data and potential risks [238]. This regulatory framework is notoriously stringent due to its extremely high safety requirements and lack of support for applicants before or during the process. Because of this, only one company has an application under review: Gourmey™ (France, July 2024). The Cultivated B™ (Germany, September 2023) submitted and then rescinded their application [239].

To complicate matters, several EU member states are attempting to ban the sale and production of cultured meat products. The Italian government passed a controversial bill in 2023 to this effect [238], whereas France, Austria, Hungary, and Romania are all trying to pass similar bills [229] [230]. These would potentially be in violation

of the European Single Market and could be challenged by the European Commission if regulatory approval is granted by the EFSA [229]. In July 2023, the Dutch government authorised tastings of cultured meat, having partnered closely with local start-ups to help fund cellular scale-up facilities for open-access research [233], highlighting the desire within the EU for this new technology.

21.2.2 United Kingdom

No longer a member of the EU, the United Kingdom (UK) regulates cultured meat products independently through the Food Standards Agency (FSA). This framework for 'Novel Foods' was similar to that in Europe, but in March 2025, the FSA launched a new two-year research programme to accelerate the approval process of cultured meat products and implement a 'regulatory sandbox' to promote collaboration between regulators and innovators [233]. This £1.6 million investment comes amid several pending applications (Aleph Farms™ and France's Vital Meat™[230]) and the recent approval and sale of cultured meat as pet food by UK-based Meatly™ [229].

At the prestigious Fortnum and Mason store, the UK's Ivy Farm™ hosted a cultured meat tasting for members of the press in early 2024. Their scotch eggs were given free of charge as a regulation workaround, highlighting possible innovative methods to use while waiting for the minimum two-year approval window [229].

21.2.3 China

The China National Center for Food Safety Risk Assessment (CFSA) is actively formulating a new set of guidelines and regulations for the cultured meat industry [227]. It is unclear how soon this will be implemented, but a US\$300 million cultured meat trade agreement with Israel (September 2024) suggests it is close. The CFSA also conferred on regulatory approval processes with FDA experts in late 2022 [240]. Both suggest a willingness for international collaboration, in addition to recent government investments in local research efforts [229].

21.3 Conclusion

It is clear that global regulatory systems are constantly evolving and these will need to be monitored and understood throughout development of the business. This evolution makes it difficult to predict exact timelines or costs of regulatory approval, regardless of the region chosen. Compliance with regulations helps build trust and acceptance in potential consumer bases [5], and will be a key part of our business focus.

The information in this section was used to justify the United States as our starting location, due to its coherent regulatory framework, collaboration with prospective companies, and pre-approval of cultured chicken products. Careful monitoring of individual states' decisions to ban or limit cultured meat will be crucial for our success, and other suitable locations will need to be identified if the North-West states change their policies.

Considering other regions and their regulatory pathways was essential for market analysis and the development of our long-term business strategy (Section 30), helping to identify future opportunities for growth and the considerations necessary to achieve this. The UK may become an attractive location after their regulatory overhaul, and with no existing competitors, it could provide a foothold for accessing the EU markets. China would be a difficult but rewarding market to target once it clarifies its regulatory process.

22 Location Optimisation - Zaheer Sidik

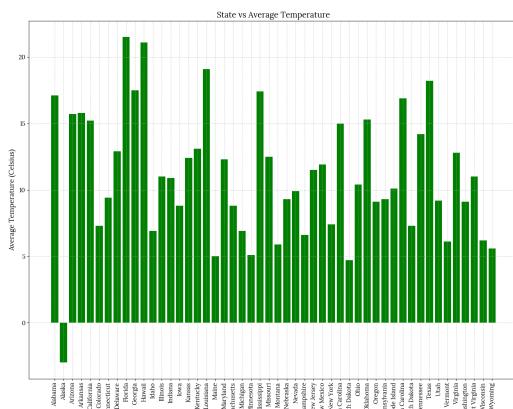
A cost optimisation was performed to determine where to establish the plant, however as we aim to initially target luxury restaurants we have also accounted for this as shown in Figure 36, furthermore as mentioned in Section 1 we aim to minimise the negative impact on the environment due to our plant, and hence we have also considered the proportion of renewable energy generation as shown in Figure 36

22.1 Regulation

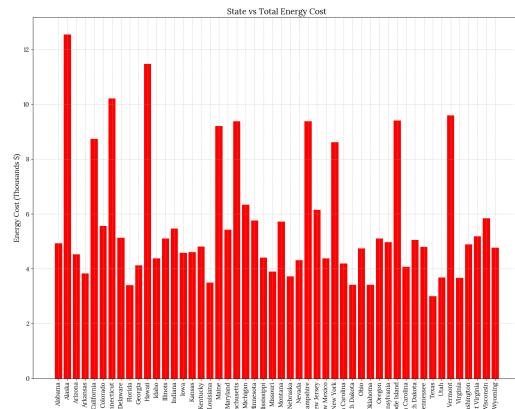
Producing and selling within the same state decreases transport costs and reduces CO₂ emissions which align with our sustainability goals, the state policies on the sale of cultured meat were taken into account to ensure that the sale of cultured meat is allowed within the state, hence states such as Florida and Alabama have been eliminated. Regulation and legislation are further discussed in Section 21.

22.2 Energy Costs

Based on our energy requirements shown in Table 50 there will be a set amount of energy per year to ensure optimal plant operation. A caveat to the total energy cost is that it depends on the temperature of the state, as our bioreactors will be in a lab that is maintained at 25C there will be an associated heating cost with the lab environment. The average annual temperature can be seen in Figure 33a, based on these values the total annual energy cost has been calculated using the cost per kWh of energy given from [113].



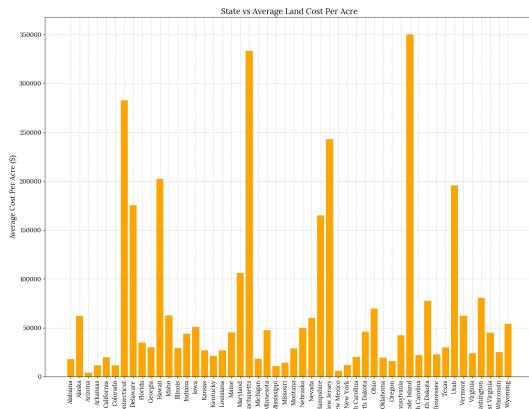
(a) Average Annual Temperatures taken from [241]



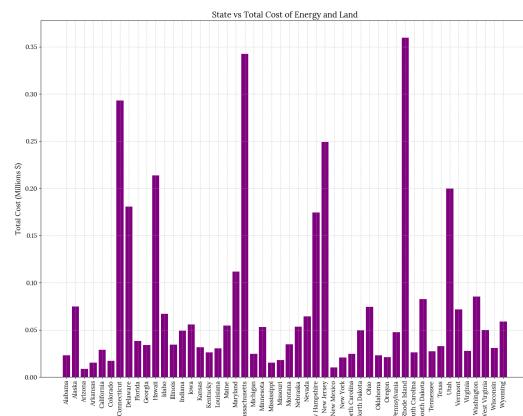
(b) Energy Costs for the Different States

22.3 Land Costs

Land costs also differ from state to state, the analysis assumes that an acre of land will be bought. On this land our warehouse, lab, car park and other facilities will be constructed, the price for an acre of land in each state can be seen in Figure 34a, the total cost for the energy and land can be seen in Figure 34b.



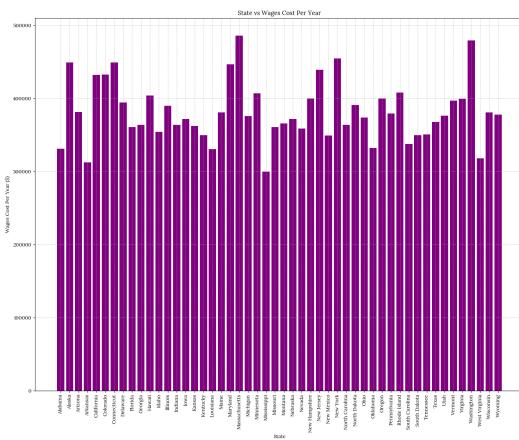
(a) Land Cost per Acre from [242]



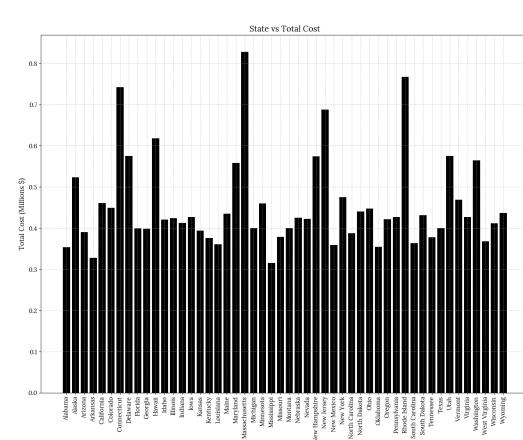
(b) Land and Annual Energy Cost for the Different States

22.4 Labour Costs

Inside our facilities workers will be required: to ensure that the bioreactors are cleaned properly and on time, deliveries are received and sent out and errors are managed effectively. This will be done by two teams of 4 individuals, each worker will work an average of 28 hours per week. As outlined in [243] and [244] it is becoming increasingly more common for companies to have periods of unmanned operation, hence we have chosen to have our plant manned for 8 hours a day with an emergency operator on standby at all times. To ensure competitive compensation the workers will receive the median wage of the state, this has been chosen to reduce the rate of employee turnover, which will reduce the frequency of having to train new employees and hence the cost associated with training will be reduced. The cost to the company for all 8 workers per year can be seen in Figure 35a. The median wages have been taken from [245].



(a) Annual Employee Costs



(b) Annual Total Location Specific Cost

22.5 Auxiliary Factors

As shown in Figure 35b, based entirely upon cost the best state is Mississippi, however there are two other factors that need to be considered, namely:

- the proportion of renewable energy generation of each state (data taken from [246])
- the number of luxury restaurants in that state (taken to be the number of michelin star restaurants in that state for this analysis, data from [247])

To find the optimal state for the pilot plant to be built a multi criteria analysis was performed with the weightings:

- Proportion of Renewable Energy Generation ($W=0.5$)
- Number of Michelin-Star Restaurants ($W=0.4$)
- Location Specific Cost ($W = 0.1$)

When compared with the costs that are fixed across the different states such as the cost of medium, bioreactors, adsorption columns etc. as mentioned in Section 15, the difference in location specific costs is almost negligible. The proportion of renewable energy generation has been given a high weighting as the core objective of our mission statement is sustainability. The number of Michelin-star restaurants is also given a high weighting as they are likely to be our early adopters and the segment we aim to service with the output of this plant.

Each criteria was scored from 0 to 5, the scores were calculated as follows: The proportion of renewable energy generation was taken to be the score out of 100. These values were then scaled from 0 to 5. The number of Michelin star was scored as, states with more than 5 restaurants receiving 5 (as with an output of 1 tonne per year we will likely only be able to service a few restaurants), those with between 0 and 5 restaurants received the number of restaurants as their score out of 5. To score the total costs, the lowest cost was taken to receive 5 out of 5, the highest cost was taken to receive 0 out of 5 and all those in between were linearly scaled, the results of this can be seen in Figure 36.

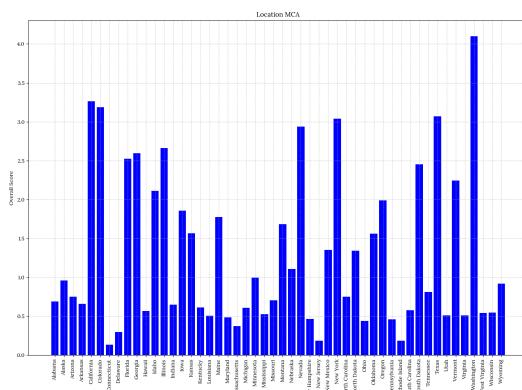


Figure 36: Location Optimisation MCA

As shown in Figure 36, **Washington** is the state the plant has been chosen to be built in, based on the location specific costs, the proportion of renewable energy generation and the number of Michelin-star restaurants.

23 TAM/SAM/SOM Analysis - Viraj Nerkar

Market analysis was performed to gauge the overall size and explore commercial potential. A TAM (Total Addressable Market) - SAM (Serviceable Addressable Market) - SOM (Serviceable Obtainable Market) was conducted to quantify the target market.

Due to the novelty of the cultured meat industry, it is difficult to accurately predict the growth rate and the size of the market in the future. Current forecasts (Section 20.1) validate the proposed numbers.

23.1 TAM

The TAM is the total market that the company could target. It is an unrealistic value, which can only be reached in principle, not in practice. However, it is a good base value to build on. For Oxfarm, this is **\$230 billion**, which represents the global chicken market size in 2024. It is expected to grow at a CAGR of 5.9% [248]. This represents the impossible scenario where the entire global chicken demand was serviced by Oxfarm only.

23.2 SAM

The SAM can be thought of as the proportion of TAM that we can logically service without taking into account competition. Oxfarm will be based in Washington (WA), US, and initially will target wealthy customers through partnerships with high-end restaurants (Section 32.1).

