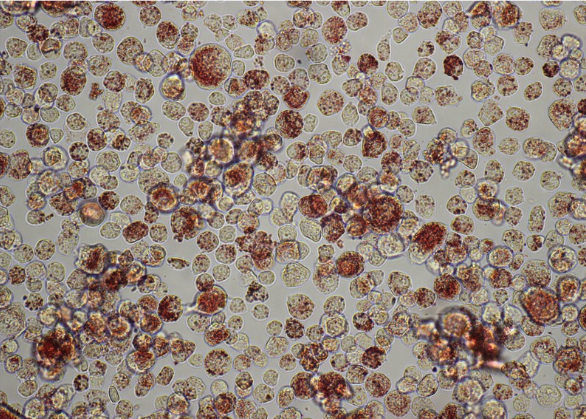
BigFat: for an innovative biotechnological process using single cells to produce 5,000 tons/year of fat



Source: Porcine Immortalised Adipose-derived Stem Cells | Dragon Biotechnologies, n.d.

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# Abstract

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This project presents the development of a process for producing porcine fat using immortalised, GMO-free adipose-derived stem cells (ADSCs) cultured in single-cell suspension. The design outlines a scalable system that integrates cell proliferation in a stirred tank bioreactor and adipogenic differentiation in an airlift bioreactor, both in perfusion mode. Adipocyte harvesting is done through top-layer decantation. The process yields 7.4 m3 of partially mature adipocytes per batch. The final product is a wet paste which can be sold to other cultivated meat producers. The proposed system can deliver pork fat similar to conventional pork fat, while offering reductions in greenhouse gas emissions with renewable energy and water use. Economic viability remains a challenge due to high medium and operational costs. Several strategies such as genetic modification of cell line, medium recycling strategies and use of yeast growth factors are discussed for future cost reductions.

# I. Introduction

## Background

The population is growing, meat demand is rising and people are increasingly becoming aware of animal welfare and environmental sustainability. All of these are reasons why the topic of meat cultivation is increasingly researched. Meat cultivation is an emerging biotechnological process where meat is produced directly from cultured animal cells instead of through traditional livestock cultivation. This can offer an alternative option to provide cruelty-free meat to the market with reduced land use, greenhouse gas emissions, and antibiotic resistance or zoonotic disease risks (Tuomisto & de Mattos, 2011, Stephens et al., 2018).

Cultivated pork is especially interesting since there is a lot of potential to improve in terms of sustainability compared to traditional livestock. Conventional pork production is the second highest in land use and carbon footprint after beef and accounted for the highest percentage of medically important antibiotic sales in USA (Sinke & Odegard, 2021; FDA 2021). Additionally, nearly half of the meat consumed in the Netherlands is pork, thus providing a large consumer market for the eventual product (Dagevos & Verbeke, 2022).

Large-scale production of cultivated meat faces considerable process and cost barriers. High media costs, low cell densities and complex downstream recovery process are critical setbacks for economic feasibility and scalability. To overcome these challenges, advances in bioreactor design, process optimization and high cell densities maintaining cell integrity are required. Although cultivated pork fat will initially incur higher production costs than traditional meat, it represents an innovative alternative meat that is more sustainable and more ethical than the conventional meat product. This is where BigFat comes in, an innovative process for the production of 5,000 tons of cultured pork fat annually.

## Project Scope and Approach

This project focuses on the development of an innovative, large-scale bioprocess for the production of 5,000 tons per year of cultivated pork fat. The final product of this process is a wet paste of partially mature adipocytes which will serve as fat ingredient for business-to-business (B2B) sale to cultivated meat companies seeking to enhance the flavour, texture and juiciness of their final meat product. In this project, non-GMO and serum-free adapted porcine immortalised adipose-derived stem cells (ADSCs) are used to cultivate pork cells through a perfusion system. The process aims to establish an animal-free, cost-efficient production system by optimizing basal medium formulation, reducing growth-factor dependence, and improving bioreactor scalability.

This report is structured to provide a comprehensive overview of the bioprocess development of cultivated porcine fat. To achieve this, substantial literature research was performed on cultivated fat, followed by cell line selection and media formulation. The next step focuses on upstream bioprocess design, fermentation and downstream process strategy which is modelled in SuperPro Designer. Following this, an economic and sustainability assessment is presented to evaluate the feasibility of large-scale production. Finally, the report concludes with recommendations for future improvements of the bioprocess design.

# II. Cell line selection

To enable large-scale cultured fat production, the ideal cell line must have high proliferative capacity, maintain adipogenic functionality, remain viable in serum-free, food-grade media, and have minimal aggregation. Some of these requirements can be met by using an immortalised cell line, which remains highly proliferative and maintains adipogenic functionality. An immortalised cell line can sustain over 60 proliferation cycles, which minimises the need for repeated animal-derived cell isolation (Wu et al., 2024).

Studies show that dedifferentiated fat cells (DFAT) and adipose-derived stem cells (ADSCs) seem to be the most robust cell lines, since they are more committed to adipogenesis and easier to differentiate in contrast to embryonic stem cells (ESCs) or pluripotent stem cells (PSCs) (Cawthorn et al., 2012; Ahfeldt et al., 2012). ADSCs are the final choice for the process, since they have high differentiation efficiency into adipocytes and are widely available from adipose tissue (Cawthorn et al., 2012). DFAT cell-line is not the final choice, since DFAT cells need to derive from mature adipocytes and revert to a stem-like state, a process which would add complexity. Also, they are less characterised mechanistically, with species dependent performance (Wei et al., 2013; Nosbusue et al., 2010).

DragonBio sells spontaneously ADSCs, which are non-GMO, demonstrate a doubling time of less than 17 hours and have been adapted to grow in single-cell suspension (*Porcine Immortalised Adipose-derived Stem Cells | Dragon Biotechnologies*, n.d.). Based on these characteristics, this cell line is the final choice for cultivation of porcine fat in single-cell suspension. The exact cell line price couldn’t be obtained and as a result the pricing is based on stem cell therapies, which range from $10,000 to $100,000 per cell-line (Aijaz et al., 2018).

## Single-cell suspension cell line creation

Alternatively, if the existing cell line from DragonBio is too expensive or exhibits issues during large scale cultivation, a new porcine spontaneously immortalised adipose derived stem cell line could be developed. Developing a new cell line takes more time than buying a cell line but allows for more freedom in the selection of desirable parameters. In this case, it needs to be an immortal cell line capable of growing in single cell suspension that can eventually differentiate into adipocytes. Immortalisation can occur spontaneously. Spontaneous immortalisation is a rare event that occurs when cells are repeatedly divided until a stable cell line is achieved after many proliferations (Pasitka et al., 2022; Gillio-Meina et al., 2000). Many spontaneously immortalised cell lines exist, including some patented porcine fibroblast cell lines (Christman et al., 2006; Gillio-Meina et al., 2000; Oh et al., 2007; Sun et al., 2006; Jin et al., 2006; Changbo et al., 2025; Yanzhen et al., 2014).

In order for the cells to grow in single cell suspension, positive selection can be used, for example by exposing the cell aggregates in shaker flasks to increase agitation and thus positively selecting for cells that can grow anchorage independent. After repeated passaging of cells that exhibit anchorage independent growth, a cell line can be obtained that can grow in single cell suspension (Pasitka et al., 2022). An overview of this process can be seen in Figure1.

A diagram of a science experiment

AI-generated content may be incorrect.

**Figure 1.** A Schematic overview is given of the adaptation process for generating a cell line capable of growth in single-cell suspension. Adherent cells are initially cultured in standard plates and then transferred to shaker flasks The agitation rate is gradually increased, and cells are selected for anchorage independence. This selection and cultivation process is repeated over multiple passages, thereby eventually generating a cell line capable of growing in single cell-suspension. Created using biorender.com

It is also possible to use genetic modification to obtain a cell line capable of growing in single cell suspension. When comparing suspended and adherent HEK239 cells, genes relating to cell adhesion and cytoskeleton organisation were upregulated in the suspended cells, especially genes of the cadherin superfamily (Malm et al., 2020; Green et al., 2025). These genes are potential targets for genetic modification of cells to improve the ability to grow in single cell suspension. However, using genetic modification is still strictly regulated and comes with its own challenges.

# III. Medium Formulation

To culture and differentiate the iADSCs into mature adipocytes, three functionally distinct media have been designed. Firstly, a proliferation medium will be used. This medium is designed to maximally expand the numbers of ADSCs. Following this, a priming medium will be applied to prepare the cells for adipogenic differentiation, while they are still proliferating. Finally, the cells will be cultured in the differentiation medium, which aims to induce terminal adipocyte formation and fat accumulation. For each medium component the aim is to ensure that it is animal free, minimizes the accumulation of toxic byproducts, and is cost efficient. The basal medium for all phases is DMEM/F12, as it provides essential amino acids, glucose (3.1 g\*L-1), vitamins and salts to maintain porcine cell metabolism. In addition to this it has been previously used for fat cultivation using porcine fibro-adipogenic progenitor (FAP) cells, which share strong metabolic similarities to ADSCs (Mitić et al., 2022).