The US chicken market was valued at \$35 billion in 2023 [249]. Our target market is the top 5% of consumers by income in the US. The top 20% earners collectively account for a total of 38% of all consumer spending [250]. Assuming that within this range, each 5% income bracket spends 20% more than the bracket below, the share of consumer spend in the top 5% bracket comes to

$$\frac{1.2^3}{1 + 1.2 + 1.2^2 + 1.2^3} \times 0.38 = 0.122 \quad (51)$$

Taking the further assumption that the proportion of general spending can be applied to chicken consumption, the SAM is **\$4.28 billion**.

23.3 SOM

The serviceable obtainable market is the fraction of the SAM that can realistically be captured after taking into account competitor dynamics and marketing strategy. Currently, there are two significant competitors in the US, Upside Foods and Good Meat (Section 25). Both of these companies are based out of California. Therefore, it is reasonable to assume that Oxfarm (through its WA headquarters) can service all the cultured chicken meat demand to the US Pacific Northwest comprising of the states of Washington (WA), Oregon (OR), Idaho (ID) and Montana (MT) [251].

From the data in Table 60, the percentage of people living in the Pacific Northwest relative to the whole country is 4.44%. According to a study, 17% (out of 1000 people surveyed) of current meat buyers in the US would switch

Table 60: Populations of the states in the Pacific NW [252]

State	Population
Washington	7,705,281
Oregon	4,237,256
Idaho	1,839,106
Montana	1,084,225
Total	14,865,868
United States	334,914,896

to cultivated meat [253]. Assuming that income and spending distributions across the country also apply in these states, the SOM value is:

- $0.17 \times 0.044 \times \$4.28 \text{ billion} = \$32.0 \text{ million}$

23.4 Analysis

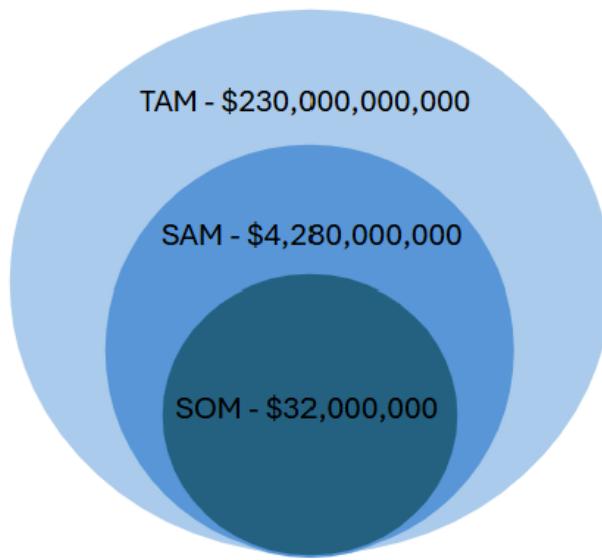


Figure 37: TAM/SAM/SOM Venn diagram

The calculated SOM value of \$32 million is not a large amount, especially compared to the current revenue (\$300 million +) of Beyond Meat in 2024 (Figure 44). This further highlights the need to develop additional revenue streams (Section 31.2) and expand to other underserved geographies (e.g. the US North East) in the future.

24 Stakeholder Analysis - Luke Nijkamp

24.1 Stakeholder Identification

A stakeholder analysis was conducted to understand the motivations, interests, and value generation potential of various stakeholders. The following stakeholder groups were identified:

- | | |
|---|---|
| 1. Governments & regulatory bodies | 6. Employees |
| 2. Early customers (of biomass or technology) | 7. Local communities |
| 3. Consumers | 8. NPOs & NGOs |
| 4. Shareholders & investors | 9. Global environment |
| 5. Suppliers | 10. Scientific bodies & research institutions |

An important distinction is made between customers - businesses purchasing the product (e.g. restaurants purchasing biomass or food brands purchasing technology), and consumers - the end users who eat the meat. Although not direct buyers, consumer preferences strongly influence customer decisions, making their satisfaction critical to driving demand.

24.1.1 Method

A stakeholder analysis template from visible.vc was used [254]. The framework included guiding questions to assess each stakeholder's interest in the business' success, their motivations, resources, and potential reasons to oppose the business.

Sources of stakeholder information included secondary research from published articles and case studies as well as primary research from our consumer survey (see Section 19.1). Our secondary research drew primarily on a stakeholder review by Amato et al. [255] and a Harvard Business School case study on Aleph Farms [212], offering insights into the perceptions of not just consumers, but also other key stakeholders, including regulators, governments, and research institutions. Team brainstorming sessions were conducted to critically evaluate potential stakeholder perspectives and their associated impacts.

24.1.2 Results

Our research revealed four common themes. First, a primary concern among consumers, customers and investors alike revolves around the perceived "non-naturalness" of cultured meat [212], along with cultural barriers to acceptance. The growing discourse around ultra-processed foods (UPFs) could further impact consumer perception of cultured meat, given its association with artificial production methods [256]. Nevertheless, amid rising food safety and security of supply concerns, especially in densely populated countries such as China, cultured meat is increasingly seen as a safer alternative to conventional meat production [255].

Secondly, the high cost of producing cultured chicken remains a major concern for customers of biomass and biotechnology, as well as for investors and suppliers [255]. In the primary research, cost also emerged as the most

significant perceived barrier among end consumers, alongside taste and texture. Despite these challenges, the industry demonstrates strong growth potential (see Section 20.1), which may motivate governments and local communities to support it for its economic benefits, particularly in job creation and regional development.

Third, stakeholders with financial stakes in the industry also highlight the lack of clear legislation as a major barrier, making it difficult to obtain approvals for novel foods, as discussed in Section 21.

Finally, themes of social and environmental responsibility also emerged. Stakeholders such as governments and NGOs are likely to express concerns about the impact on traditional farming communities [212]. Additionally, high production costs during early large-scale production may limit market growth and increase the risk of monopolisation by large companies [255]. In our primary research, potential consumers identified a reduction in carbon emissions, animal cruelty and land use as the biggest selling points of cultured meat.

24.2 Stakeholder Mapping

Stakeholders were prioritised using various tools, as illustrated in Figure 38 above.

Mendelow's matrix [257] was employed to categorise stakeholders based on their power and interest. Investors, early customers, and R&D partners, were positioned as high power and high interest due to their direct involvement and potential to shape strategic decisions. Governments and regulatory bodies were classified as high power but lower interest, as they can significantly impact operations through policy but are less involved in day-to-day developments. Local farmers, employees, and research institutions were considered highly interested but with limited power, reflecting their vested interest in the project's success (or failure) without direct control.

HIGH POWER	Keep Satisfied <ul style="list-style-type: none">GovernmentRegulatory bodies	Manage closely <ul style="list-style-type: none">InvestorsEarly CustomersTechnology/ R&D partners	Potential for Cooperation	HIGH	Supportive (Involve) <ul style="list-style-type: none">Environmental organisationsAnimal rights groupsResearch institutionsProgressive policymakers	Mixed Blessing (collaborate) <ul style="list-style-type: none">Major equipment/ ingredient suppliersInvestors with interest in traditional and alternative proteinsRegulators
LOW POWER	Monitor <ul style="list-style-type: none">Raw material suppliers, standard part suppliersLocal communities	Keep Informed <ul style="list-style-type: none">EmployeesLocal farmersAcademic bodiesConsumersEnvironmental and agricultural NPOs			Marginal (Monitor) <ul style="list-style-type: none">General consumerLocal government agenciesSmall independent restaurants/ farmers	Non-supportive (Defend) <ul style="list-style-type: none">Traditional poultry farmersAgricultural NPOsSceptical policymakersConsumer advocacy groupsReligious institutions regulating dietary laws
LOW INTEREST		HIGH INTEREST	Potential for Threat	LOW	LOW	HIGH

(a) Mendelow's Matrix, adapted from [257]

(b) Stakeholder attitudes, adapted from [258]

Figure 38: Comparison of stakeholder analysis frameworks

Stakeholders were also classified based on their expected stance toward cultured meat and their influence (Figure 38b). Environmental groups, animal rights advocates, and research institutions were considered supportive due to shared goals in sustainability and ethics. Investors and regulators posed both opportunities and risks, given their essential roles in funding and oversight. Traditional poultry farmers, agricultural NPOs, and religious institutions were seen as potential opponents, likely to resist due to cultural, economic, or regulatory concerns. General

consumers and small independent producers were viewed as having limited influence or engagement, reflecting their lower capacity to support or hinder industry development.

These matrices were used to inform our stakeholder engagement below.

24.3 Value Mapping

The Cambridge Value Mapping Tool [259] was used during the development of our business plan to evaluate how value is delivered, missed, destroyed, or could be created across all stakeholders, not just customers.

Value captured	Product environmental benefits; health benefits; reduced risk of contamination; strong industry growth potential; animal-free serum
Value destroyed	High production costs, potential displacement of traditional farming jobs; carbon dioxide emissions; technological hurdles
Value missed	Misinformed consumers; underutilised waste streams; not using plant while awaiting regulatory approval; restaurants not the only places selling expensive food
Value opportunities	Process innovation (e.g. co-culturing with algae); strategic partnerships (e.g. with suppliers during R&D); consultation from animal rights groups/ NPOs; site visits; selling lactate and ammonia waste products; hiring people from agriculture industry

Table 61: Summary of Value Dimensions

This group exercise revealed that while our cultured meat product captures key benefits such as reduced environmental impact and animal welfare improvements, there are still aspects with negative implications. As illustrated in Section 17.1, our decision to use Washington's grid supply over a pure renewable energy source results in comparatively high emissions, which offset some of the aforementioned environmental benefits. Furthermore, the high production costs limit our meat to only the wealthiest consumers, reducing market size and inclusivity.

The tool also identified areas of missed value. These included consumer reluctance driven by misconceptions about cultured meat, and unmonetised opportunities such as valuable waste streams and underutilised infrastructure during regulatory approval phases.

These insights informed opportunities for value creation. For instance, while cultured meat may displace traditional farming jobs (as noted under value destroyed), it could simultaneously enable local employment through new roles in biomanufacturing and supply chain support. As proposed by Didier Touba of Aleph Farms, this opens up opportunities for a just transition, whereby displaced agricultural workers are integrated into the cultured meat industry through targeted reskilling [212]. Another similar opportunity involves hosting site visits to production facilities, growing brand awareness, whilst also educating consumers and dispelling misconceptions.

24.4 Stakeholder Engagement

Collaboration, early engagement, and transparency should be central to our stakeholder management.

First, it is essential to engage closely with the most influential stakeholders (those in the top row of Figure 38b). This includes forming strong partnerships with suppliers to ensure strategic alignment, and working closely with regulators to streamline the approval process.

Moreover, early engagement with potentially disruptive stakeholders, particularly those in the bottom right

quadrant of Figure 38b is crucial. Involving them at this stage can provide valuable insights to inform development and help prevent resistance or conflict later on.

Finally, the business should focus on transparency through targeted communication, particularly for stakeholders in the 'keep informed' and 'non-supportive' categories outlined in Figure 38. This can be fostered through initiatives such as site visits and comprehensive reporting, which help build trust among the general public and local communities.

25 Competitor Analysis - Viraj Nerkar

When establishing a business venture, it is vital to analyse and understand the competition in the market- both direct and indirect. As cultivated meat is still a nascent technology, there are very few direct competitors (firms which sell a similar product or service and have the same ideal customer profile). On the other hand, all traditional meat sellers such as farms and butchers along with firms using other alternative protein technologies (plant-based and fermented) can be considered as indirect competitors.

25.1 Upside Foods

UPSIDE Foods™ is a cultivated meat start-up headquartered in Berkeley, CA. It was founded by Uma Valeti, Nicholas Genovese, and Will Clem under the name Memphis Meats in 2015. In April 2022, the company had annual revenues of \$137.4 million [260].

25.1.1 Business Model

The firm's product line-up includes chicken sandwich, sausages, and potstickers. It has also developed beef meatballs and duck with the aim of eventually offering every major meat in its product range. Currently, Upside Foods operates a B2B sales strategy, partnering with upscale chefs and restaurants in the US [261].

This allows it to command a premium, thus compensating for the high production costs associated with cultured meat. It has plans to develop B2C by selling through supermarkets and on its website. It has also acquired Cultured Decadence- a cultured seafood company in 2022 to penetrate the seafood market.

25.1.2 Investments

Upside has gone through 10 funding rounds, from an Incubator round in 2015 to Late Stage/ Series C round in 2022/23. It is one of only two cultured meat companies to achieve "Unicorn" status (valuation of over \$1 billion). It is backed by some of the most powerful venture investors such as Abu Dhabi Growth Fund, SoftBank Vision and Temasek Holdings [260].



Figure 39: UPSIDE Foods

25.1.3 Timeline

Figure 40 shows the key milestones in Upside Foods' journey.

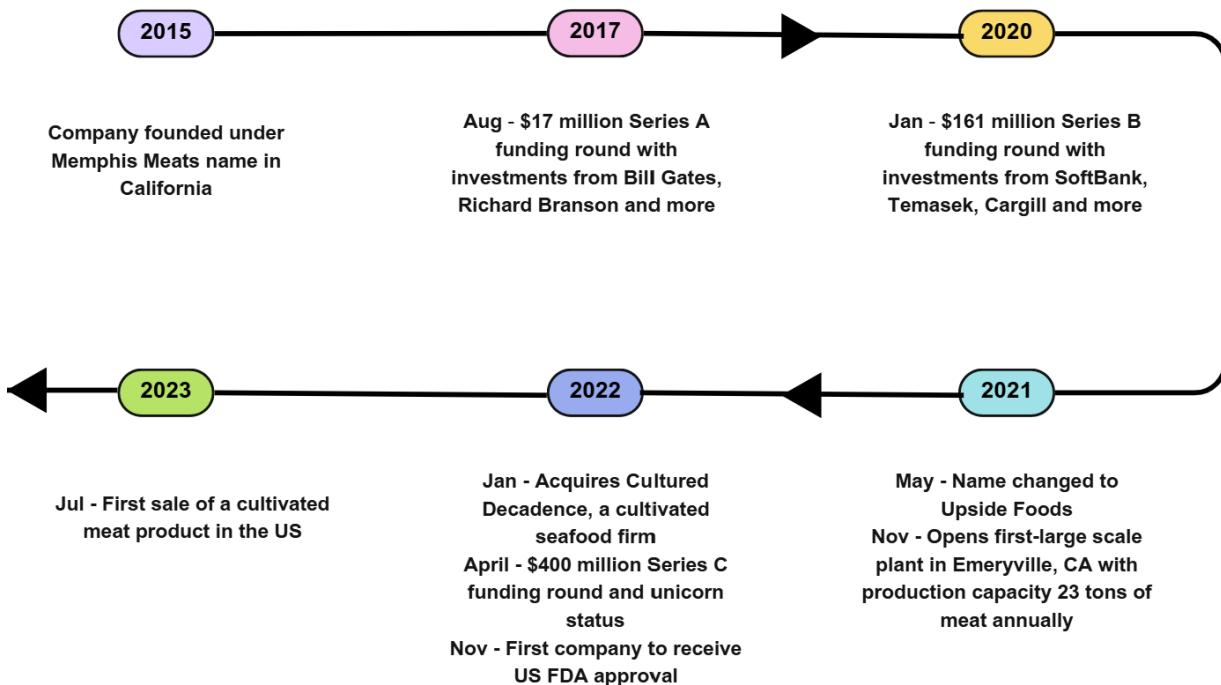


Figure 40: Upside Foods timeline (Made with Canva) [261]

25.2 Eat Just

Eat Just Inc.TM is an alternative protein company with headquarters in Alameda, CA. The parent company was founded in 2011 by Josh Tetrick and Josh Balk in Alameda, CA. Since then, it has achieved Unicorn status in 2016. The JUST EggTM brand focuses on plant-based alternatives to chicken eggs using mung beans. GOOD MeatTM is a separate division that produces cultivated meat.

25.2.1 Business Model

Eat Just's plant-based meat and cultivated meat brands operate separately. Just Mayo (plant-based mayonnaise) was its first product, launching in 2013 in the US retail [262]. Just Egg (its most famous product) launched in 2018, also in the US, followed by Good Meat, which gained regulatory approval from Singapore for its cultured chicken product in late 2020.

Eat Just's business model has a clear focus on diversification. The distinct nature of its product lines,



Figure 41: GOOD Meat

both plant-based and cultivated, has allowed it to hedge its bets. The company had an annual revenue of \$85.6 billion in 2023 [263]. The vast majority of this comes from its plant-based products. Good Meat currently only sells at a single retailer, Huber's Butchers in Singapore. It has also partnered with Jose Andres, a world-renowned chef, and his Washington DC-based restaurant, China Chilcanos. However, as of March 2025, tastings of cultivated chicken are paused at the venue, and therefore Good Meat does not sell any product in the US [264].

The firm, in general, is not profitable; however, its more mature plant-based meats are close to breaking even [265]. Good Meat is much further away from commercial success, with profitability not in sight for many years. The firm plans to release a cheaper product called Good Meat3 which will have a much smaller percentage of meat in the final product. The additional ingredients will consist of wheat and soy.

25.2.2 Investments

Eat Just has undergone several funding rounds, the latest of which took place in 2023. It has raised a cumulative total of \$465 million. It is supported by many prominent investors, including the Qatar Investment Authority [266]. Good Meat has separately raised \$267 million from 3 Series D rounds with notable investments coming from UBS Asset Management [267].

25.2.3 Timeline

Figure 42 shows the key milestones in Eat Just's journey.

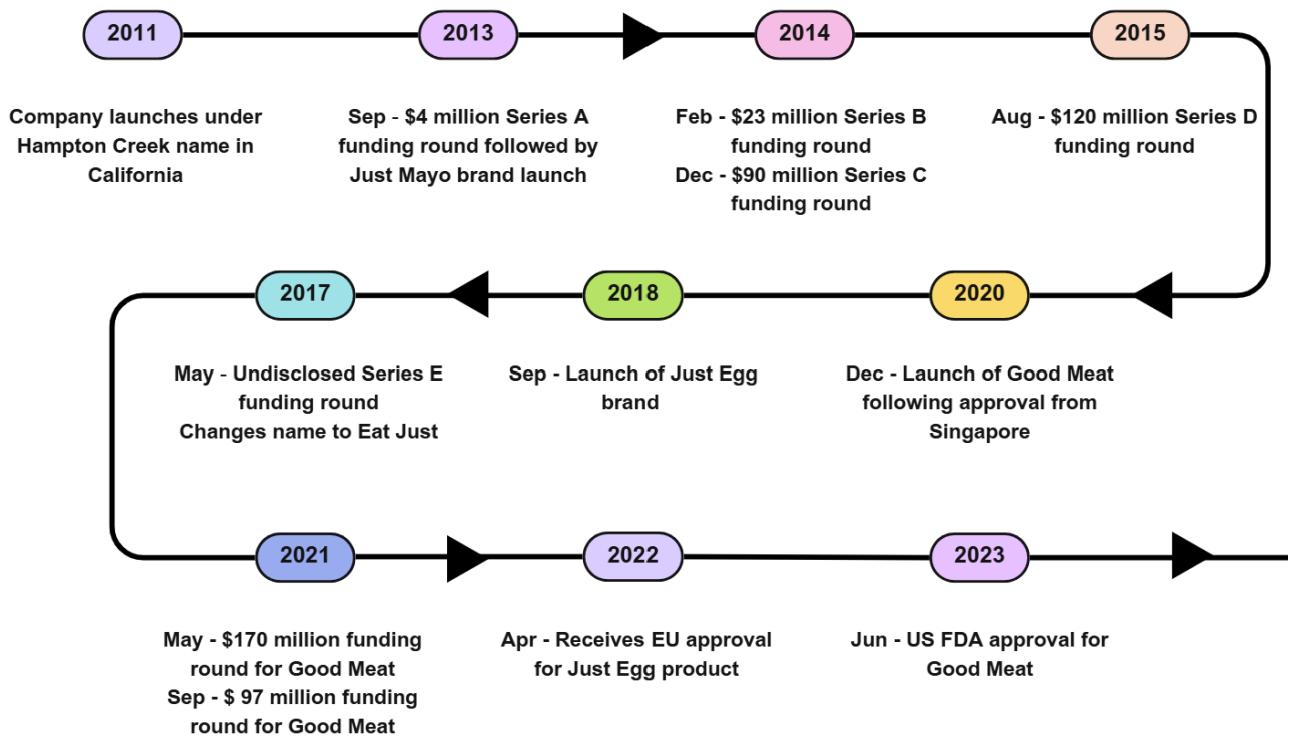


Figure 42: Eat Just timeline (Made with Canva) [262][266]

25.3 Beyond Meat

Beyond Meat™ was a pioneer in the plant-based meat industry. It was founded in 2009 by Ethan Brown in El Segundo, CA. It is most well known for its Beyond Burger product, made from beetroot extract. Its product line-up also includes chicken strips, sausages, meatballs and jerky. Beyond Meat was the first alternative protein company to be listed publicly. It IPO-ed in May 2019 (NASDAQ: BYND) at a valuation of \$3.9 billion. As of March 2025, it has a market capitalization of \$232 million.

25.3.1 Early Success

Beyond Meat built on the traditional veggie burger which was targeted at the vegetarian/vegan market. Its aim was to capture a slice of the much larger beef burger market pie. The main part of its early success came from branding and strategic partnerships.



Figure 43: Beyond Meat

Traditional meat had a stereotypical association with strength, power and masculinity, while a plant-based diet did not. To expand its customer base and resonate with the broader population, Beyond Meat used athlete endorsements through NBA players such as Chris Paul and Kyrie Irving, who were also investors in the company. This partnership allowed it to show that plant-based protein is good enough to fuel elite athletes [268].

Furthermore, Beyond Burger's nutritional value was supported by many doctors and nutritionists. Early adoption by the fast-food and quick-service industry allowed many customers to try the product. Investor bullishness was based on the hope that plant-based meat can make similar inroads into traditional meat as plant-based beverages (e.g. oat milk, almond milk, etc.) made into the cow's milk market [269]. Perhaps its greatest branding success came when Whole Foods, one of the largest grocery chains in the US, agreed to place its products in the meat aisle, indicating the success of its marketing strategy [268].

25.3.2 Recent Headwinds

Following a successful IPO in 2019, Beyond Meat's valuation has since crashed by 98% from its maximum value of \$14.8 billion in July 2019. Its stock price has plummeted to less than \$3 a share, while its revenue (Figure 44) has declined YOY (year-over-year) since 2021. This change in fortune can be attributed to 3 primary factors.

Consumer behaviour is primarily determined by cost. As much as Beyond Meat succeeded in branding, they failed to reduce the unit cost to be comparable to animal meat. Furthermore, macroeconomic factors such as the Ukraine war-induced recession reduced the appetite of consumers to spend extra on a more sustainable option [271].

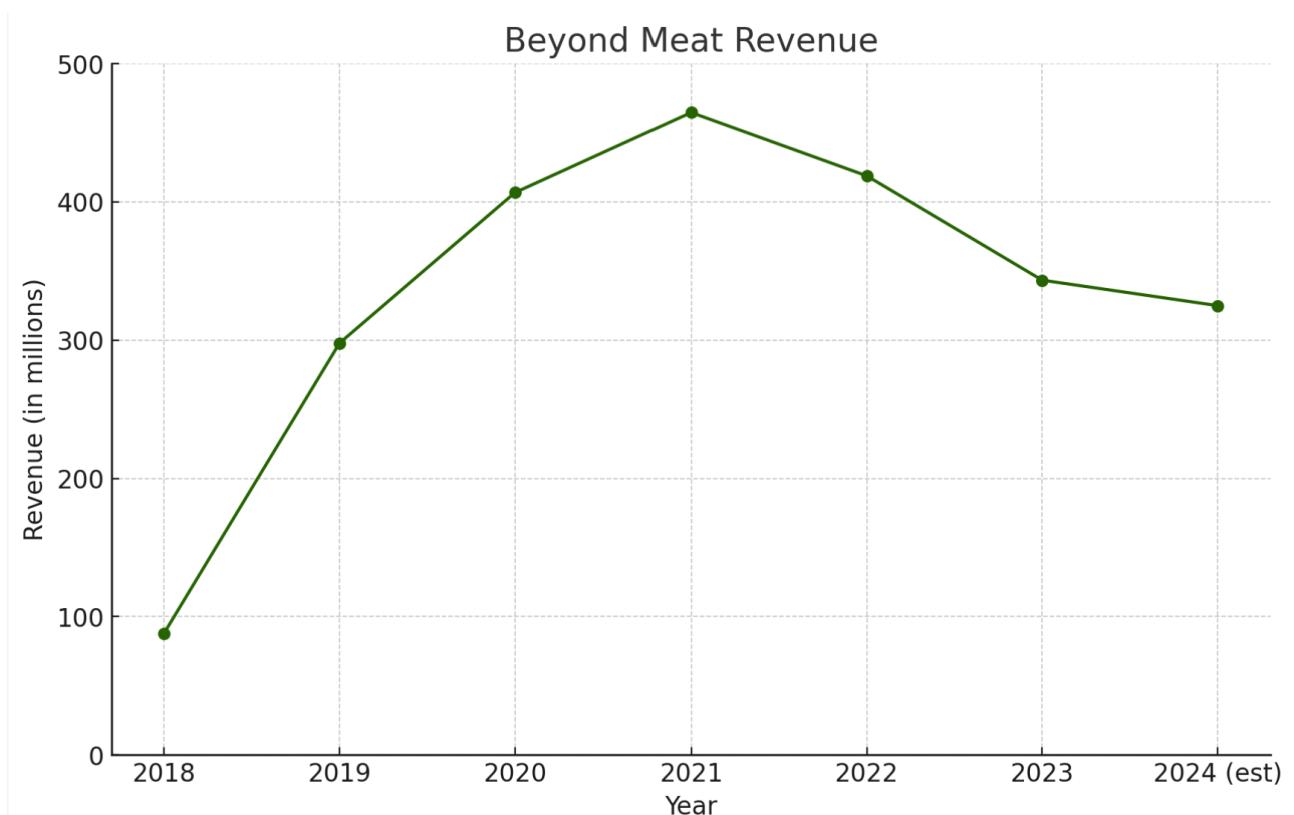


Figure 44: Beyond Meat Revenues [270]

Although its initial hype and IPO success led many to try out their product, taste was a clear factor in poor customer retention [272]. Beyond Meat did not deliver the taste or satisfaction required to lure consumers away from traditional meat.