## Waste accumulation

However, it has been shown that in porcine mesenchymal stem cell expansion high medium glucose concentrations of around 4.5 g\*L-1 can lead to the accumulation of lactate when compared to more physiological glucose levels of 0.9 g\*L-1 (Ferrari et al., 2014, Sabater et al, 2014). Lactate accumulation is unwanted as it results in reduced cell densities. However, physiological glucose levels could result in glucose depletion. To prevent this, the cells could be fed fresh medium with high glucose concentrations through cultivation in a perfusion or feed and bleed reactor, supplying fresh medium while potentially removing waste products. In addition to lactate, ammonia can cause growth inhibition at concentrations as low as 2 mM, and therefore it is beneficial to minimize production of ammonia. To prevent this, we add the non-ammoniagenic compound pyruvate (1.1 g\*L-1), as pyruvate is directly metabolized into the tricarboxylic acid (TCA) cycle without requiring amino acid deamination, which is the process that generates ammonia, thereby preventing glutamine driven ammonia accumulation (Hubalek et al., 2023). In addition to this, supplementation with pyruvate also decreases lactate production (Genzel et al., 2005).

## Single cell suspension

Besides limiting accumulation of wasteful products, it is essential to maintain single cell suspension and to minimize shear stress. To achieve this, the food-grade compound methylcellulose (300 g\*m-3) is added as an anti-aggregating and anti-shear agent (Goldblum et al., 1990; Hua et al., 1993; Michaels et al., 1992). Methylcellulose aids in protecting cells from shear stress and maintaining single cell suspension, by decreasing the aggregate formation of the cells allowing for better nutrient uptake (Pasitka et al., 2023).

## Proliferation

The proliferation medium contains a series of growth factors essential for serum-free expansion of the iADSCs. These growth factors consist of fibroblast growth factor-2 (FGF-2) epidermal growth factor (EGF), and platelet-derived growth factor-BB (PDGF-BB). FGF-2 is the principal mitogen for cell proliferation. It helps to maintain proliferative capacity and adipogenic potential. Both EGF and PDGF-BB further assist in sustaining cellular proliferation and viability. The selected concentrations of FGF-2 (5 mg\*m-3), EGF (5 mg\*m-3), and PDGF-BB (10 mg\*m-3) are within the ranges of previous literature, showing that these concentrations support serum-free proliferation of mesenchymal and adipose-derived stem cells (Lai et al., 2018; Rodrigues et al., 2010; Tarapongpun et al., 2023). The various growth factors, their concentrations and costs in the proliferation media is shown in Table 1.

**Table 1.** An overview of the concentration, cost and suppliers of the growth factors present in the proliferation medium.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Final Concentration (mg\*m-3) | Cost per kg (€) | Cost per m3 (€) | Supplier/source |
| EGF | 5 | 17,240,000 | 86.20 | (plant-derived recombinant protein; ORF Genetics, Iceland) |
| PDGF-BB | 10 | 30,170,000 | 301.70 | (plant-derived recombinant protein; ORF Genetics, Iceland) |
| FGF-2 | 5 | 21,550,000 | 107.75 | (plant-derived recombinant protein; ORF Genetics, Iceland) |

## Priming

The last 3 days of proliferation will be performed using a priming medium. This medium is designed to proliferate the cells, while priming the cells to differentiate more efficiently. It has been found that three days of pretreatment with FGF-2 led to significantly enhanced lipid accumulation and the expression of key adipogenic transcription factors such as peroxisome proliferator-activated receptor gamma (*PPARγ*) during the following differentiation step (Tarapongpun et al., 2023). When both EGF and FGF-2 were present during this stage a significantly lower expression of these transcription factors was observed, compared to exclusively FGF-2. For this reason, the priming medium will consist of the basal medium alongside FGF-2 (5 mg\*m-3). In addition to this, it was also found that if FGF-2, or EGF were maintained during the differentiation stage this led to reduced lipid droplet formation and expression of *PPARγ*. Therefore, the differentiation medium will exclude any of the growth factors present in the proliferation media.

## Differentiation

To differentiate our ADSCs into mature adipocytes, a specialized medium is needed. Based on prior literature, the cells are grown in this medium for a timeframe ranging from 7 to 11 days (Bohan et al., 2014; Liu et al., 2024; Pasitka et al., 2023; Thrower., 2025). This medium often contains compounds such as IBMX, dexamethasone, or rosiglitazone. While these compounds are effective at initiating adipogenic differentiation, each of them is considered a pharmaceutical drug by regulatory organizations that are unsuitable for food-grade applications (U.S. Food and Drug Administration, 2011). The development of a defined and food-grade differentiation medium depends on substitution of conventional pharmaceutical inducers with compounds that comply with food safety regulations. According to recent literature successful differentiation to adipocytes depends on the combination between anabolic signalling, activation of transcriptional regulators, and the availability of lipid substrates (Mitić et al., 2023; WO2023003470A1, 2023). To increase the glucose uptake and activate anabolic pathways, insulin (5 g\*m-3) will be added to the medium. To replace synthetic PPARγ agonists such as rosiglitazone, the plant derived magnolol (2.66 g\*m-3) and honokiol (2,66 g\*m-3) will be added. Magnolol functions as an agonist of PPARγ, thereby upregulating lipid metabolism and adipogenic gene transcription (Zhang et al., 2019). Honokiol provides additional activation of PPARγ, as well as contributing to antioxidant and anti-inflammatory effects which support homeostasis during differentiation.

To induce fat accumulation in the adipocytes, oleic acid will be added (14200 g\*m-3). Oleic acid acts as both a substrate for triglyceride formation, but also as a mild PPARγ agonist. This combination leads to increased expression of adipogenic genes as well as contributing to lipid droplet accumulation (Belal et al., 2018; Yanting et al., 2018). A further addition to the differentiation medium is lecithin (12 mg\*L-1), which acts as a lipid carrier and emulsifier. This leads to increased solubility and cellular uptake of oleic acid, magnolol, and honokiol, while additionally contributing to medium stability during agitation (WO2023003470A1, 2023). Table 2 presents an overview of the adipogenic induction compounds, their concentrations and costs in the differentiation medium.

**Table 2.** An overview of the concentration, cost and suppliers of the adipogenesis inducing components present in the differentiation medium.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Final Concentration (g\*m-3) | Cost per kg (€) | Cost per m3 (€) | Supplier/source |
| oleic acid | 14200 | 1.35 | 0.08 | (Dahua Industry HK Limited, China) |
| magnolol  and honokiol | 2.66 | 25.86 | 0.07 | (purified plant extracts; Made-in-China.com, China) |
| lecithin | 12 | 12.93 | 0.16 | (food grade, sunflower  derived; Nature Foods,  Europe) |
| insulin | 5 | 21,334.50 | 106.67 | (recombinant *E. coli*; Gotham et al., 2018) |

## Cost reductions

One of the major limitations in the large-scale production of cultivated meat is the high cost of research grade basal media and growth factors. Commercially available DMEM/F12 media is sold by suppliers such as Thermo Fisher Scientific at approximately €134 per 50 L, corresponding to €2,689 m-3 of medium. Although relatively expensive, the price of the basal medium is overshadowed by the high costs of growth factors such as FGF-2, which is sold at approximately €1,728,310 per gram (Specht, 2020). These costs are acceptable for small scale research but are not feasible for large scale cultivation.

To address this, all components of the basal medium will be purchased separately in bulk and mixed together into a final medium which contains high similarities to DMEM/F12. This bulk-prepared medium only consists of food grade components and requires in-house mixing and sterilization. According to Specht (2020), buying components in bulk is expected to reduce the total medium cost from €2,689 to €198.3 m-3. An overview of the composition and the cost of each compound in the basal medium is shown in Table S1.

In addition to cost reduction through bulk purchasing, plant-derived recombinant growth factors supplied by ORF Genetics (Iceland) can be used instead of mammalian derived growth factors. These are produced using a barley expression system, providing a completely animal-free and cheaper alternative compared to mammalian expression systems. The price of the plant derived growth factors needed for our process range between €15-30 per mg. The main cost driver in the differentiation medium is insulin. Although the price of recombinant insulin produced through *E. coli* or yeast expression-systems is around €21334 kg-1, (approximately 1000 times cheaper than the proliferation growth factors), it remains costly as the required insulin concentration for differentiation is roughly 1000 times higher than that of the proliferation growth factors (Gotham et al., 2018).

By incorporating these cost reduction strategies, the total price of each medium can be determined by adding up the cost of the basal medium with additional costs for either growth or differentiation factors. After adding up the costs, the proliferation medium is estimated to cost €773.30 m-3, the priming medium €323.3 m-3, and the differentiation medium €322.4 m-3.

## Medium sterilisation

The process should adhere to the safety standards and prevent contamination. As a result, medium sterilisation is necessary, with the most robust methods being the usage of heat and filters. Heat sterilisation will be performed with steam at 121 oC. Steaming at 121 oC for 15-30 minutes is able to kill bacteria endospore, fungi and most viruses (Sastri et al.,2014). Then hormones, growth factors and vitamins, all of which are heat sensitive, will be sterilised separately through a 15-20 nm nanofiltration. Nanofiltration is able to remove bacteria, fungi and viruses. After the heat sterilisation finishes and the water cools, the compounds will be mixed (Berovic et al.,2005; Johnson et al., 2022; Liu et al.,2000).