To protect their market share, the animal meat industry targeted the nutritional value of plant-based meat. Although the word "plant" is used in its description, the product is heavily pumped with salt, sugar, and industrial chemicals (binding agents) to make it taste and feel like meat. The lack of a "natural feel" caused significant damage to the alternative protein industry at a time when consumers are clamouring for organic food options and a simpler ingredient list [272].

25.4 Summary and Key Takeaways

Beyond Meat's advertising campaign displays a roadmap of how to overcome certain customer concerns. A successful sales and marketing strategy is essential for the growth of a start-up. Upside Food's ability to pivot to the seafood market through the acquisition of Culture Decadence has put it in a strong position to capture the US cultivated meat market. Furthermore, Eat Just's agile business model of accessing both plant-based meat and cultivated meat markets has lowered its exposure to significant obstacles in any particular market. Oxfarm must show marketing prowess, create an agile business model, and possess the willingness to pivot to other revenue streams to succeed in a promising but challenging industry.

These companies have received hundreds of millions of dollars in venture investments by venture capital firms, angel investors, established corporations (e.g. Cargill) and sovereign wealth funds. Due to the capital-intensive

nature of the industry, a lot of money will need to be raised to facilitate capital expenditure in building the production facilities. Obtaining interest from investors in a similar business model to the competition will be tough for multiple reasons. Many of the target investors have already committed to one of the other alternative protein firms and might be hesitant to support a competitor. In addition, based on the financial trajectory of Beyond Meat, investor sentiment in this industry is likely to have waned.

Therefore, competing head-on with firms that have had a 5-10 year head start in a slow-moving field and without substantial investor support could be a recipe for failure. Hence, it is essential to consider a business plan that can navigate these challenges. Potential solutions to be explored include a B2B operation where the customer is another food company looking to expand into cultivated meat (e.g. Bird's Eye™) 31.2 and exploring ancillary revenue streams by monetizing the cell medium and any other proprietary technology formulated in-house 31.4.

26 Historic Development Timeline - Zac Smith

The concept of producing meat for human consumption by cultivating cells 'in vitro' was proposed as early as the 1930s by Winston Churchill [227]. Today, technological and regulatory advances made in the last century have created a business opportunity for cultured meat [7]. The timeline below (Figure 45) shows its most important milestones and is useful for analysing past trends and possible future outcomes.

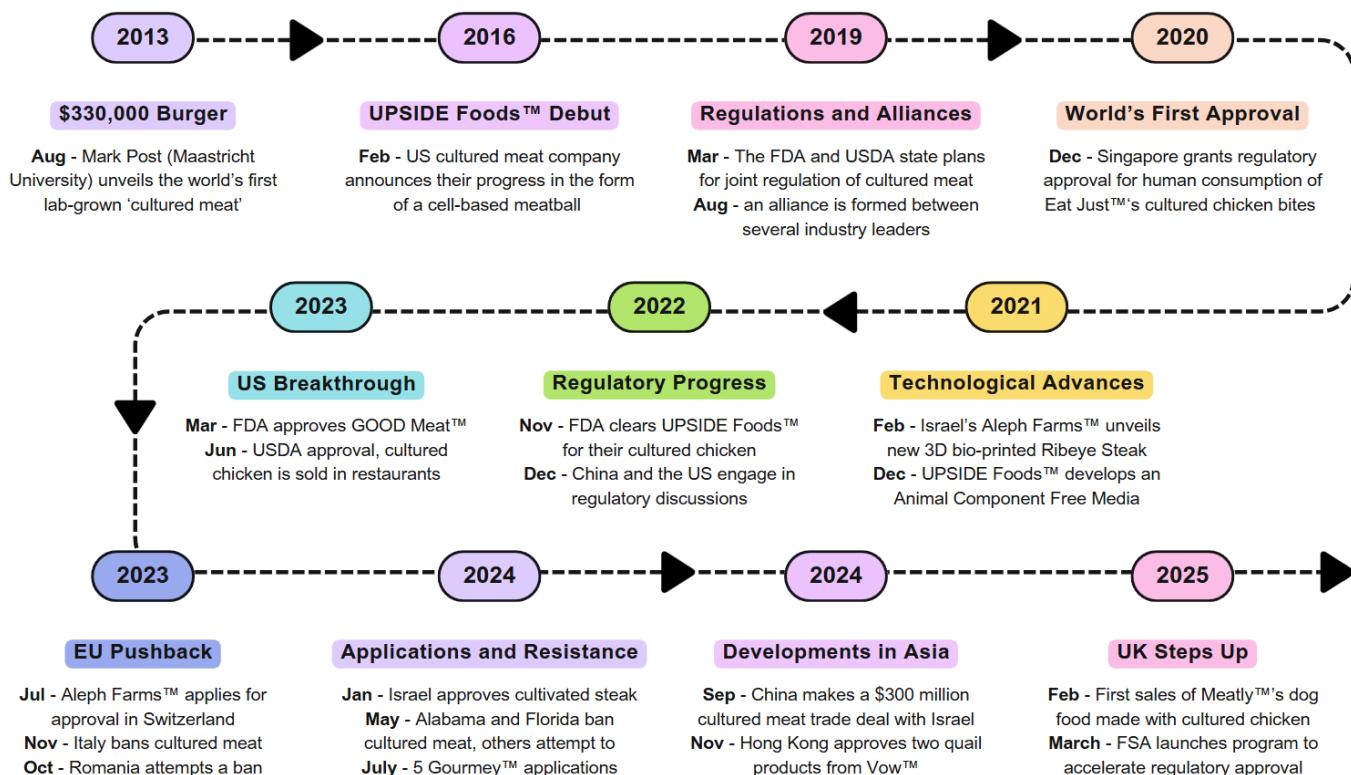


Figure 45: Timeline of the Development of the Cultured Meat Technology and Industry (Made with Canva)

Figure 45 starts with the first example of cultured meat, where Mark Post and Mosa Meat™ unveiled a commercially unviable hamburger patty[31]. Three years later, UPSIDE Foods™ (formerly Memphis Meat) presented a lab-grown meatball for only \$1,000 [273]. The alliance formed between UPSIDE Foods and other industry leaders

aimed to apply pressure to regulatory bodies [273]. The first sale of cultured meat in a Singaporean restaurant was a major breakthrough in 2020 [228], and several technological improvements followed a year later [273].

All regulatory milestones are covered in Section 21: China's regulatory discussions, landmark US approval in June 2023, pushback from Europe and America, approval in Israel and Hong Kong (2024), and development of UK regulations. The application from Aleph Farms™ in Switzerland was Europe's first [235], and France's Gourmey™ swiftly followed by applying for regulatory approval in Switzerland, Singapore, and the UK, US and EU [239].

27 Marketing Strategy - Zaheer Sidik

If we pursued a B2C model we would need to consider who we aim to target with our marketing before designing our adverts, this follows from Section 19, as mentioned in [212], the early adopters would be luxury restaurants and hence novel foodies and the early majority would be younger consumers in the age group 18-35, with a keen interest in environmental preservation. From a business standpoint this would be ideal, as younger consumers make up the next generation and are likely to have a longer consumer lifetime. Hence we would aim to capture a significant portion of that market. In Section 19.3, environmentally conscious individuals are mentioned as a market we would also intend to capture. Based on behavioural patterns we can predict that they would be likely to recommend cultured meat to their family and friends.

This section attempts to compare the marketing and distribution of both the B2C model and B2B model proposed in Section 30 to better understand the requirements associated with each of these models.

As mentioned in Section 19.5, a B2C model would require educating the public as the current public perceptions of cultured meat include:

- Uncertainty about the taste and quality of the product
- Uncertainty about the health benefits and safety of the product
- Neophobia (Uncertainty about trying new food and changing the norm)

Furthermore, the public is also ill-informed about the environmental impact of traditional farming methods, hence a well structured marketing campaign would have to focus on each of these points and convey the message to the target audience in a clear and simple manner.

27.1 Marketing Channels

27.1.1 B2C Model

To address the public misconceptions about cultured meat and to target the desired market segments the campaigns would involve:

- Public taste tests of cultured chicken, videos would then be recorded of these interviews and uploaded to social media such as TikTok and Instagram

- Celebrity endorsements as mentioned in [212], we would most likely aim to strike partnerships with celebrities with large followings and interest in the food industry as well as social media influencers that specialise in food content
- Tours of the cultured meat plant offered to the public
- Partnerships with high end restaurants to offer exclusive menu items
- Advertisements highlighting the environmental benefits of the product in a clear manner e.g. billboards, posters and commercials

As mentioned in Section 21.2.2, Fortnum and Mason advertised the product of Ivy Farms through offering free taste tests as the product is not yet able to be sold commercially in the UK. This workaround would allow us to build a positive brand reputation in countries before legislation allows us to sell there, potentially allowing us to capture portion of the market early. Through the variety of channels and methods we would aim to reduce the neophobia associated with cultured chicken and help the public gain an understanding of the environmental impact of traditional farming methods.

27.1.2 B2B Model

In the B2B model the primary driver for other companies to adopt our technology would be the proof-of-concept. Hence our marketing channel would be proving to companies that our plant is a viable solution and that consumers want cultured chicken, this would be done by using the output of our 1 tpa plant to service high-end restaurants in Washington state.

27.2 Distribution Channels

27.2.1 B2C Model

Assuming that the price eventually becomes competitive with traditional chicken we would intend to roll out to supermarkets and restaurants. When this occurs it would be imperative that our product is seen as a direct substitute to traditional chicken and hence we would request that it is placed in the same sections as traditional chicken, rather than with plant based alternatives. When rolling out to restaurants an optimistic timeline would be as follow:

- Exclusive cultured chicken product launch e.g. Mc75 (75% less impact on the environment)
- Gradual phasing of existing menu to use cultured chicken rather than traditional chicken (assuming widespread demand of initial product)
- No more traditionally farmed offer by restaurant (very optimistic)

27.2.2 B2B Model

A B2B model would involve advertising on our website for a tailored solution and reaching out to big brands/restaurants to generate interest/awareness about our value proposition. This would then be followed up by consultations and meetings with members of the company to ensure their desires/needs are met. An optimistic timeline is as follows:

- Company executive reaches out for a consultation through website portal
- Meetings to discuss plant output and location
- Detailed plant design
- Orders for different components sent to suppliers
- Construction and commissioning of plant outsourced to an engineering company

Depending on the client we would also propose media formulation/sourcing and yearly maintenance.

27.3 Conclusion

As outlined above the B2C model would be more costly for us as a fledgling company, with large social media campaigns and public education being necessary to attract customers/consumers, whereas the B2B model allows for us to pass on the costs of marketing, social media and brand reputation onto the firm we will be supplying. This, in conjunction with the conclusion of Section 19 means that we will primarily focus on the B2B with the pilot plants whilst still servicing a high-end restaurant to show proof-of-concept to businesses looking to expand into the cultured meat sector as outlined in Section 31.

28 Supply Chain Strategy - Zaheer Sidik

This section aims to look into the different types of uncertainties and quantify the risks and costs faced by Oxfarm in pursuing the B2B or B2C ventures. Through the exploration of both strategies we can better inform our choice of business strategy as outlined in Section 30. If we pursue a B2C model as our company grows and develops multiple product lines we would expect the supply chain to become increasingly complex, hence supplier and retailer relationships require due consideration. We would expect our supply chains/networks to be focused on efficiency as we would intend to run each of our plants at maximum output all of the time.

28.1 Supplier Uncertainty

28.1.1 B2C Model

As shown in Table 62 delayed or poor quality components from suppliers would be detrimental to current and/or future batches. It would also result in periods of plant activity which would lead to lost revenue. Hence, as a business we would tend to stay away from the JIT manufacturing methods of Toyota and hold as much of any given component as possible based on shelf life and process requirements. This would lead to increased storage

Table 62: B2C Supplier Uncertainty

Risk	Impact	Probability	Cost
Supplier delay	Production halted	Medium	Medium
Quality Variation	Batch fails inspection	Low	Medium
Increased Price	Either decreases profit margin or increases cost to consumer	Medium	Low

costs, which would be trivial compared to the cost associated with losing a batch. However the upside is that the plant scheduling shown in Figure 4 would be likely to be followed at all times and the proportion of wasted batches and downtime is minimised when compared to other supply chain methodologies.

28.1.2 B2B Model

Table 63: B2B Supplier Uncertainty

Risk	Impact	Probability	Cost
Supplier delay	Plant Assembly Paused	Medium	High
Quality Variation	Complaint from business and negative impact on brand reputation	Medium	Medium
Increased Price	Either decreases profit margin or increases cost to business	Medium	Low

If selling the plants as a solution as mentioned in Section 30, we would not intend to hold any physical inventory. The plants would be specifically customised to the users needs and hence production and assembly would begin as soon as demand is received (pull-based supply chain). This means that we would rely heavily on our suppliers being able to provide the products ordered quickly and to a consistently high quality (any issues in the components reflects badly on our company) , we would attempt to ensure this by prioritising our relationships with our suppliers e.g. exclusive bioreactors from ThermoFisher. As they play a key role in the success of Oxfarm.