Using filtration alone is less compelling, since applying nanofiltration to the whole medium would be financially unsustainable, due to high operational costs of the membranes (Berovic et al.,2005; Na et al., 2022). Another possibility is using reverse osmosis and ultrafiltration to sterilise the water and add the preferred salts afterwards, since that also offers protection of the water from viruses. However, due to high costs this option will not be explored further (Lee et al.,2017; Burke at al., 2025).

# IV. Fermentation

General considerations

### Stainless steel vs. Single-use

The most common types of bioreactors are single use and stainless steel. The main advantages of single use technologies are increased flexibility, low initial capital costs and reduced downtime (Boyd Biomedical, 2018; Pristine clean bags, n.d.). Single-use bioreactors are sterile when they arrive, so no cleaning or sterilisation processes are required, in unlike stainless steel bioreactors. For cleaning in place (CIP) the standard procedure is rinsing with hot water followed by alkaline and acid wash and final rinsing to remove chemical residues. For sterilization, steam in place (SIP) using saturated clean steam is the best option, since it is effective, standard and does not leave chemical residues (Seiberling et al., 2018; Eibl et al.,2019). The advantages of a stainless-steel bioreactor are that it is reuseable, more cost efficient over the long term and can scale up to larger volumes (Bioterms, 2023). The aim for this project is a long term cost efficient and large scale bioprocess, so stainless steel bioreactors will be used.

316 grade stainless steel bioreactors were chosen since they have added molybdenum, which provides superior resistance in the presence of chloride ions (Cl−), which, when combined with oxygen and water, can cause erosion. This is highly important, since Chloride (Cl−) homeostasis is a critical aspect of animal cell physiology and the media contains chloride salts (Zhang,J. et al., 2023; Wang, D. et al., 2025).

### Seed Train

To obtain a sufficient number of cells for the large-scale bioreactors, the cells first need to proliferate. This seed train process will be done in 5 steps, each time increasing the volume 10-fold. First, the ADSCs will be grown in a shake flask of 280mL and 2.8L consecutively. After this, stirred tank bioreactors of 28L, 280L and 2800L are used (see Appendix K). We assume that the stirred tank bioreactors behave similarly to the final proliferation tank, so the scaling down of this seed train should not lead to complications. Finally, the scaled up ADSCs will be transferred to the proliferation bioreactor.

### Large scale bioreactor considerations

The most important considerations in a suspension bioreactor at large scale are the shear stress as well as nutrient and oxygen availability. At scales of 20 m3 or higher, stirred tank or airlift bioreactors are commonly used at large scales, so these are considered (Varley & Birch, 1999).

Either nutrients or oxygen will be the limiting factor in the bioreactor, it is assumed that oxygen will become the limiting factor for cell growth, because there is a maximum to the amount of oxygen that can be added while more nutrients can always be supplied in a perfusion tank. The maximum cell concentration is calculated by dividing the Oxygen Uptake Rate (OUR) by the specific oxygen uptake rate. First, the gas flow rate for oxygen is calculated from the decay rate and the total volume of the reactor. This is then used to calculate the specific surface area of the gas bubbles. Based on these calculations, the Oxygen Transfer Rate (OTR) is calculated. The OTR is equal to the OUR. It was found that the maximum cell concentration increases with a larger reactor volume. The calculations for both the proliferation and differentiation are explained in their respective paragraphs. The full calculations can be found in appendix D.

One operation mode that is possible to use is a feed and bleed batch. Using this process, the proliferation and differentiation steps are separated into two batches. The proliferation would consist of two batches of 72 hours, and the differentiation of 65 hours. Using this system, the medium requirement is much less than when using perfusion, which reduces the cost. Due to time limitations, this option was briefly explored in this report, but it is recommended to investigate it further in later research.

## Proliferation

For proliferation, a stirred tank bioreactor of 20 m3 in continuous perfusion mode will be used. The height to width ratio is 5:1. To deal with the shear stress generated in the bioreactor, flow breakers, low shear impellers and a retreat impeller, which minimises shear stress (White Mountain Process, n.d.), will be used. The goal is to harvest 1/16th of the cells every 4 days. The first day after harvest, the medium supplied will contain the following growth factors: FGF2, EGF and PDGF-BB. After the first day of proliferation, the medium that is supplied through the perfusion system will only contain FGF2. Because the doubling time of the cells is roughly 1 day (*Porcine Immortalised Adipose-derived Stem Cells | Dragon Biotechnologies*, n.d.), 1/16th of the cells will then be a ‘full’ tank, which can be harvested, and the cycle can continue again. In future research, different harvesting times can be investigated to see if they can produce more biomass and become economically feasible.

A stirred tank bioreactor was chosen, because a stirred tank bioreactor is seen as the “gold standard” of large-scale cell production (Rees et al., 2015). It has been proven to provide good mixing of nutrients and oxygen (Hanga et al., 2020; Meyer et al., 2017). Because ADSCs are fragile, the increased shear stress from the impeller is a large concern. A stirred suspension bioreactor could use flow breakers, low shear impellers and a low stirring speed to minimize shear stress (Chang et al., 2017; Strobl et al., 2020). Currently, GOOD Meat is building a cultivated meat facility that will contain ten 250,000L stainless steel stirred bioreactors, showing that very large scale stirred suspension bioreactors for meat production are possible (GOOD Meat, 2022).

For the stirred tank bioreactor, a volume of 20 m3 was chosen. It was found that stirred tank bioreactors commonly scale to about this volume (Kelley, 2007). The calculations in appendix D indicate that the maximum cell concentration for a stirred tank bioreactor of this size is 4.485 kg \* m-3.

A continuous bioreactor has been chosen over batch because the upscaling process only has to be performed once. After this, the cells can continuously proliferate. Part of the cells will be harvested once they reach the maximum concentration, and the rest can keep proliferating in log phase. Another advantage over batch is that fresh medium can be continuously supplied. This means that cells should not have any restrictions due to nutrient availability, and toxic components such as lactate can be removed before they accumulate (Xiaoxia & Buser, 2016; Fisher et al., 2019; Yang et al., 2020; Yongky et al., 2019).

Continuous perfusion has been chosen over fed batch, because it allows for more cell growth in the same time span. Based on the doubling time, we calculated the maximum specific growth rate (μ). The cell balance took the μ and cell concentration at a specific moment into account and based on this the cell concentration over time was calculated (appendix E). For the fed batch, the total amount of cells in the reactor instead of the number of cells per volume was calculated, because the volume of a fed batch system increases over time. The calculations showed that this leads to less cell growth than a perfusion system (appendix E). This makes sense, because it can continuously exchange medium instead of adding it. Some biopharmaceutical companies have intensified fed-batch processes by replacing two fed-batch scale-up bioreactors with a single perfusion system, in proliferation, thereby shortening production duration and increasing productivity (Yongky et al., 2019).

Perfusion is chosen over chemostat, because chemostat does not allow for cell retention (Novick & Szilard, 1950). Because of this, the cells will flow out of the reactor and will be wasted. Adding perfusion is an addition to retain the cells in the bioreactor and achieve higher cell concentrations because waste is prevented.

A sugar balance was formulated for the reactor. Based on the medium formulation, consumption based on literature and the cell concentration previously calculated, the flow rate for the proliferation phase (4 days) was calculated. The calculations showed that a 96-hour proliferation phase would require 57,667 m3 of medium (appendix F).

The total gas flow rate was calculated based on the oxygen concentration. This was performed by multiplying the oxygen consumption per cell by the cell concentration. By integrating over the required 96 hours, because the cell concentration changes over time, we find that 2,527\*103 m3 of gas are required for the proliferation (appendix G).

## Differentiation

For differentiation an airlift bioreactor of 67 m3 in continuous perfusion mode will be used. The goal is to get the cells halfway through the differentiation. Typically, this takes ~11 days (*Porcine Immortalised Adipose-derived Stem Cells | Dragon Biotechnologies*, n.d.), so 5.5 days (130 hours) is chosen as the time for differentiation. This means that they have grown substantially but will not have large limitations due to shear stress. Because the differentiation takes longer than the proliferation, more differentiation reactors than proliferation reactors are required.

A major problem in the differentiation of the cells is that ADSCs become buoyant due to lipid build-up. The cells naturally float to the top of the reactor and interfere with the nutrient and oxygen flow, decreasing yield (Daquinag et al., 2013). Because of this, the reactor for differentiation needs to create a downward flow that is faster than the upward velocity due to the buoyancy.

In a stirred tank bioreactor, stirring creates a vortex which drags the cells down to counteract the buoyancy. However, this will also create shear stress because of eddy currents created by the impellers. If the eddy currents are smaller than the adipocytes, the cells will rupture due to shear stress. The maximum agitation speed for a given volume at which the eddy currents are smaller than the adipocytes was calculated based on the Kolmogorov length (appendix x). According to the calculations, the maximum agitation speed in a reactor of 5 m3 is ~2.2 rpm, while in a reactor of 20 m3 it is ~1.6 rpm. This shows a downward trend in which a larger bioreactor can only have a lower stirring speed. The buoyancy was calculated according to stokes’ law (appendix J). The downward velocity in the tank was based on the maximum agitation speed and on the flow number and downward flow of a low shear impeller (appendix C). These calculations showed that the cells will flow to the top of the reactor faster than that they are pulled down in a stirred tank bioreactor, so the cells will accumulate on top. This calculation is not very precise, but does give an order-of-magnitude estimation of the feasibility. Because the upward flow is orders of magnitude larger than the downward flow, it is assumed that the process is not feasible. For a more precise calculation of what happens in the bioreactor, a computational fluid dynamics analysis should be performed.

For an airlift bioreactor, an order-of-magnitude estimate could not be calculated due to the complex hydrodynamics. In literature, a computational fluid dynamics analysis for an airlift bioreactor of 300 m3 was found. In this analysis, downward flow in the reactor has been shown to be 1.5 m\*s-1, and the average flow is around 0.5 m\*s-1 (Li et al., 2019). Because the buoyancy of the adipocytes will be in the order of centimetres per second, the buoyancy should not become a problem for an airlift bioreactor of this or slightly smaller scale. An airlift of 1,500 m3 has also been built previously, indicating that airlift bioreactors are possible at extremely large scales (Westlake, 1986). Because of this, an airlift bioreactor is recommended for the differentiation process.

Advantages of an airlift bioreactor are that it consumes less power than most bioreactors because of efficient gas dispersion while also being able to generate relatively lower shear stress, making them suitable for cultivating ADSCs in single-cell suspension cultures (Mutaf & Oncel, 2023; Li et al., 2020). A problem with airlift bioreactors is the foam formation at the top of the reactor, which reduces the cell viability (Frahm et al., 2009). Therefore, anti-foaming agents are required to ensure smooth operation (Mutaf & Oncel, 2023; Zhang et al., 2021). Recent studies with microporous ring spargers and microbubbles in airlift bioreactors show improved mixing efficiency, further supporting their application in large-scale adipocyte cultivation (Pajčin et al., 2022; Mahmood et al., 2015).

For the airlift bioreactor, a volume of 67 m3 was chosen, because based on the calculations (appendix D) this would allow all of the cells to be transferred from the stirred tank to the airlift bioreactor. Even if less cells will be transferred, the process is still viable. This meant that the 67 m3 airlift bioreactor is the theoretical largest volume that would be needed for the bioprocess. Based on the calculations, the bioreactor allows for a final cell concentration of 151 kg\*m-3 (appendix D). In later research, the optimal relationship between harvesting proliferation and differentiation should be investigated. Based on this, the bioreactor can be scaled down accordingly.

For an airlift bioreactor, batch, fed-batch, chemostat and perfusion modes are possible. The downside of batch and fed-batch is that the lactate accumulation becomes limiting. Chemostat and perfusion avoid these problems by removing the lactate. The problem with chemostat is that it also removes the cells, leading to increased biomass waste. Perfusion is able to achieve high cell densities, remove lactate and minimise biomass waste (Doris, 2022). For these reasons, the differentiation will be performed in perfusion mode.

The total gas flow rate was calculated based on the oxygen concentration. This was performed by multiplying the oxygen consumption per cell by the cell concentration. By integrating over the required 130 hours, because the oxygen consumption changes over time, it was found that 7,426\*103 m3 of gas are required for the differentiation.

A sugar balance was formulated for the reactor. Based on the medium formulation and consumption during differentiation based on literature, the flow rate for the differentiation phase (5.5 days) was calculated. The calculations showed that a 130-hour differentiation phase would require 7,684\*103 m3 of medium.

# V. Downstream Processing

The recovery of partially mature porcine adipocytes for cultivated fat production requires a method that preserves the fragile, lipid-filled cells and ensures high yield, while enabling industrial scale-up. To achieve adipocyte recovery, tangential flow filtration (TFF) centrifugation and decantation have been considered. Eventually it was decided that decantation is the best option as it causes the least amount of shear stress to the cells, thus preserving the integrity of the cells (Xue et al., 2020; Conde-Green et al., 2010). Centrifugation can cause damage to the fragile lipid-cells, thus significantly lowering the yield which is why it is ruled out as a method (Xue et al., 2020). Additionally, tangential flow filtration (TFF) is not recommended. It is a pressure-driven process, where the feed flow moves to the membrane surface with transmembrane pressure (Agrawal et al., 2023; Veje et al., 2024; Gerth et al., 2014). Single-pass TFF is a type of TFF that runs continuously, that can minimize high shear forces on shear sensitive cells by keeping them viable and intact (Chaubal et al., 2025). However, it has high operating costs and is not proven for large-scale production, thus it is ruled out as a method (Viegas et al., 2019). Therefore, in this project, a decantation-based approach was selected for the recovery of cultivated porcine adipocytes because of its gentleness.

## Decantation

Decanting followed by a holding tank and then a second decanting is the main downstream recommendation, since it exploits the natural buoyancy of the cells, while being a gentle method that prevents cell-lysis (Viegas et al., 2019; Rombaut et al., 2007). Passive decantation, which is a form of gravity separation, is ideal since there is minimal shear stress exposure compared to mechanical separation (Herndon et al., 2005; Condé-Green et al., 2010). The partially mature adipocytes float to the top of the suspension and pass through an enclosed sterile tubing system into a holding tank. The holding tank will contain isotonic water, which will dilute the remaining medium. It is followed by a second decantation to obtain the final product.

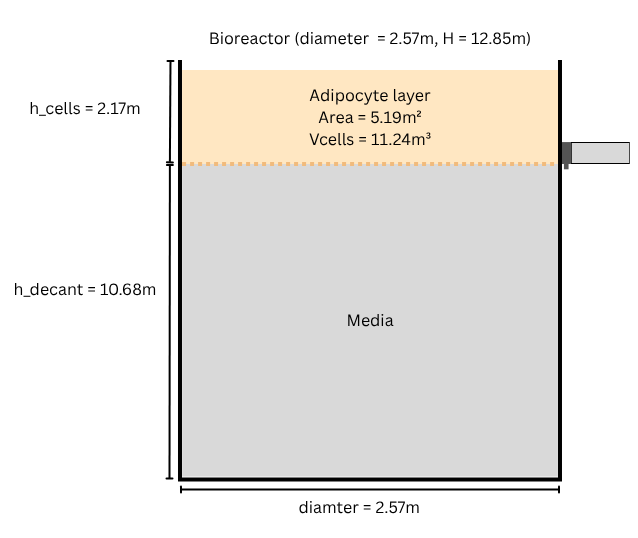
Finding out an accurate percentage of efficient separation for decantation was complicated, since large-scale animal cell literature was scarce. Studies done on microalgae in ideal conditions show separation efficiency and recovery rate of over 90% (Joel et al.,2019; Zhou et al., 2018; Ndikubwimana et al., 2016). Plastic surgery studies that performed decantation on lipid stem cells, show adipocyte cell recovery of around 73% (Condé-Green et al., 2010; Mecott et al.,2019).Based on these studies and considering the sensitivity of animal cells, an 80%-90% separation efficiency is estimated for the decanting. As a result, it is assumed that the separated mixture from the first decantation will consist of 10% medium and 90% cells. The mixture will flow into a tank with approximately 9x more water than the remaining medium. In this tank, decantation will again be performed. Now, the mixture will consist of around 90% cells, 9% water and 1% medium. This is expected to be an acceptable composition for the finalised wet paste. Although additional decanting can be performed for further cleaning, this is not recommended right now as the loss in adipocytes is not deemed worth the lower concentration of medium, since the medium is all food grade.

To effectively harvest the mature adipocytes, it is essential that only top layer of the bioreactor is decanted which will contain a high concentration of adipocytes as they float to the top once differentiated. To achieve this goal, the decantation pipe must be placed at a height which corresponds to the top layer of cells. The total reactor volume (V) of our airlift differentiation bioreactor is equal to 67 m3, and the ratio of diameter to height is 1 to 5. To calculate the diameter (d) and the height (Htotal) of the bioreactor, the formula

can be used, resulting in a diameter of 2.57 m and a height of 12.85 m. The circular cross-sectional area (A) calculated as , is equal to 5,19 m2. The volume of the cells can be calculated by dividing the total kilograms of cells per batch with the cell density of adipocytes, this results in a total cell volume:

The top amount of height that is occupied by the cell layer can then be calculated by dividing the total cell volume with the reactor surface area:

Consequently, the decantation pipe should be positioned at . This will ensure that only the cell-containing layer is removed while leaving excess medium behind. The decantation pipe will have an automated valve which remains closed during the differentiation step. Once the differentiation is complete the valve will be opened under controlled conditions to selectively harvest the differentiated, floating adipocytes.



**Figure 3.** The decanter used in this process is designed for efficient adipocyte harvest via collecting the top layer of the tank filled with adipocyte cells. The harvesting will be done with a tube positioned at the interface between the aqueous phase (media) and the cell layer, and will direct the cells into a separate collection tank when the valve opens.

## Design overview

Following the literature review and decision making, a design has been developed for the bioprocess for the cultivation of pork fat. This design includes a seed train for scale up, the proliferation and differentiation bioreactors, and the downstream processing based on decantation and medium dilution. A schematic overview of the bioprocess design is shown in Figure 4.

A diagram of a chemical process

AI-generated content may be incorrect.