28.2 Manufacturing Uncertainty

28.2.1 B2C Model

Table 64: B2C Manufacturing Uncertainty

Risk	Impact	Probability	Cost
Component Failure	Production Halted	Low	High
Process/Technological Innovation	Plant/process becomes outdated	Medium	High

Table 64 shows that in the case of technological innovation that allows for cheaper or quicker manufacturing of the cultured chicken product our plant/process would become obsolete very quickly. Hence to mitigate this risk we would ensure that we are up to date with the innovations within the field, by prioritising R&D within our company as we would hope to be pioneer process innovation in this field. Ultimately these changes will affect all companies within the field, so if we can be the ones to find the innovation we will gain a head start on our competitors. Component degradation is a part of any manufacturing process and to reduce the effects we would ensure regular maintenance and inspection of components which ties in with the safety and risk of the plant mentioned in Section 16. Supplier relations are also crucial when dealing with component failure/degradation as we would look to receive replacements as soon as possible.

28.2.2 B2B Model

Table 65: B2B Manufacturing Uncertainty

Risk	Impact	Probability	Cost
Process/technological Innovation	Plant/process becomes outdated	Medium	Medium

A key benefit of selling the plant as a solution to other companies is that we wouldn't face manufacturing uncertainty (in producing cultured meat), as we would only be in charge of plant design and assembly. However in the case of technological innovation we would have to update our process/plant design to ensure that it remains competitive, this would lead to different construction/manufacturing processes of the plant and hence an inherent uncertainty as staff/contractors would have to be trained in a new method of construction, this could potentially increase assembly costs and time.

28.3 Demand Uncertainty

28.3.1 B2C Model

Table 66: B2C Demand Uncertainty

Risk	Impact	Probability	Cost
Changing consumer demand	Difficult to predict and adapt production	Low	Medium
Different Product Lines	Difficult to predict demand and effect on other product lines	Low	Low

As the pilot plant only produces 1 tonne per year of product we would expect that insufficient demand will not be an issue at this stage. However, as we design new product lines we would expect that demand for a new product may result in a change for the demand of a pre-existing product as mentioned in Section 66. To cope with the inevitable changing of consumer preferences, excess supply would be used in celebrity/influencer promotions, as mentioned in Section 27 (we would send our product to them for free in exchange for them reviewing it on their platform). Furthermore, to improve brand image we would also donate to homeless shelters and food banks.

28.3.2 B2B Model

Table 67: B2B Demand Uncertainty

Risk	Impact	Probability	Cost
Changing business needs	Redesign production process	Medium	Medium
Demand fluctuation	Periods of high strain on supply chain	Medium	High

We can expect demand for the B2B solution to vary greatly in plant type as we can expect different companies to have different final product specifications, as well as that we can expect surges in demand e.g. McDonald's suddenly wants 50 plants along the West Coast. In periods of high demand we would rely heavily on our suppliers to provide components quickly and to a high quality. In contrast to the B2C model, in periods of low demand we will not lose money on unsold products as we will hold no inventory (pull-based supply chain); assembly/production would only begin once an order has been placed.

28.4 Conclusion

As outlined above and in Sections 19, 27, 30 it is economically more favourable to pursue a B2B venture as oppose to the B2C model, due to lower levels of uncertainty and risk. The strategy for this can be seen in Section 31 and the finances supporting this conclusion can be seen in Section 32.

29 Porter's Five Forces - Viraj Nerkar

Porter's five forces is a model for understanding the strengths and weaknesses of a company. It was proposed by Michael E. Porter of Harvard University [274]. The five forces are competitive rivalry, threat of new entrants, threat of substitutes, buyer power, and supplier power. A score (out of 5 with a higher score indicating a higher threat) is assigned to each force to quantify its impact on Oxfarm.



Figure 46: Porter's Five Forces [275]

29.1 Competitive Rivalry

This force aims to assess current competition in the industry. From Section 25, there are two main competitors based in the US, Upside Foods and Good Meat. Both have a similar product offering through partnerships with high-end restaurants. The key distinction is geography. Good Meat currently sells through a restaurant in Washington DC and neither firm has a presence in the Pacific NW. Due to the vast size of the US and the distribution challenges of transporting food across long distances, this presents a potential gap in the market. **Score: 4/5**

29.2 Threat of New Entrants

It is important to investigate the difficulty of entering an industry for a new rival as it can affect market share. The harder it is to enter, the more secure your position is. Cultivated meat is an emerging industry. It requires a significant initial investment in equipment, technology, and research. This creates a hurdle which is likely to be too

big to overcome for smaller players focusing on a niche who do not have institutional investor support. However, established companies in the food industry could expand their product offerings to include cultivated meat. These firms will have the resources, strategic partnerships, brand awareness, and a pre-existing customer base to achieve economies of scale. **Score: 2/5**

29.3 Threat of Substitution

The threat of substitutes is defined as the (potential) availability of similar goods or services that a customer can purchase instead [276]. The lack of substitutes gives businesses significant leverage in pricing dynamics. Cultivated meat itself is a substitute for animal and plant-based meat. It combines the taste and nutritional value of traditional meat with the sustainability and animal-friendly benefits offered by plant-based meats. On the other hand, newer technology such as fermented protein, can disrupt the growth of cultivated meat in the future, although it is still in an early development stage. **Score: 3/5**

29.4 Buyer Power

Buyer power is concerned about the ability of consumers to drive down prices. Strong buyer power can be the result of the ease of switching to alternative products and price-sensitive buyers [277]. An example of strong buyer power is the airline industry. It, famously, has laser-thin margins driven by price-sensitive customers and next-to-no cost of switching to a competitor. Neither of these criteria apply to us (customers are wealthy and are looking for a premium and novel experience vs value for money, and due to geographical reasons they cannot switch to competitors). However, cultivated chicken will initially be presented as a luxury rather than a necessity, thus reducing leverage over pricing, and high-end restaurants have the ability to choose an alternative cultivated meat provider instead. **Score: 3/5**

29.5 Supplier Power

Supplier power looks at the supplier's ability to increase costs or reduce component quality. Over reliance on a specific supplier (often caused by a lack of feasible alternatives) can be a point of weakness for a business. Initial acquisition costs regarding equipment are one-off and therefore can be excluded. Sources of variable cost (e.g. medium components, oxygen supply, etc.) all have numerous alternative suppliers. **Score: 1/5**

29.6 Analysis

Porter's five forces provides a useful template in examining the business plan in the context of the overall industry. A total score of **13/25** highlights the challenges of operating in this market. Points of strength are geographic location that does not directly compete with incumbents (although the Pacific NW is a much smaller and less affluent market than California or the North East) and the lack of reliance on specific suppliers. The areas of concern include the decade-long lead possessed by the current competition, the possibility of powerful players entering an emerging field, and technological developments in adjacent products (e.g. fermented meat).

30 Business Strategy - Zac Smith

The business strategy defines the guiding principles of the company to influence its priorities and actions. It ensures that maximum value can be created and that the business has a clear long-term vision and identity [278]. This enables resources to be allocated and used effectively while opportunities and threats can be identified and managed.

30.1 Identity

The business identity is crucial to defining the success of our company. It allows all stakeholders, including investors and future employees, to understand our motivation. Our company vision is the reference point for how each decision should be made. Our vision is as follows:

**Reduce the ethical concerns and environmental impacts associated with intensive animal farming by
delivering high-quality, safe, and healthy cultured meat.**

Our identity is maintained through a set of core company values that dictate how we want to conduct business:

- **Transparency** - We will endeavour to be open and honest with customers, collaborators, and regulators about all aspects of our business to help build trust in our products and our company.
- **Care** - Every action will prioritise sustainability, animal welfare, and people. We will strengthen and nurture all of our internal communities and external business relationships.
- **Innovation** - Continuously improve our technological capabilities, encourage creative ideas, and support each other in new endeavours as we strive to improve our product and our company.

30.2 SWOT Analysis

The first tool used in our strategic planning was a SWOT analysis. This was performed to evaluate our current (and future) competitive position by investigating four key performance indicators: our internal strengths and weaknesses, plus external opportunities and threats. The SWOT analysis illustrated in Figure 47 is wide-ranging to provide a complete and thorough overview of the business.

Several strengths come from our detailed engineering work that focused on environmental and ethical practices. The multiple revenue streams and pivot options are detailed in Section 31, while the existing valuable relationships include Thermo Fisher and The Cultivated B (biotech suppliers we have liaised with), and Oxford University itself. Most of the weaknesses are due to our lack of physical work and the absence of a Minimum Viable Product (MVP).

Some opportunities come from the increase in demand for ethical/sustainable meat products and the growing interest in this sector [21]. The abundance of possible markets and government aids (Section 21) plus collaborators such as universities, investors, and NPOs (Section 24.2) all present several opportunities for growth.

However, there are several external threats. Established brands like UPSIDE Foods and GOOD Meats are ahead in both time (regulatory approval, plant size), financing, and brand perception, so they could monopolise the market

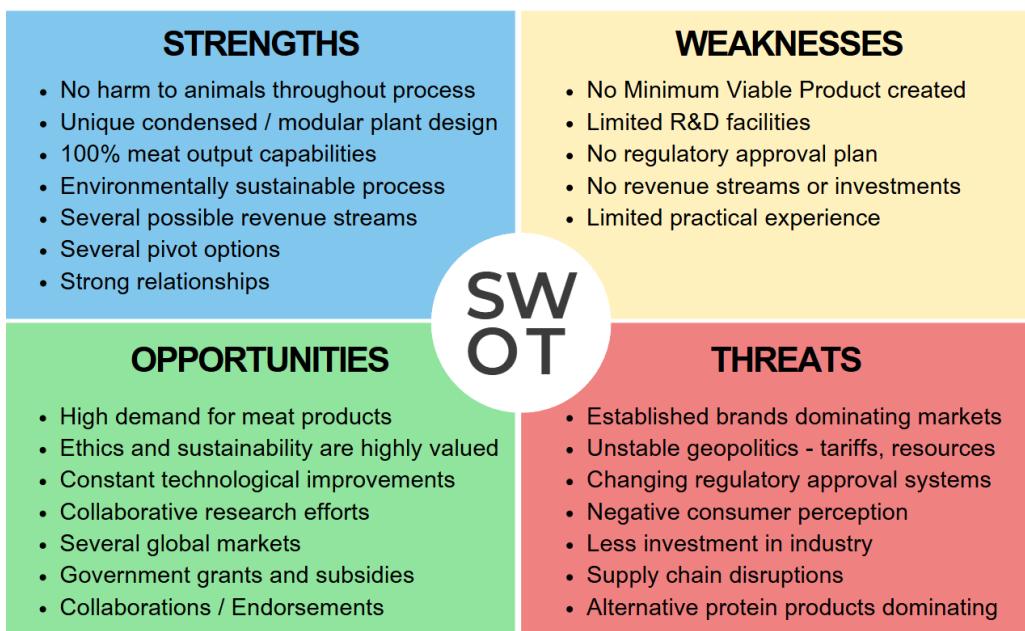


Figure 47: Current SWOT Analysis (Made with Canva)

before we can launch. Unpredictability in global politics, regulations, supply chains, and competing products will all be difficult to navigate. Consumer perception could be irreversibly damaged by industry scandals or poor media coverage. Finally, there has been a decline in investment in the industry (Section 25.4) which was highlighted in the Q&A with The Cultivated B (Section 31.1).

30.3 VRIO Analysis

The VRIO framework (developed by James Barney [279]) is used to analyse whether an internal resource or capability is valuable, rare, costly to imitate, and if the business is organised to capture the value. A VRIO analysis was performed for the meat product detailed in the engineering section and then for the plant design itself.

30.3.1 Cultured Chicken Nugget

Valuable: A cultured chicken product is of considerable value for several reasons. It is more ethical and environmentally friendly than traditional (intensively farmed) meat, which benefits the planet but may also open the market to vegetarians/vegans who oppose meat for those two reasons. It is not considered a meat substitute like most alternative proteins (Section 20.2), as it can mimic the taste and texture of traditional meat more closely. Currently, the estimated extreme cost of production (Section 15.4) means that this would not yet be a valuable product as it is not commercially viable. This cost of production is expected to fall (Section 31.3).

Rare: Cultured meat is a developing technology with extreme barriers to entry. Despite the increasing number of start-ups in this industry, only a handful of companies are currently producing cultured meat at scale. Even fewer are authorised to access any markets (Section 21.1). The arduous process of gaining regulatory approval anywhere in the world currently keeps this product scarce, but advancements made by GOOD Meats and Eat Just (cultured chicken sales in the US) prove that this is no longer rare.

Inimitable: As discussed, it is extremely difficult to enter the food industry and even more difficult to produce

cultured meat. It is very costly to imitate this product directly and imitations are more likely to be achieved from other alternative proteins that are substitutes and not copies. Other cultured meat start-ups may be able to catch up which limits the sustainability of any competitive advantage gained through this resource.

Organised: The small team that launches this product must be well structured to realise its competitive advantage. An organised framework that blends R&D, production, logistics, and management is essential to allow this cultured chicken product to succeed.

Conclusion: This analysis shows that the pure product is not currently valuable (high price point), rare, or inimitable, and therefore there may not be enough of a competitive advantage from this resource alone to extract significant value. This prompted a second resource to be analysed.