**Figure 4.** A simplified schematic overview of the bioprocess design for the production of porcine adipocytes. The first step is the expansion of immortalized adipose-derived stem cells through a seed train. Following this, the cells enter a perfusion reactor for proliferation, after which the cells are differentiated in an airlift bioreactor. Both the perfusion and airlift reactors can operate in feed-and-bleed or continuous perfusion mode with cell retention. The adipocytes accumulate fat during differentiation, leading to buoyant mature adipocytes which float to the top of the tank. The cell harvesting is performed by using an automated decantation valve that opens once the differentiation phase is complete. The secondary decantation tank receives the cell-rich fraction, which contains approximately 2-10% residual culture medium from the first tank. To decrease medium content in the final product, the cells are transferred to a secondary decantation tank which is filled with water. From this tank the decantation step is repeated. The decanted adipocytes are then transferred to a storage tank and packaged as a fat paste, which is sold to other cultivated meat companies as a fat ingredient.

# VI. SuperPro Designer

SuperProDesigner was used to create a model of the envisioned process (see Appendix L). Based on the cost analysis in chapter VII, the break-even cost of this model is €675 kg-1. This is much higher than expected to be economically feasible and thus two alternative models were made in SuperPro Designer (see Tabel 3). The 2nd model has the same perfusion operating system as model 1 but has a tenfold increase in glucose concentration in the medium, while keeping the other medium components at the same concentration.. This means that on paper, much less medium is needed for a similar cell growth in the reactors. This concentration is not feasible in reality as glucose can be toxic at concentrations of 15 g\*L-1 and higher, but the analysis was still done with this concentration to get an idea of the impact on costs. The 3rd model has a feed and bleed system rather than a perfusion system. Since the medium is frequently refreshed in this system, lactate accumulation is less of an issue and thus the glucose concentration can be higher in this system too, once again reducing the amount of medium required per number of adipocytes produced. In each of these models the medium preparation, the seed train and the downstream processing is the same. The three models will be described here and are followed by a cost-analysis.

**Table 3.** An overview of the three models made in SuperPro Designer with their operating mode, medium glucose concentration and break-even selling price.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Operating mode** | **Glucose concentration medium** | **Break-even selling price product (kg-1)** |
| **Model 1** | Perfusion | 3.1 g\*L-1 | €675 |
| **Model 2** | Perfusion | 31 g\*L-1 | €95 |
| **Model 3** | Feed and Bleed | 7.8 g\*L-1 in proliferation  31 g\*L-1 in differentiation | €130 |

**Medium preparation; P1-P12:**

The medium is mixed and sterilised to the concentrations described in chapter III, with adjustments in glucose concentrations per model (see table 3). Heat sensitive components such as vitamins, growth factors and amino acids are sterilised through nanofiltration (P1), while the salts, the majority of the water and the glucose are sterilised with steam at 121 °C (P3). The medium is then mixed together and transferred to holding tanks (P8, P9, P11, P12) for each step of the process where growth factors specific to each step are added.

**Seed train; P13-P17:**

The seed train consists of five steps: 280 mL shake flask (P13); 2.8 L shake flask rack (P14); 28 L bioreactor (P15); 280 L bioreactor (P16) and a 2800 L bioreactor (P17). In each step the same basic procedure is followed. First the medium is added from the holding tank and heated to 37 °C. Then the reactor is inoculated with the contents of the previous step (in the first step this inoculation occurs with 0,5 g biomass from the cell line). The reactor is incubated for 60 hours (approximately 3 doubling times) and the following reaction occurs:

1 glucose + 0.5 amino acids + 2.7681 O2 à 2.7681 biomass + 2.7681 CO2 + 5 H2O

After the final step in the seed train a biomass density of 1.18 g\* L-1 is reached with which the proliferation bioreactor is inoculated.

**Perfusion Proliferation & Differentiation (Model 1 & 2); P18-P20:**

The proliferation occurs in a stainless steel stirred tank bioreactor (P18) with a working volume of 20 m3. It is initially filled with 13.5 m3 of medium, which is heated to 37 °C. After inoculation, perfusion occurs with either a 600 L\*h-1 (model 1) or 60 L\*h-1 (model 2) of medium pumped through. Model 2 has a lower flowrate because with a higher glucose concentration, less medium is needed for the same amount of growth. After one day, the medium composition is changed slightly by removing EGF and PDGF-BB, while maintaining FGF-2. Perfusion continues for an additional 3 days with the FGF-2 priming medium. The biomass growth follows the same reaction as in the seed train. Aeration consists of regular air. After 4 days a biomass density of 4.85 g\* L-1 is reached in model 1 and 15.5 g\* L-1 in model 2.

Before inoculating the differentiation reactor, the medium of the proliferation phase is partially removed through microfiltration (P19). 16 m3 of spent medium is removed, concentrating the biomass to 24.28 g\* L-1 in model 1 and 77.6 g\* L-1 in model 2. The differentiation occurs in an airlift with a 67m3 working volume (P20). It is initially filled with 60 m3 of medium which is heated to 37 °C, after which it is inoculated with the biomass from the proliferation step. Perfusion occurs for 130 hours in which 59,107 m3 \* hour-1 (model 1) or 5,9107 m3 \* h-1 (model 2) of medium is being pumped through. Biomass differentiates to adipocytes in a 1 on 1 ratio. After 130 hours an adipocyte concentration is reached in 63 m3 of 145.3 g\*L-1 in model 1 and 156.9 g\*L-1 in model 2 in 63 m3. This is then cooled to 4 °C for preservation.

**Feed and Bleed proliferation & differentiation (Model 3); P18-P20, P24 & P25:**

The start of the batch process is the same as the perfusion process. In the proliferation phase a 20 m3 bioreactor (P18) is filled with medium, heated to 37 °C and inoculated with biomass from the seed train. A batch phase of three days is run, after which the spent medium is removed and fresh medium is added for a second 3-day batch. Since the medium is refreshed, lactate accumulation is expected to be less of an issue and thus a higher glucose concentration can be used than a regular batch, 7.8 g\* L-1, and thus more biomass growth can occur per batch. After the two batches, a biomass concentration of 5.4 g\* L-1 is reached. This is concentrated with microfiltration (P19) to 27.1 g\* L-1 with which the differentiation reactor is inoculated.

The differentiation still occurs in a 67 m3 airlift reactor (P20), where just like in the proliferation, two batches are run and the medium is refreshed in between the batches. Each batch lasts 65 hours and a glucose concentration of 31 g\* L-1 is used. A difference with the proliferation is the scaling out aspect. Rather than a single airlift reactor per batch, 10 airlift bioreactors are used so the cells can be at a lower density for the differentiation phase and enough medium can be supplied to the cells. At the end of the differentiation an adipocyte concentration of 12.5 g\* L-1 is reached in 714 m3.

**Downstream processing; P21-P23:**

The adipocytes are decanted in P21. In reality this would occur in the differentiation tank, but this is not possible to simulate in Superpro Designer thus it was chosen to represent this with a separate tank. 90% of the adipocytes are decanted and placed into a water-filled tank (P22) to dilute the remaining medium and are decanted again (P23), once again with 90% efficiency. This means that the final product is an adipocyte paste with a concentration of 822.5 g\* L-1 in model 1; 808,9 g\* L-1 in model 2 and 716,1 g\* L-1 in model 3. Per batch 7.5 MT of adipocytes are produced in model 1, 8 MT in model 2 and 7.2 MT in model 3.

**Superpro Designer limitations:**

There are several restrictions in the SuperPro simulation that limit how accurate the model can be. Firstly, is the limit of 25 process components per file, thus it was chosen to simplify the depiction of the medium sterilisation. In the actual process, all media will be sterilised but in the model only the sterilisation of the perfusion medium for the differentiation is shown, with the remainder of the medium depicted as a simple incoming stream.

Additionally, the process is designed to have medium sterilised and mixed when it is demanded in a specific part of the process, e.g. the proliferation medium. This means no large holding tanks should be necessary, as the medium is created ‘on demand’. However, SuperPro still requires the tanks (such as P-6) to be able to hold the full content of the incoming and outgoing streams, thus they are much larger in the simulation than they would be in reality.

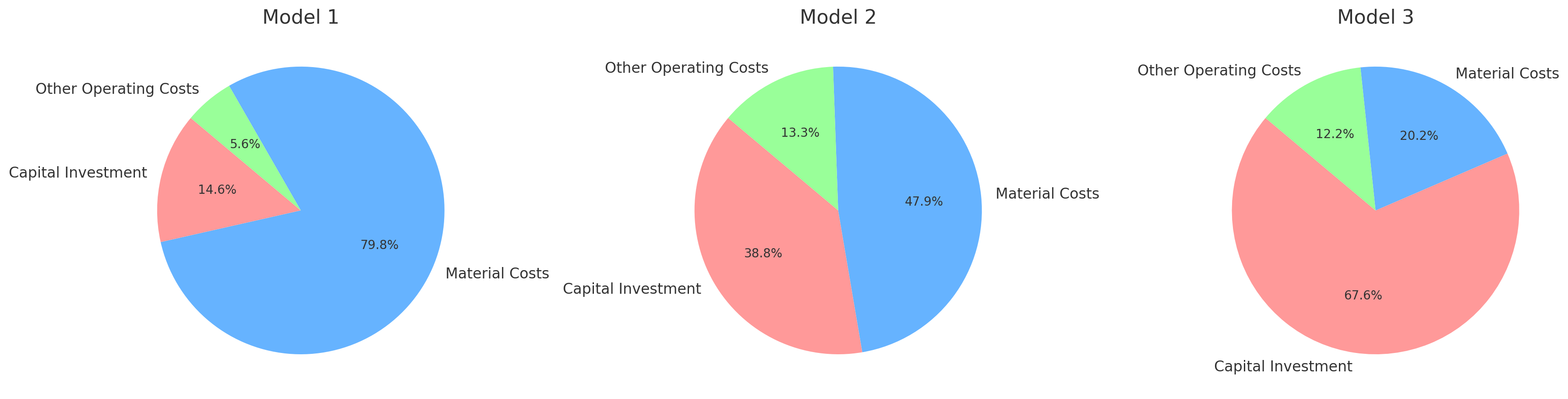
A simplified version of the medium composition is entered into SuperPro, which includes glucose, vitamins, growth factors, amino acids and salts. Of these components, only glucose and amino acids are actually used in the reactions in SuperPro, as the exact consumption of the other components is not clearly known. These components are therefore present in the same quantities in the in- and outgoing streams, something that would not happen in a real process where these components will be consumed by the cells. It was still chosen to depict these components in the medium in SuperPro model to get a more accurate cost depiction.