30.3.2 Processing Plant Design

Valuable: An entirely animal component free (ACF) plant has been designed that utilises perfusion and medium recycling to condense the size of the working plant, increase sustainability, and reduce costs of production. This process is easier to scale out and can be tailored to individual requirements.

Rare: Cultured meat production plants are becoming more common, but most companies are focusing on scaling up their processes to use larger (10,000 L) bioreactors. The process is unique in its approach to tackling the technical challenges and is more suited to scale-out methods.

Inimitable: The plethora of established and start-up companies working in this industry means that several will have the capability to replicate the engineering work we have done. Intellectual property (IP) protection may help slow the creation of suitable imitations, but the concept itself cannot be patented, and therefore only grants a temporary competitive advantage.

Organised: As mentioned before, a well-structured team is crucial in extracting value. This is more pertinent here, as implementing a business plan that captures the value of this capability is less straightforward than that of the cultured chicken product.

Conclusion: Conducting this second analysis showed that utilising the technological process design and not just its output is more likely to generate at least a temporary competitive advantage.

30.4 Conclusion

The cultured meat industry is still very unstable: heavy reliance on international politics and legislation; uncertainty in technological and regulatory timelines; consumer perception that fluctuates unpredictably. However, the tools used in this section indicate that more value can be extracted from the technological process we have designed because it is more resistant to these issues. Combining this with the conclusions drawn from the competitor analysis, marketing strategy, supply chain strategy and the Porter's five forces analysis (Sections 25, 27, 28 and 29) emphasises the need to investigate alternative business models to the standard B2C system.

Therefore, this business strategy is further developed in Section 31 by considering multiple business models and then incorporating the optimal commercialisation method to create a top-level strategic timeline (Section 31.3).

31 Business Model - Zac Smith

Due to the unpredictability of the market, technological advancements, and the regulatory climate, several potential value capture methods (business models) have been developed that could be implemented as part of the business strategy. This section outlines potential directions our business could take, with enough variation between them to allow for much needed flexibility if changing circumstances require a pivot.

31.1 Primary Research

Before creating our potential business models, we conducted an online interview with The Cultivated B, a German biotechnology company that specialises in alternative protein service solutions. Erez Shani (Head of Global Sales) and Peter Mazzi (Production Marketing Manager) kindly answered several scientific and business questions, but the key responses were the following:

Q: What provoked the change in company direction from a cultured meat producer to a biochemical support provider?

"We started as the sister company of a large firm that sold traditional and plant-based meats, but the tedious legislative process and the negative reaction of the EU market prompted an evolution into a generic biochemical company that specialises in A to Z process support."

Q: What stage is the cultured meat industry in, and what are its greatest challenges?

"Despite early indicators of promise, there has been a decline in recent years as sales have slowed in Singapore and are struggling to gain traction in the States. Most of these are as little as 5% hybrid products to achieve a commercially viable price point. The science is not yet at the industrial scale and a lot of companies are overinflating their capabilities, resulting in lower international investment as companies underperform. [Confirmed by Competitor Analysis - Section 25.4]

The first hurdle to overcome is international legislation and regulation issues, but these are harder to control or influence. Achieving price parity is essential, but existing scale-up solutions are not currently viable, as large vessels produce challenging growth environments that are difficult to control." [Confirmed by Research [101] [58]]

This interview helped explain why they pivoted from a cultured meat producer (attempting to gain EFSA approval) to a more generic biochemical company that specialises in A-Z process support, including fermentation and pharmaceuticals. It was incredibly useful and led us to develop several commercialisation strategies that would require less investment, focus on scaling out our technology, and provide other pivot options.

31.2 Commercialisation

Having completed the VRIO analysis (Section 30.3) and discovered that there is potentially more value in the processing plant itself, several commercialisation strategies were developed that could extract value in different ways. We considered how to configure a product service system (PSS) as opposed to being a one-dimensional seller of goods and have outlined and developed the most likely of several ideas generated.

B2C Option: We scale up (and out) our plants to produce much larger quantities of our cultured chicken products which we sell directly under our own brand. This requires a significant marketing budget and funding for large capital investments but we retain control over branding and final product quality.

B2B Product Option: We raise capital to scale up (and out) our plants to produce much larger quantities of our cultured chicken products which we sell to other businesses (restaurants, supermarkets) for them to use and distribute to consumers under their own label. We secure larger contracts and avoid the expensive procedure of establishing and maintaining a positive public brand.

B2B Process Plant Option: We develop a more modular and self-contained version of the process plant to sell to food production companies or businesses that want to produce their own cultured meat. This decreases the amount of funding needed for capital investments and allows us to implement multiple revenue streams (supplying cell lines, culture media, and maintenance).

B2B Service Option: We leverage our experience and facilities to become a service provider that designs novel production plants, undertakes consultancy work, or leases out our existing plants for R&D purposes (similar to The Cultivated B).

Of these four ideas, we believe that the **B2B Process Plant** option holds the most promise when considering the VRIO analysis, marketing and supply chain strategy, and existing competitors. It also requires less investment, following The Cultivated B's advice (Section 31.1). It can be manipulated into a product, use, or result orientated PSS through combinations of consultancy, leasing, pooling, or tailoring to specific functional results targets, to name a few possible variations. Furthermore, customers would be required to regularly purchase our cell lines and cell media to maintain the regulatory approval granted to our process.

Potential strategic business partners or clients would be large-scale food manufacturers, such as American holding company Conagra Brands, Inc. who own meat consumer brands (Birds Eye™), create ready meals, and directly supply institutions and restaurants [280]. Combining with companies of this magnitude enables us to growth hack by leveraging their existing supply chains and brand awareness.

31.3 Top-Level Timeline

To enable maximum extraction of the temporary competitive advantage, a long-term and flexible business plan is necessary. Therefore, an optimistic but feasible timeline for the implementation of our B2B business model strategy was developed by outlining crucial milestones and rough indications of when they should be achieved. Assuming that we start in mid 2025, this is an overview of the steps our company must take:

- **US Pilot Plant Operational** - Successful proof of concept for engineering design that generates minimum viable products of both the cultured meat output and the functional processing plant [Late 2026].
- **Regulatory Approval** - Receive FDA and USDA certification that our cultured chicken product (and production process) is safe to be sold throughout the US [Mid 2027].

- **Sales Launch** - Generate our first income stream and garner public interest by launching our first product in collaboration with a high-end restaurant / celebrated chef [Late 2027].
- **Technology Scale Up** - Begin designing and constructing a larger and more commercially viable production plant (10tpa), using technological advancements to reduce production costs [Late 2027].
- **Commercialisation** - Assess the state of the market and our internal capabilities to ensure our B2B process plant model is the best to implement to cross the chasm from early adopters to early majorities [Early 2030].
- **Diversification** - Assess the state of the market and our internal capabilities to decide which business diversification model(s) is best to implement [Late 2031].

31.4 Diversification Options

In order to facilitate the long-term growth of our company and provide pivot options to help navigate an uncertain market, multiple diversification methods have been considered. Several of these options could be explored in tandem.

- **Varied Product Line-up** - Utilise different cell lines and make small adjustments to our processing plant to allow the growth of different meat types at different price points (e.g. pheasant, duck, foie gras). Experiment with different hybrid components and percentages.
- **Technological Improvements** - Develop new sophisticated cell growth techniques that allow the production of more complex meat products, including chicken breasts (with skin) or structured steaks [235].
- **Other Animal Products** - Use current capabilities to grow non-edible cell products (e.g. collagen, leather).
- **International Aims** - Apply for regulatory approval in different locations to attempt to break into existing or emerging markets. The UK's new regulatory framework should be implemented by the time we want to diversify (Section 21) which could give a route into the European market. This is desirable for the 27 countries to which we could sell, but also for the increase in reputation: EU regulatory approval is the "global gold standard" of novel food safety regulations [239].

31.5 Conclusion

Restricting the company to a single business model at this early stage of the development process is likely to be damaging in an industry as unpredictable as cultured meat. The plethora of growth strategies listed in this section highlight our creative and expansive approach to this challenge, but to further our analysis, the most promising model was identified: the **B2B Process Plant Option**.

To secure the value of this method, considerable time and resources must be allocated to protect the intellectual property (IP) rights of this technological process and our internally-developed media (Section 4.3). Early and continuous consultation with IP lawyers to produce patents is crucial.

32 Financial plan - Luke Nijkamp

The financial plan evaluates the financial attractiveness of the proposed business concept described above. The project scope only considers a business model of becoming an equipment and service supplier of a modular 10 tpa solution. It was hypothesised that, given the uncertain future of the cultured meat market, both from a consumer acceptance and medium cost standpoint, this model would be perceived as less risky by investors.

The financial projections aim to give an overview of the predicted costs, revenues, profits and payback period of the venture from which its financial attractiveness and funding requirements can be assessed. The framework for these mostly follows that given in the book "Fundamentals of Entrepreneurial Finance" [281], and all financial values are given in US Dollars (\$).

32.1 Timeline

Building on the top-level timeline in Section 31.3 an eight-year development plan is proposed to guide the commercialisation of our cultured chicken production process:

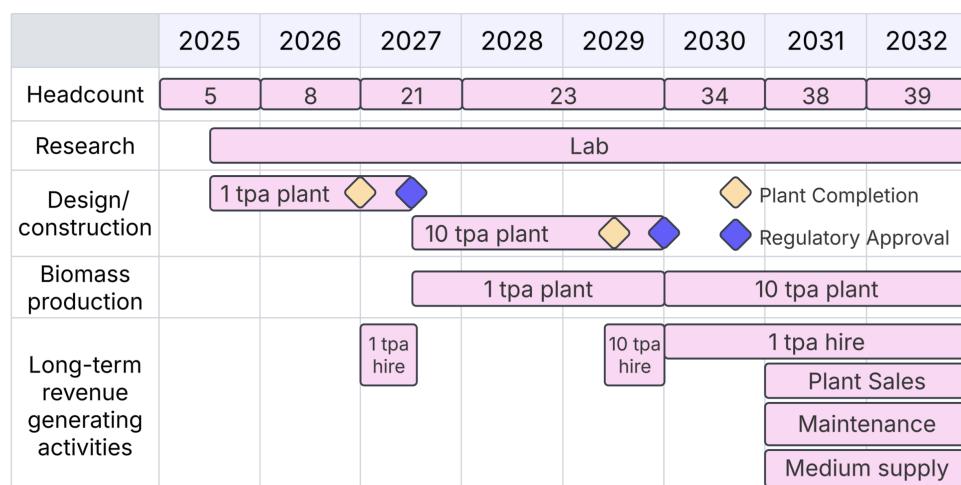


Figure 48: Business Timeline - Created with Lucidspark

Pilot Plant

The second half of 2025 will be dedicated to laboratory-scale testing, with the subsequent commissioning of a 1 tpa minimum viable product (MVP) pilot plant, as outlined in this report. Detailed engineering design is expected to commence in parallel with lab testing in 2025. Allowing approximately one year for construction and operational testing, the plant is projected to be operational by early 2027.

It is assumed that regulatory approval processes will be initiated during the plant design phase. However, final regulatory clearance is anticipated to require an additional six months following plant completion. A decision was made to pursue regulatory approval for the pilot plant despite the associated time and cost. Considerations steering this decision included being able to sell the product and gather user feedback early, important factors for attracting early adopters and convincing investors. This early regulatory engagement would also likely facilitate a more efficient and streamlined approval process for the subsequent 10 tpa commercial plant, with first commercial

biomass output anticipated for mid-2027.

Initial product distribution will target early adopters, specifically high-end restaurants in Washington State. In the interim period between plant commissioning and product approval, it is proposed to lease unused capacity to complementary biotechnology firms for research purposes, at a fixed daily rate, thereby generating extra revenue.

Scale-up Plant

The design of the 10tpa, scale-up facility will run concurrently with initial production from the pilot plant, informed by operational learnings from the 1 tpa plant. It is assumed that one year of successful output from the pilot plant will create sufficient investor confidence to fund the detailed design and construction of the 10 tpa facility, with construction forecasted to begin by mid-2028.

Given the expectation of a more streamlined regulatory process for the second facility, both plant construction and regulatory approval are projected to be completed by early 2030. At this point, commercial production will shift to the larger plant, while the 1 tpa facility will be repurposed for internal research and development and leased to third-party firms.

Shift to Technology Supplier

As previously noted, following the 10 tpa plant completion the business model will transition from biomass production to an equipment-focused, engineering service model. While, limited sales of cultured chicken from the 10 tpa plant will continue (to maintain consumer engagement and market credibility), the core revenue strategy will centre on the production and sale of modular 10 tpa facilities to other companies. Additional revenue will be generated through annual maintenance and support contracts, and the supply of proprietary cell lines and media formulations.

32.2 Estimating Revenues

32.2.1 Biomass Revenue

Revenue from selling biomass was first estimated using a bottom-up projection, considering current industry retail prices and the assumed annual production capacity. At the end of 2023, a 30 g cooked (40 g raw) serving of a competitor's cultured chicken was sold for \$45 at a high-end U.S. restaurant [282]. Assuming chicken accounted for 90% of the ingredient cost, this implies a retail price of approximately \$1000/kg. Using the industry-standard restaurant gross profit margin of 60–70% [283], and taking the conservative lower bound, we estimate a wholesale revenue of \$625/kg. As the industry scales, we assume the price restaurants are willing to pay will decline by 10% annually.

A top-down estimate was also considered, based on the achievable market share. It was projected that each high-end restaurant would sell an average of 15 cultured chicken portions per day. Using a filtered Trip Advisor search, 97 high-end restaurants in Washington State were identified. Based on full market adoption, this corresponds to an annual market size of approximately \$1.3 million as of the end of 2023. While as mentioned, wholesale prices for cultured chicken are anticipated to decline over time, serving sizes are assumed to increase proportionally, keeping the price per portion constant. This trend is expected to continue until serving sizes reach that of a conventional

chicken breast, averaging 174 g [284], which is not predicted to occur within the first eight years.

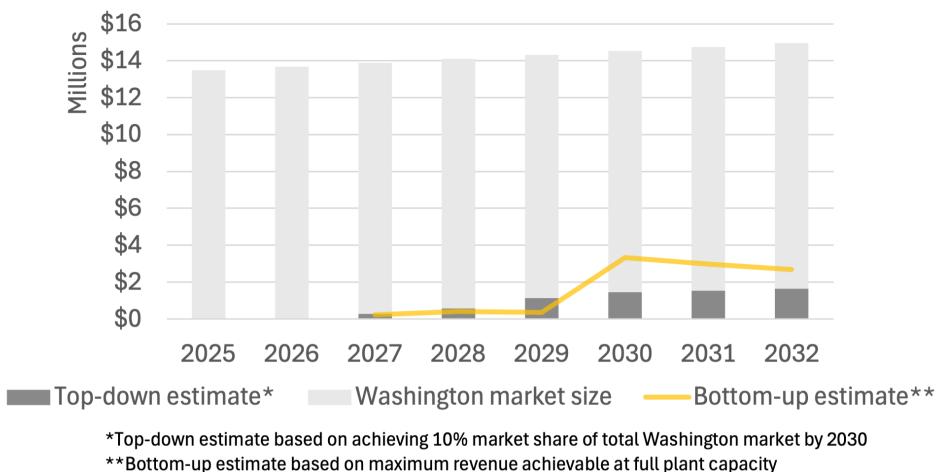


Figure 49: Top-Down vs Bottom-Up Revenue Estimates

The Washington market was projected to grow at 1.5% annually [285]. The project's anticipated market share was assumed to rise rapidly to 10% by 2030, before levelling at 11% to reflect the likelihood that some restaurants would remain hesitant to adopt cultured chicken. Comparison of the top-down and bottom-up estimates indicates that by 2030, when the 10 tpa plant comes online, the Washington State market alone would no longer be sufficient to absorb plant production. At this stage, it is suggested to expand distribution to other states identified in the SOM (see Section 23.3) such as Oregon and Idaho, both of which score highly in our location analysis (Figure 36). As production costs decline and hybrid products are developed, it is anticipated that demand will expand beyond high-end restaurants to a wider range of food-service markets.

32.2.2 Equipment Revenue

Since equipment supply would largely be limited by start-up funds available to the project, only a bottom-up estimate was conducted. Rather than undertaking the construction and detailed design of the plants in-house, it is proposed to outsource these activities to an Engineering, Procurement, and Construction (EPC) contractor. Revenue projections are based on an assumed 30% markup applied by the EPC contractor to the raw plant cost, followed by an additional 10% markup applied by the business. The raw cost of a 10 tpa plant was estimated by scaling the raw cost (see Section 15.3) using the following equation:

$$C_2 = C_1 \left(\frac{Q_2}{Q_1} \right)^{0.6} \quad (52)$$

Where C denotes cost and Q denotes output capacity. The scaling exponent of 0.6 reflects the presence of economies of scale, acknowledging that capital cost does not increase linearly with plant capacity [286]. Larger facilities benefit from more efficient use of equipment and infrastructure, resulting in a less than proportional increase in cost as size increases. Plant capital costs were also assumed to decrease by 5% each year through reductions in bioreactor and other equipment costs [212], as well as improved process efficiency [287].

Table 68: Estimated Costs and Revenues

Source	Metric	2025	2026	2027	2028	2029	2030	2031	2032
Biomass sales	Medium costs (\$/kg)	14.7k	8.13k	4.47k	2.46k	1.35k	744	409	225
	Biomass sold (kg)	0	0	500	1000	1000	10.0k	10.0k	10.0k
	COGS (\$M)	0	0	2.24	2.46	1.35	7.44	4.09	2.25
	Revenue (\$M)	0	0	0.228	0.410	0.369	3.32	2.99	2.69
Plant Sales	Plants sold	0	0	0	0	0	0	1	3
	Plant unit cost (\$M)	10.9	10.3	9.81	9.32	8.85	8.41	7.99	7.59
	COGS (\$M)	0	0	0	0	0	0	7.99	22.8
	EPC margin (\$M)	0	0	0	0	0	0	2.40	6.83
	Revenue (\$M)	0	0	0	0	0	0	11.4	32.6
Plant maintenance	Plants maintained	0	0	0	0	0	0	1	4
	Maintenance cost (\$k)	326	310	294	280	266	252	240	228
	COGS (\$k)	0	0	0	0	0	0	240	923
	Revenue (\$M)	0	0	0	0	0	0	0.571	2.17
Plant hire	Revenue (\$M)	0	0	0.137	0	0.137	0.548	0.548	0.548
Medium & cell lines	COGS (\$M)	0	0	0	0	0	0	4.09	9.01
	Revenue (\$M)	0	0	0	0	0	0	5.32	11.7
Totals	Total COGS (\$M)	0	0	2.23	2.46	1.35	7.44	16.4	34.9
	Total Revenues (\$M)	0	0	0.365	0.410	0.506	3.87	20.9	49.7
	Gross Profit (\$M)	0	0	-1.87	-2.05	-0.847	-3.57	4.44	14.7
	Margin (%)	-	-	-513	-500	-167	-92.4	21.3	29.6

Maintenance contracts were taken as 5% of plant revenue (including EPC and the assumed margin), while the costs for spare parts (COGS for maintenance) were estimated at 3% of the raw plant cost.

As noted in Section 32.1, it is planned to generate pre-approval revenue by renting the 1 tpa and 10 tpa plants to other companies for testing at a daily rate of \$5,000. Given the early stage of the industry, it is assumed that the plant will be leased for only 30% of the available days. After the 10 tpa plant is approved, it is estimated that half of the 1 tpa plant's operating time will be used for in-house R&D, with the remainder available for leasing.

Sales of medium and other process consumables like adsorbents represent an additional revenue stream. It is anticipated that, particularly in the early years, businesses purchasing a 10 tpa plant will also source their culture medium from Oxfarm, as this may facilitate faster regulatory approval for their plants and ensure process consistency. Medium requirements were assumed to scale linearly with unit costs decreasing by 45% each year, an assumption based on a recent McKinsey report [288]. The initial 2025 cost was derived by subtracting labour costs from the total material requirements outlined in Section 15.1. A margin of 30% was established for sales of

cell culture medium.

32.3 Estimating Costs

32.3.1 Cost of Goods Sold (COGS)

All the COGS and revenue estimates are summarised in Table 68. COGS for biomass refers to the direct production costs, including only medium, adsorbent, and oxygen, while excluding operational costs such as utilities and labour, as well as capital costs. For the plant sales section, the COGS represents the cost of all the equipment that makes up the plant, whilst for maintenance, COGS comprises the cost of spare parts per year.

As illustrated, significant losses are anticipated on biomass product through to 2030. While this may appear risky, it aligns with the strategies of other industry players, such as Good Meat, who are also incurring substantial losses to encourage consumer adoption and trial of their products [289].

32.3.2 Employee Payroll

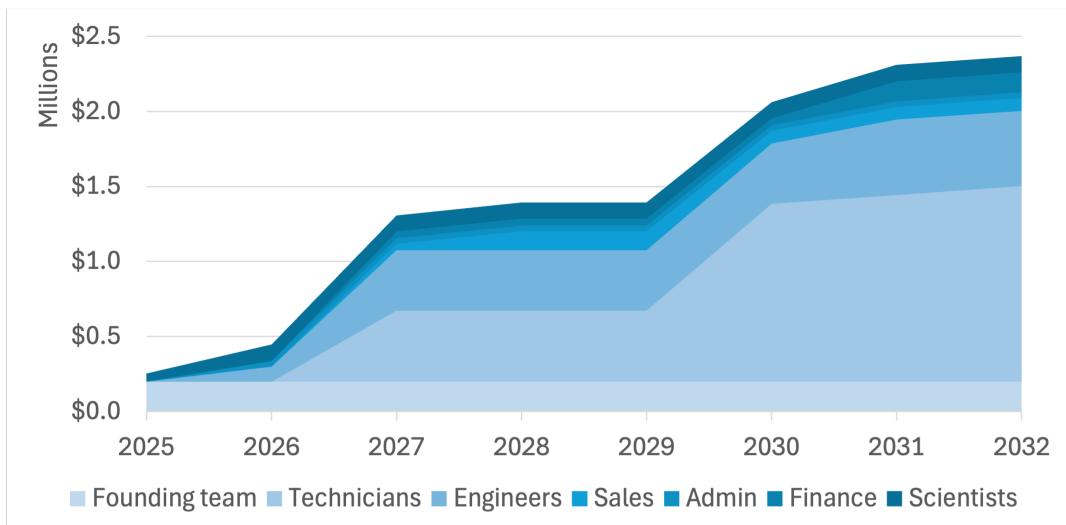


Figure 50: Hiring Strategy

Figure 50 illustrates the proposed hiring strategy as the business scales. Salary estimates were derived from average Washington State figures reported on Indeed.com, adjusted downward by 60% to reflect typical compensation levels in early-stage startups.

The Oxfarm founding team will comprise four key members: a Chief Executive Officer (CEO), a Chief Technical Officer (CTO), a Head of Marketing, and a Lead Cell Biologist. In addition, a Chair of the Board will be appointed; an individual with strong industry connections and a well-established reputation, to enhance investor confidence and lend external credibility.

In 2026, it is planned to expand the team by hiring a bioprocess engineer to support the preliminary design of the pilot plant, as well as one additional scientist and an administrative staff member. By 2027, coinciding with the commissioning of the 1 tpa pilot plant, eight technicians will be recruited to operate the facility and four engineers to initiate design work on the 10 tpa scale-up plant. With the start of revenue generation, a finance assistant and

a sales assistant will also be recruited to support core business functions.

During 2028 and 2029, the team will remain largely stable, with modest additions to the sales team to accommodate increased biomass output.

In 2030, following the commissioning of the 10 tpa facility, a significant expansion of the technical workforce is anticipated, increasing the number of technicians to 20 to support the scale-up in production. Further growth is expected in subsequent years as the company begins to service external maintenance contracts.

From 2031 to 2032, the engineering and finance teams will continue to grow in line with the transition to a technology-based business model. Conversely, the sales team is expected to downsize slightly, reflecting a shift toward fewer, higher-value business-to-business contracts.

32.3.3 Non-payroll Operating Expenses

In addition to payroll, other operating expenses have been accounted for, as detailed in Table 69.

Table 69: Non-Payroll Operating Expenses, values in k USD

Category	2025	2026	2027	2028	2029	2030	2031	2032
Maintenance	0	0	81.9	81.9	81.9	326	326	326
Utilities	3.15	3.15	9.57	10.0	10.5	41.9	44.1	46.3
Prof. services	80.0	88.0	96.8	106	117	129	142	156
Lab & facility	75.2	80.9	80.9	86.4	97.6	97.6	97.6	97.6
R&D	100	120	144	300	360	400	420	441
Sales/Marketing	50.0	55.0	60.5	66.6	73.2	80.5	60.0	66.0
Travel	40.0	44.0	48.4	53.2	58.6	64.4	70.9	77.9
Admin	20.0	23.0	26.5	30.4	35.0	40.2	46.3	53.2
Vehicle leasing	5.00	5.00	5.00	5.00	5.00	10.0	10.0	10.0
Total	373	419	553	740	839	1190	1220	1270

Expenses such as utilities, laboratory consumables, facility rental, and R&D were modelled to increase in conjunction with the commissioning of each new facility. Conversely, sales and marketing expenditures are expected to decline after 2030, reflecting the shift from a biomass supply model to an equipment and engineering services model.

Other more general costs such as professional services, administration and travel are more difficult to predict and so constant annual growth rates for these were assumed of 10%, 10% and 15% respectively. Additionally, maintenance costs for the two owned plants have been assumed at 3% of the raw capital cost per year. Finally, it was assumed that one company vehicle would be required (two from 2030), which would be leased for an annual rate.

32.3.4 Capital Costs

The primary capital costs arise from the construction of the two owned production facilities. These are detailed in Table 70, along with projected annual depreciation. The pilot plant is assumed to have a useful life of 10 years, and the scale-up plant 15 years, each with a residual value of 10% of the original capital cost.

Additional capital expenditures, including laboratory equipment, office furnishings, and other infrastructure,

Table 70: Capital expenditures

Capital Expenditure	Cost (\$)	Purchase Date	Ownership (yrs)	Residual Value (\$)	Yearly Dep. (\$)
Lab equipment	30,000	2025	10	3,000	-2,700
Pilot plant	3.55M	2026	10	0.355M	-0.319M
Scale up plant	14.1M	2028	15	1.41M	-0.848M
Office furniture	3,000	2026	5	0	-600
Office furniture	6,000	2030	5	0	-1,200

have also been accounted for.