# VII. Cost Analysis

A cost comparison was done between the three SuperPro models discussed in section VI. The results of these are summarised in Table 4. Model 1 and 2 have the same perfusion operating system but different glucose concentrations in the medium, resulting in less medium required in model 2, the system with high glucose concentration. The 3rd model has a feed and bleed operating system with higher glucose concentrations in the medium as well. The costs are based on a production process of 5000 MT of adipocytes annually. For the first model a break even selling price of €675 kg-1, in the 2nd model this is €95 kg-1 and in the 3rd model €130 kg-1.

**Table 4.** An overview of the costs associated with all three SuperPro models based on the annual production of 5,000 MT of cultivated fat.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1** | **Model 2** | **Model 3** |
| **Break-even selling price** | €675 kg-1 | €95 kg-1 | €130 kg-1 |
| **Selling cost required for 10 year**  **payback time** | €687 kg-1 | €103 kg-1 | €164 kg-1 |
| **Total capital investment** | €579,163,000 | €312,707,00 | €1,363,045,588 |
| **Total equipment costs** | €43,626,000 | €42,987,000 | €210,646,000 |
| **Annual material costs** | €3,155,613,006 | €385,829,385 | €407,471,905 |
| **% materials costs in overall costs** | 79.78 | 47.93 | 12.22 |
| **Annual operational costs without**  **material costs** | €219,380,000 | €107,484,000 | €246,277,000 |



**Figure 6.** Pie chart displaying cost distribution for Model 1, Model 2 and Model 3. Each pie chart shows the percentage distribution of Capital, Operational and Material costs to the total cost of the respective model.

## Capital Costs

Capital costs usually consist of large upfront investments, such as purchasing and installation of equipment, utilities and construction (Kelada et al., 2021). For capital costs difficult to be found, the built in SuperPro Designer costs have been used instead.

The capital costs of the bioreactors were estimated using the MATCHES equipment cost estimation platform, with 316 grade stainless steel bioreactors and top entering axial impeller being chosen. In Table 5 the costs of the main bioreactors in the SuperPro model are given.

**Table 5.** An overview of various bioreactor costs with propellers according to MATCHES

|  |  |
| --- | --- |
|  | Cost of reactors |
| Inoculum tank 28 L | €10,584 |
| Inoculum tank 280 L | €35,453 |
| Inoculum tank 2800 L | €71,508 |
| Fermenter 20 m3 | €289,071 |
| Airlift 67 m3 | €525,522 |

As shown in table X, the total equipment costs from the two perfusion models are nearly the same, around €43 million, as nearly the same equipment is used in both models. The third model that uses a feed and bleed system has a much higher equipment cost of over €210 million euros. This steep increase is because more reactors are used in the differentiation phase, since much higher volumes of medium is used per adipocyte produced.

The costs for the cell line were not taken into account in the SuperPro model for simplicity reasons. Literature of stem cell lines for advanced stem cell therapies claiming a purchase cost, that ranges from $10,000 to $100,000 per cell-line (Aijaz et al., 2018). The DragonBio cell-line is spontaneously immortalized and suspension adapted. However, it is not a custom made gene therapy, so it is safe to assume that it won’t be in the higher price bracket.

## Material costs

In each of the three models, the main driver of the costs is the raw materials needed, specifically the medium. In the 1st model, the material costs make up 93% of the annual operating costs of the system, over €3 billion is spend on medium for the production of 5000 MT of fat per year. By increasing the glucose concentration tenfold in model 2, less medium is needed for the production of the same amount of adipocytes, thus reducing the material costs to €386 million for 5000 MT of adipocytes. In the feed and bleed model, the glucose concentration is higher too, thus here too the material costs for the production of 5000 MT of adipocytes is much lower at €406 million. the proliferation medium is estimated to cost €773.30 per m3, the priming medium €323.3 m-3, and the differentiation medium €322.4 per m-3.

the proliferation medium is estimated to cost 773.30€ per m3, the priming medium €323.3 m-3, and the differentiation medium €322.4 per m-3.

## Operational Costs

The operational costs of a cultivated fat production process stem from the continuous resources and supervision required to maintain efficient process operation. Besides material costs, these consist of labour, energy consumption, process water, and waste treatment.

The level of these costs can vary greatly, depending on the complexity, size and automation of the unit operation. Equipment such as bioreactors, which requires continuous monitoring and aseptic control, demand more labour hours and energy supply than simpler units like storage tanks, or filtration. Increasing the automation of complex operations can lead to reduced operational costs, although it usually results in increased capital investment. Waste treatment depends on the total media usage, the amount of side stream recycling, and the level of cleaning required (Humbird, 2021; Risner et al., 2021; Garrison et al., 2022).

Without taking raw material into account the operational costs for each model are €219 million for model 1, €107 million for model 2 and €246 million for model 3.

# Selling price

For each model, two selling prices were considered, a break-even price, at which the gross margin is just above 0%, and a selling price at which the payback time for the investment is approximately 10 years. For Model 1 these prices are respectively €675 kg-1 and €687 kg-1, for Model 2 €95 kg-1 and €103 kg-1 and for Model 3 €130 kg-1 and €164 kg-1. This shows that the 2nd model, using a perfusion system and a higher glucose concentration is economically the most feasible. It uses less medium than Model 1, thus greatly reducing the annual operating costs. However it also uses higher glucose concentrations than the cells are able to handle, thus it is not deemed technically feasible right now. It is recommended to do more research in the perfusion system and determine the maximum glucose concentration the system can handle in order to minimise the medium used in the process and with it minimising the production costs.

# VIII. Comparison with Livestock Method

## Sustainability Comparison

Conventional pig farming has a large environmental footprint due to feed production, manure management, and energy use. Life-cycle assessments (LCAs) estimate that producing pork releases about 6–8 kg CO₂-eq kg-1 of meat, with manure (≈43%) and feed (≈28%) being the main emission sources (de Vries & de Boer, 2010; MacLeod et al., 2013). Land use is also high with about 8–12 m2 per kilogram of pork, mostly used for farming soy or maize for feed. Moreover, global water footprint assessment by Mekonnen and Hoekstra (2012), reports that producing one kilogram of pork requires on average about 6,000 litres of water. However, about 80–85% of the water comes from rainfall, while 10–15% (“blue” water) originates from irrigation. Only a small fraction (<5%) is “grey water”, related to pollution from manure and feed runoff.

For cultivated meat, the earliest model by Tuomisto and de Mattos (2011) predicted positive outputs, with 78–96% lower greenhouse-gas emissions and about 90% less land use than European pork, assuming renewable energy and efficient medium recycling. Later research by Mattick et al. (2015) found smaller benefits, with reductions between 7% and 45%, depending on how the system boundaries and energy inputs were defined.

A more recent and detailed analysis by CE Delft (2021) showed that environmental outcomes depend strongly on the electricity source. Using conventional power, cultivated meat could emit up to 14 kg CO₂-eq kg-1, significantly higher than conventionally farmed pork. But when powered by renewable energy, emissions could drop to about 2 kg CO₂-eq kg-1. CE Delft also found that cultivated meat uses 0.2–1.8 m² kg-1 of land, about 80–85% less than pork, and less blue water (~40 L kg-1 of “blue” water) (Sinke & Odegard, 2021). The Good Food Institute (2024) reached similar conclusions, emphasizing that sustainability benefits grow as production scales and cleaner energy sources are used.

Based on process modelling in SuperPro Designer, the BigFat bioprocess (model 1) designed for 5,000 tons a year emits approximately 10.1 x 106 kg CO₂-eqyr-1 corresponding to about 2.03 kg CO₂-eqkg⁻¹ of cultivated fat. However, this data only represents the emissions mainly from aeration and sterilisation energy and excludes upstream electricity generation. This aligns with CE Delft report of 2 kg CO₂-eq kg⁻¹ of cultivated meat with renewable energy. According to the SuperPro energy simulation, the BigFat process consumes about 318 kWh kg⁻¹ of cultivated fat. The usage is mainly from aeration and perfusion in the proliferation and differentiation stage (~76%) followed by medium sterilisation and heating operation (~24%). Assuming conventional grid electricity of 0.4 kg CO₂-eq kWh⁻¹, this corresponds to 127 kg CO₂-eq kg-1 of cultivated fat of energy related emissions, resulting in total of approximately 129 kg CO₂-eqkg-1 of cultivated fat. A full life cycle assessment would be needed to determine the most accurate total emissions, however based on the simulated process, the energy related CO₂ emissions are substantial, highlighting the critical importance of using renewable energy to ensure the process is more sustainable than conventional pork farming. Lastly, the simulated process consumes roughly 2.7 × 108 kg yr⁻¹ of process water, equating to about 54 L\*kg-1 of fat, which is comparable with predicted results from CE Delft (2021).

## Cost Comparison

Conventional pork production has been optimised over the decades with breeding, feed conversion, and automation, resulting in farm-gate prices of around $2–4 per kg of carcass weight (FAO, 2020). In contrast, lab-cultivated pork is still in early development and remains expensive to produce. Several published techno-economic analyses (TEAs) vary in their cost estimates, mainly because they use different assumptions for cell densities, growth-factor prices, and energy sources.

The model developed by Humbird (2021) simulate a 100 m³ stirred-tank bioreactor operating a mammalian-cell suspension culture using serum-free, food-grade medium and projected a production cost of about $63 kg-1 of wet cell biomass, excluding packaging. Humbird’s sensitivity analysis showed that more than 80% of total cost was associated with growth-medium components. A comparable result was reported by Risner et al. (2021), where an 8.6-kilo-ton-per-year facility was evaluated. Their analysis also identified growth-factor and basal-medium costs as the primary bottlenecks, with total production costs between $40 and $100 kg⁻¹, depending on medium recycling efficiency and energy price. Both studies emphasized that achieving higher cell densities and reducing media prices would be essential to reach commercial viability. The CE Delft (Sinke & Odegard, 2021) techno-economic model evaluated several scenarios ranging from current technology to highly optimized production in the future. Estimated costs ranged widely, from about $60 kg⁻¹ under conservative assumptions, to $6–7 kg⁻¹ in an optimistic scenario using food-grade media, efficient recycling, and high cell density.

Based on SuperPro Designer simulations, the current BigFat process exhibits an estimated 9.5 × 10⁴ cells mL⁻¹ with material cost of 3.37 billion yr⁻¹ at an output of 5000 t yr⁻¹ of cultivated fat. This corresponds with €675 kg-1 fat. Growth factors represent by far the largest expense, accounting for ~93.5% of total medium cost as supported by analyses done by Humbird (2021) and Risner et al (2021). Amino acids and glucose contribute comparatively little (~6% and 0.5% respectively). The Good Food Institute (2024) also expects a gradual cost reduction as component prices fall and manufacturing processes progress.

**Table 6.** Display of comparison of sustainability parameters between conventional pig farming, cultivated meat (literature values) and the BigFat cultivated fat process (SuperPro model 1).

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Conventional**  **Pig Farming** | **Cultivated Meat**  **(Literature)** | **BigFat Cultivated Fat**  **(SuperPro Model 1)** |
| **GHG emissions**  **(kg CO₂-eq kg⁻¹ product)** | 6 – 8 | 2 – 14 *(depends on energy source)* | 2.03 (process-only)  129 (with grid electricity) |
| **Main energy contributors** | Feed production,  manure management | Bioreactor heating & mixing | 64 % sterilisation & heating, 28 % aeration & perfusion |
| **Land use**  **(m²\*kg⁻¹ product)** | 8 – 12 | 0.2 – 1.8 | – |
| **‘Blue’ Water use**  **(L\*kg⁻¹ product)** | 600-900 | 40 | 54 |
| **Key emission sources** | Feed (≈ 28 %),  manure (≈ 43 %) | Electricity &  medium production | Aeration and steam  generation |
| **Production scale (MT\*yr⁻¹)** | – | pilot | 5,000 MT\*yr⁻¹ |
| **Cost per kg of fat** | $2–4 | $40-100 | €675 (~$784) |

# IX. Societal Aspects

There are different societal perspectives on the consumption of cell-based cultivated meat. Consumer acceptance of cultivated meat depends heavily on sensory quality, the terminology used on product labels, and the way information is framed. By addressing each of these aspects, it allows to develop effective strategies to increase consumer trust and therefore sales for the cultivated fat process. Moreover, an important societal aspect is the current regulatory affairs around cultivated meat and whether the proposed bioprocess is in line with regulation.

One of the major barriers to acceptance is concerns about cultured fat having poorer sensory characteristics such as taste, texture and flavour (Benny et al., 2022; Anomaly et al., 2024; Rubio et al., 2020; Sugii et al., 2023). By feeding the cells glucose, the cells metabolise it into fat and according to literature, the composition is similar to subcutaneous pork fat (Lew et al., 2024). In the proposed bioprocess design, oleic acid will be put in the medium and although it is mainly used for inducing differentiation it also has some effect on the taste, since it increases lipid droplet formation and enhances monounsaturated fatty-acid accumulation (Belal et al., 2024; Zhang et al., 2019; Yuen et al., 2023).

The process will be positioned in the Netherlands, where cultured meat is classified under the Novel Food Regulation (EU) 2015/2283. In the bioprocess, spontaneously immortalized cell line will be used, since genetic modification is not currently allowed in EU and it also faces societal resistance (Odenwald, 2024). The European Commission (EC) has not yet granted any authorization for cell-based food isolated from animals, however two applications are currently in the early phases. Before cultured meat can enter the market, a stringent scientific risk assessment must be conducted by the European Food Safety Authority (EFSA), regarding short and long-term risks. The assessment includes genotoxicity, toxicological, nutritional and allergenicity tests and EFSA recently introduced a new nine-month deadline for risk assessments, which will accelerate the process (European Food Safety Authority, 2024; Lanzoni et al., 2024). The cultivated fat process does not contain non-food grade components, allergens or toxins so it is a fair assumption that it will positively pass the assessment.

After the assessment, an authorization from the EC must also be obtained and labelling must be transparent for the consumer (Monaco, 2025; Post et al., 2020). For marketing purposes of the cultivated fat bioprocess labels like “animal-free” and “clean-meat” are recommended, since they gain more positive consumer responses than terms like “lab-grown”, “fake-meat” or “kweekvlees” (cultured meat) (Rubio et al., 2020). Those kinds of negative terminologies tend to increase feelings of unnaturalness among consumers and can even stimulate associations with disease risks (Morais-da-Silva et al., 2022).

Animal free media will be used in this process, which promotes animal welfare and is positively shaping acceptance (Benny et al., 2022; Bryant & Dillard, 2019). Furthermore, this process is performed in sterile conditions, thus eliminating antibiotic residues and pathogen risks and improving product consistency and safety (FAO, 2020).

# X. Discussion

The project aimed to design a scalable cost-effective process for producing cultivated pork fat using non-GMO, single cell suspension adapted immortalised porcine ADSCs purchased from the company dragonbio. A three-step medium strategy (proliferation, priming, differentiation) was developed using animal-free components, replacing DEX and IBMX with plant-derived *PPAR-Y* agonists magnolol and honokiol. To prevent shear stress methylcellulose was added. For the fermentation process a stainless-steel stirred-tank and airlift bioreactors were operated in perfusion mode to prevent waste accumulation. For downstream processing, decantation was chosen over centrifugation or TFF, as this resulted in the lowest shear stress. This design was modelled in SuperPro, which lead to the finding that perfusion operation led to high media usage. To account for this a feed and bleed operating system was additionally evaluated in SuperPro.

The two main assumptions and risks associated with the medium formulation are the estimated costs of the bulk medium components and the addition of methylcellulose and oleic acid. The medium cost estimations were based on an article by Specht (2020), which was an economic analysis rather than scientific literature. In addition to this many of the component prices were sourced from Alibaba, which is notorious for providing varying cost-estimations and product-quality. Because of this, the costs for the basal medium provides a useful baseline for techno-economic modelling but likely does not accurately reflect realistic costs for food-grade bulk medium components.

Methylcellulose and oleic acid intended to reduce shear stress and improve suspension stability; however they are also shown to increase viscosity. This could potentially reduce molecular diffusion rates, leading to gradients in nutrients, dissolved gases, and waste metabolites. However, these risks can be mitigated by improving mixing efficiency in the bioreactor. To ensure that the medium composition and viscosity is viable, a pilot-scale testing phase is recommended in which the parameters such as cell growth, oxygen transfer and gradient formation are measured, before moving to full-scale implementation (Chen et al., 2024).

The harvesting time and amount of proliferation can be further optimised. Right now, 15/16th of the cells are harvested when they are at the maximum concentration, so after 4 days (thus 4 doublings), the cells are at a maximum again. If 3/4th of the cells were harvested every two days (two doublings), a new differentiation cycle could be started every 2 days instead of 4. When trying this, the impact of the proliferation and priming medium should be considered. Further research should investigate at how these media can be optimally used if the duration at which the medium is used is changed, and what the optimal harvest times and amounts would be. It should also be investigated if increasing or decreasing the differentiation time will impact the quality of the product. If an increase in differentiation is chosen, the shear stress sensitivity will increase, which should be considered in the bioprocess design.

The use of the seed train should also be investigated. If the cells are kept in the proliferation phase for a long time, it will not be required to inoculate this tank every time. However, it is likely not feasible to keep them in the proliferation phase indefinitely. It should be investigated how the proliferation efficiency will decrease over time. It should also be investigated how to get the most cost effective scheduling for maximal cell utilisation while maintaining high efficiency.