32.4 Income

The projected income, as shown in Table 71 and Figure 51, illustrates the company's profitability over time, accounting for operating expenses, capital depreciation, and taxation. Washington State imposes only a sales tax, with an average rate of 9.38%, which has been applied to all revenue projections [206]. As shown, the company is not expected to reach profitability until 2032.

32.5 Cash Balance

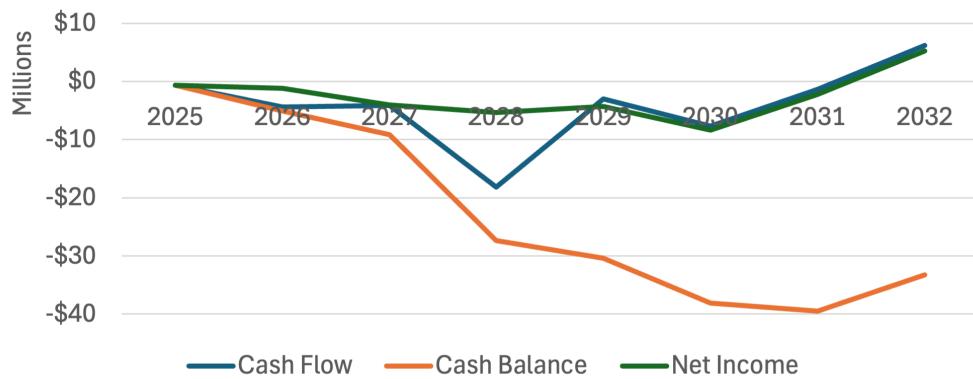


Figure 51: Hockey Sticks

It is proposed to manage Oxfarm's supply chain with maximum efficiency to maintain a low net working capital position. Net working capital is defined as:

$$\text{NWC} = \text{Operating Current Assets} - \text{Operating Current Liabilities} \quad (53)$$

[281]. Inventory levels will be kept to a minimum, for instance, holding no more than one month's supply of growth medium, both for internal use and for sale. Similarly, during the equipment supply phase, the spare parts inventory will be limited to components sufficient for servicing a single plant over one year.

Project payment terms required that customers pay on delivery, thereby minimising accounts receivable. Wherever feasible, payments to suppliers will also be made promptly. However, some liabilities will remain unavoidable, including monthly payments for utilities, professional services, and marketing-related subscriptions (e.g., web hosting and sales platforms).

32.6 Cash Flow

A projection of the business' cash flow was created to understand the financing needs of the business, again shown in Figure 51. Cash flow is described via the following equation [281]:

$$\text{Cash Flow} = \text{Net Income} - \text{Change in Net Working Capital} - \text{Capital Expenditures} + \text{Depreciation} \quad (54)$$

Table 71 summarises the cash flow over the next 8 years, which informed the funding strategy below.

Table 71: Profit and Cash Flow Projections 2025–2032, values in M USD. Adapted from Hellman et al. [281].

Category	2025	2026	2027	2028	2029	2030	2031	2032
Profit after tax	-0.630	-1.19	-4.09	-5.39	-4.30	-8.36	-2.21	5.25
Accounts Receiv.	0	0	0	0	0	0	0	0
Inventories	0	0	0.373	0.205	0.113	0.620	0.922	1.17
Accounts Payable	0.024	0.013	0.014	0.016	0.017	0.022	0.021	0.023
Net Working Cap.	-0.024	-0.012	0.358	0.189	0.096	0.598	0.901	1.14
Change in NWC	-0.024	0.011	0.371	-0.169	-0.094	0.503	0.302	0.242
Cap. Expenditure	0.030	3.55	0	14.1	0	0.006	0	0
Depreciation	0.003	0.323	0.323	1.17	1.17	1.17	1.17	1.17
Cash Flow	-0.633	-4.43	-4.14	-18.2	-3.03	-7.70	-1.35	6.18
Cash Balance	-0.633	-5.06	-9.20	-27.4	-30.4	-38.1	-39.5	-33.3

32.7 Funding Strategy

32.7.1 Funding Requirement

As shown in Figure 51, this venture requires substantial investment to avoid a negative cash balance. A three-part funding strategy is proposed to meet the business's financial needs over the first 8 years:

- 2025 – Seed round: \$1M
- 2026 – Series A round: \$10M
- 2028 – Series B round: \$30M

The numbers above represent the minimum required to maintain a positive cash balance (as shown in Figure 52 below). Consequently the project aims to raise additional funds to mitigate the risk of bankruptcy.

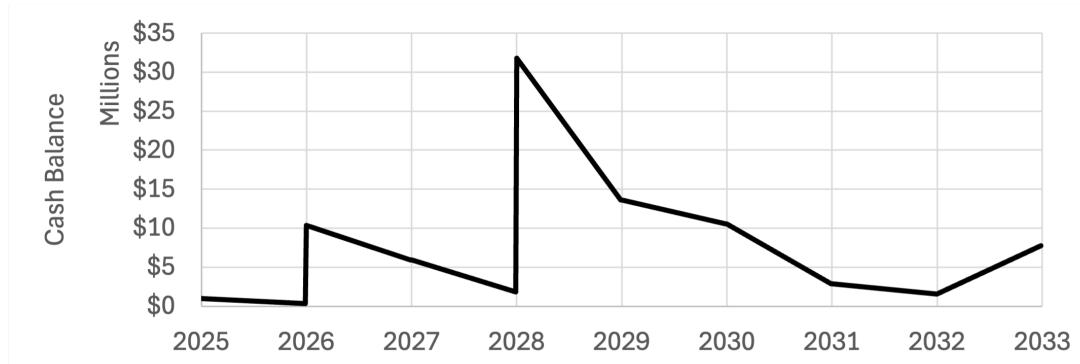


Figure 52: Cash Balance with Investment

32.7.2 Choosing Investors

A range of funding sources can be considered for a start-up seeking financial backing. These include venture capital firms that professionally manage institutional funds, personal investment from founders and their networks, and business angels who contribute independently. Corporate investors may provide funding for strategic gains, while fintech platforms enable public crowdfunding. Traditional bank loans are an option for startups with credible financials, though banks are typically risk-averse. Public funding, such as government grants and tax relief, as well as university grants, can be particularly valuable for early-stage startups in scientific fields.

To identify the most relevant investors at each stage of this project, the 'FUEL' framework from Hellmann et al. [281] was applied, which considers investors' fundamental structure, underlying motivations, expertise and networks, and investment logic and style. For example, universities offer extensive scientific networks and patient, mission-driven capital, while corporations provide industrial connections and market access. Venture capital funds generally have shorter investment horizons and require evidence of traction, focusing on rapid scaling, whereas angel investors are often more willing to accept higher risk in early-stage ventures. These distinctions, combined with research into the funds of current industry players (Table 72) informed the funding strategy below.

Table 72: Investment rounds for selected cultured meat companies.

Company	Seed Round	Series A	Series B	Source
Mosa Meat	Sergey Brin (Google co-founder)	M Ventures, Bell Food Group	Blue Horizon Ventures, Bell Food Group, Nutreco, Merck Ventures, Agronomics	[290] [291]
Ivy Farm	Oxford University	-	-	[292]
Aleph Farms	Strauss Group's The Kitchen Hub, Israel Innovation Authority	VisVires New Protein, Cargill, Strauss Group, and others	L-Catterton, DisruptAD, Skyviews Life Science, Cargill, Thai Union, BRF, CJ Group, and others	[212]

32.7.3 Investor Strategy

Seed Round: It is proposed to establish a partnership with a leading university to access academic expertise and laboratory facilities across relevant departments. Seed funding will be sought from biotechnology and sustainable food-focused investors, including angel investors interested in alternative proteins. In parallel, the project team will pursue non-dilutive funding from government programmes such as Innovate UK, and consider joining foodtech accelerators for capital and mentorship.

Series A: To support pilot-scale production and initiate offtake agreements, the project aims to seek strategic investment from major food and agriculture companies (e.g., Cargill, Conagra Brands). These partnerships can provide capital, supply chain access, and market validation. Government grants and subsidies will also be pursued to reduce the risk of scale-up and regulatory approval.

Series B: Significant funding will be required for the 10 tpa commercial-scale facility. VCs with expertise in biotech and foodtech will be targeted, as well as infrastructure funds and strategic investors with bioprocessing experience. Engineering firms, equipment suppliers, and corporates in related sectors may be engaged as technical

partners. International investors focused on sustainability and food security may also be approached to support global expansion. In addition capital-sharing partnerships with other biotech firms could be considered.

33 EEM Conclusion - Luke Nijkamp

The goal of the EEM chapters was to formulate a well-informed commercialisation strategy for Oxfarm.

The emerging conclusion from the market and stakeholder review is that, although the industry shows strong growth potential, it still faces significant uncertainties related to regulation, technology, and consumer adoption. The cultured chicken sector faces even greater challenges, compared to products like beef and lamb, due to its status as a traditionally low-value meat, limiting profit margins. Furthermore, its relatively lower environmental impact means that there is less incentive for consumers to switch to cultured chicken for environmental reasons.

As a result, the business plan focuses on leveraging the value of the technology itself rather than relying on the direct sale of the biomass product. The financial plan suggests the strategy is viable, achieving profitability and positive cash-flow by 2032. However, it demands substantial investment (an excess of \$40M over 4 years) and future work should test this conclusion with sensitivity analysis under more optimistic or conservative assumptions. Funding may also be difficult to secure in an early-stage industry lacking proof of concept, especially as break-even isn't projected within the first 8 years. This plan also relies on other, better funded companies being prepared to shoulder the risk of medium costs and consumer uptake whilst using our technology.

Nonetheless, there is potential to capture substantial market share by providing companies with a quick and reliable entry point into the sector, once the market has matured. The main financial opportunity lies in integrating these clients into Oxfarm's culture medium supply chains and securing long-term service agreements. By delivering a fully integrated solution rather than a standalone product, this also creates a high barrier to entry for competitors.

34 Project Conclusion - Luke Nijkamp

This project assessed the feasibility and viability of cultured chicken production by designing a 1 tpa pilot plant and developing a supporting business plan. The design meets the project aims of using an animal component-free medium and incorporates a perfusion-loop recycling system that reduces medium consumption by over 78%. The business plan, grounded in analysis of the alternative protein market, stakeholders, competitors, and regulatory landscape, also explores funding strategies that offer added value beyond financial support.

The current and future financial analysis revealed that selling biomass alone does not present a viable business case in the near or medium terms, even assuming aggressive reductions in medium cost. Consequently, marketing the technology and associated services represent Oxfarm's core, long-term offering. Washington was identified as an optimal starting location based on regulation, cost and emissions.

Future developments will rely on advances in key technologies and resources, including biocompatible adsorbents, biocatalysts, cell separation systems, and bioreactors. While this project adopts a single culturing approach, further work should explore alternatives such as continuous harvesting and incorporate advanced methods like thermal

manipulation, cell line adaptation and co-culturing. On the business side, analysis should include sensitivity testing of different scenarios and models to ensure the financial plan is robust. Additionally, a more rigorous evaluation of optimal market selection, facility location and funding strategy, together with a comprehensive stakeholder engagement plan, are required.

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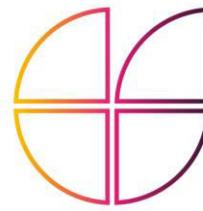
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All web URLs were last accessed on 18/05/2025.

3YP - Engineering in Society Supporting Statement



DEPARTMENT OF
ENGINEERING
SCIENCE



Please complete this supporting statement as a group/team and include one copy with your report submission at the end of your report. (This statement does not count towards your page count). This statement is not assessed, but ensures that your group have considered these important topics

Project Title:	11. Cultured Meat Production: a Food Supply-chain for the Future
Student Name:	Date:
Zac Smith	13/05/2025
Viraj Nerkar	13/05/2025
Zaheer Sidik	13/05/2025
Luke Nijkamp	13/05/2025

Please indicate exactly where in your project submissions (Report and/or Logbook) the following topics have been considered.

Technology Strategy – Societal, User, Business and Customer Needs:

Discussed in the introduction and Section 17, 20, 27.

Key decisions included switching from microcarriers to suspension (28 November minutes), and using FBS alternative (6 February minutes), customer survey (3 January minutes), stakeholder value mapping (24 February minutes)

Project Financing:

Current project costs are detailed in Section 14 of the report. Financial projections can be found in section 30. Individual costings can be found in logbooks under the engineering section.

Meeting with ThermoFisher (20 February) for key component and medium costs.

Sustainability and the Environment:

An environmental impact assessment and LCA are conducted in Section 16, further environmental considerations are given in the report introduction.

Key decisions included using FBS alternative (6 February minutes), perfusion system (31 December)

Diversity and Inclusion:

The team agreed to an inclusion statement in MT Wk 1 as detailed at the beginning of the logbook. Team leaders were rotated each week to ensure fair distribution of roles and voicing of opinions.

Inclusion statement can be found on page 2 of all logbooks.

Considerations of Health and Safety:

Covered by Section 15 of the report.

Refer to page numbers, section numbers, etc. Please refer to the example Supporting Statement.