In SuperPro Designer, three versions of the process were simulated: the original perfusion process (Model 1), a perfusion process with a higher glucose concentration (Model 2) and a feed and bleed system with a higher glucose concentration (Model 3). Model 1 has the highest break-even selling cost at euro €675 kg-1, but is also deemed the most technical feasible since the other two models use medium glucose concentrations above the expected toxic concentration. Further research into the actual glucose toxicity and the upper limit of glucose concentration in the medium should be performed, to reduce the medium costs as much as possible. For the same reasons, more research should be done into the actual consumption of medium components so the composition of spent medium is known and can be recycled when possible. Furthermore, lactate and ammonia production is not taken into consideration in the SuperPro models, as it is expected that this can be kept to a minimum through controlling the glucose concentration in the medium. It is recommended to do more research into this as well to confirm that no toxic buildup of these compounds occurs. Overall, more research needs to be done for SuperPro to be an accurate representation of the expected process.

When compared to traditional livestock cultivation, Model 1 of SuperPro simulation for cultivated fat production shows significant reduction in GHG emission when considering process only contributions and substantially lower water consumption. However, including grid electricity, the GHG emissions exceeded those of traditional livestock cultivation highlighting critical need to integrate renewable energy sources. The price of the cultivated fat is significantly higher than traditional livestock cultivation, suggesting that Model 1 is not feasible for industrial translation yet. The sustainability aspect of Model 1 aligns with previously studied cultivated fat production methods, suggesting a good validation of the model itself.

# Innovative Aspects of BigFat

### Single-cell Suspension Cultivation

A key innovation of this project is the use of single-cell suspension culture for iADSC Traditional approaches to culturing fat often rely on 3D scaffolds or aggregates which help adipocytes grow and proliferate. However, these methods can create gradients in nutrient availability and limit the uniformity of cell growth and differentiation. Maintaining adipocytes as single cells ensures that each cell receives consistent access to nutrients and differentiation, resulting in a more homogeneous adipocyte population. This approach also simplifies scaling, monitoring and process control providing a flexible platform for optimisation.

### Process Upscaling

A notable innovation of this project is the integration of design considerations for large scale production addressing a gap in many studies that produce cultured fat at lab scale. Bioreactor choice, perfusion strategies, and process parameters have been optimised to allow a transition from small scale to industrial scale production. By tackling challenges associated with mixing, nutrient delivery and waste removal in larger volumes, the project demonstrates a technically viable path toward commercial production.

### Decanter-based Harvesting

The harvesting method itself represents a novel aspect of the process. This method deviates from the traditional methods of harvesting cells such as centrifugation or filtration methods. Custom engineered harvesting method was developed to ensure that the cells are harvested with minimal shear stress to keep cells intact. This approach provides a solution to one of the major bottlenecks in cultured fat production.

# XI. Additional recommendations

# Side stream handling

Finding ways of recycling the used water and steam is important, as consistent and high amounts of water are needed for cleaning and sterilisation of equipment in this process. The condensed steam from the SIP in process equipment can be collected and after that, fed back into a clean-steam generator or a boiler feedwater tank. This closed-loop system will be able to reduce water consumption. As far as CIP is concerned, CIP systems consume high amounts of water in food and biotech plants. A common practice in pharma and food industry is for the final CIP rinse to be collected and be used as a pre-rinse for the next CIP cycle. Lastly, heat exchangers can be used to recover heat from hot rinse water, to preheat incoming cold rinse. All those steps can greatly minimise water consumption (Strade et al., 2020; Pereira et al., 2020). Although, these recycling steps were not included in the SuperPro due to time-constraints, it is highly advisable to be performed.

Another major issue is the handling of the water with nutrients as a waste. These streams will contain organic carbon, nitrogen compounds and salts. Organic wastewater cannot be discharged without pretreatment. Firstly, for the inactivation of mammalian cells and bacteria it is highly recommended to perform heat inactivation to 80oC for 1 minute (Gregoriades et al., 2003). Afterwards, the medium will be handled off-site by wastewater treatment companies, the costs of which have been included in the SuperPro cost analysis. On-site biofuel production from the water-nutrient waste is also an environmentally friendly and possibly less costly option in the long run. However, from building the plant to labour and biogas handling the on-site option is not currently financially attractive and it is more of a future recommendation when the plant is established and can afford this investment (He et al., 2024).

## Renewable energy

Currently renewable energy is in the recommendation section, since the exact costs of adding it are not clear, but it is strongly recommended, since it will decrease the CO2 emissions and as a result it will make the process more sustainable. A power purchase agreement (PPA) could be a useful deal for implementing renewable energy. PPA is a long-term contract where power generators deliver green energy to industries and organizations (Stanitsas et al., 2023; Pombo-Ronero et al., 2024).

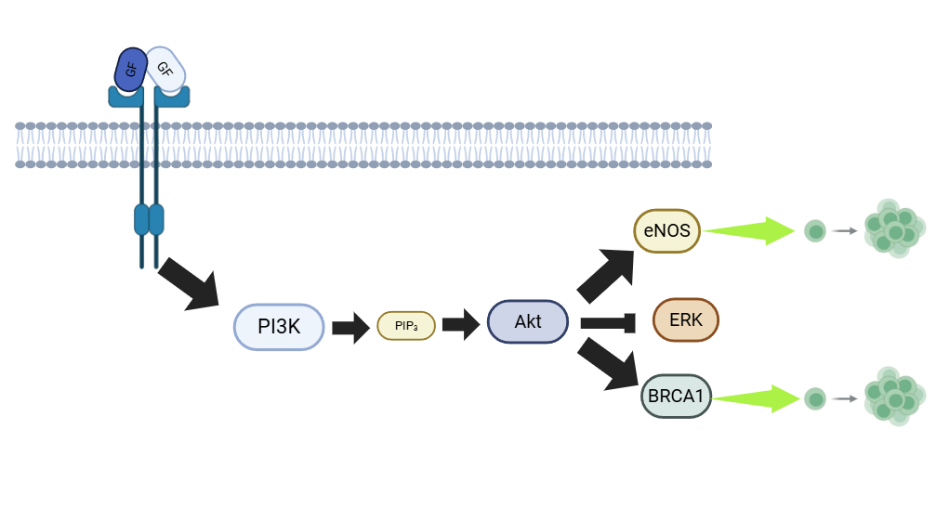
According to the central bureau of statistics wind, sun and biomass already supply more than 50 % of total electricity production in the Netherlands (CBS, 2023). Studies showed that combining solar and wind energy is the most economically feasible solution that also achieves a big reduction in overall CO2 emissions (Muller et al., 2023). Solar energy alone fluctuates a lot and the solar-wind combination provides higher stability (Kreuwel et al., 2020; Muller et al., 2023). Moreover, grid connection and battery storage are also extremely important as a back-up plan on cloudy, windless days (Bakhtvar et al., 2021). Lastly, as a future consideration, geothermal energy has high potential for heating and sterilising in the process, but it is not used for large-scale biomanufacturing yet (Willems et al., 2019; Figueira et al.,2024).

## GMO

Studies done on genetically modifying human and rat ADSCs to directly overexpress the required growth factors, successfully demonstrated an increase in cell proliferation of ADSCs achieving effects comparable with that of exogenous supplementation of the same growth factors (Mao et al., 2020; Zhang et al., 2014; Wang et al., 2019). Genetic modification involved simply integrating the growth factor gene sourced from humans to transform a lentivirus, followed by transfection and overexpression in human ADSC (Mao et al., 2020; Zhang et al., 2014; Wang et al., 2019).

It is important to explore the genetic similarity of human growth factor genes with porcine genes to assess the translatability. From Appendix L, sequential and structural similarity obtained from KEGG and AlphaFold databases can be inferred, except for PDGF, which has low sequence similarity, the other growth factors have both sequence and structural similarity, suggesting that they perform the same functions (Kanehisa & Goto, 2000; Jumper et al., 2021). Growth factors are predominantly signalling proteins, these have cleavage sites that are removed before post-translational modifications (PTMs). The presence of signal peptides and having the same cleavage sites were explored using SignalP 6.0, and based on the results, except for FGF2, a non-secretory protein lacking a signal peptide, the other two growth factors in both humans and pigs had similar signal peptide compositions (Teufel et al., 2022).

Further, UniProt was used to identify the PTMs to show that both organisms have similar modifications. This reveals that when the human gene is overexpressed in a pig cell, increased production of the protein can be expected. Unfortunately, only the PTMs of EGF have peer-reviewed data in the database, but on a positive outlook, the growth factors in both organisms show similar PTMs. Finally, KEGG was used to look into whether the proteins mentioned have similar metabolic pathways. It was found that the proteins from the two organisms had the exact same metabolic pathways except for PDGF. The main pathway these growth factors follow is the PI3K pathway (Kanehisa & Goto, 2000). As seen in Figure 7, the growth factor binds to the tyrosine kinase receptor (RTK), which activates phosphoinositide 3-kinase (PI3K), which subsequently phosphorylates phosphatidylinositol trisphosphate (PIP3). This activates the protein kinase B (AKT) protein, which inhibits extracellular signal-regulated kinase (ERK) formation while also promoting cell proliferation by activating breast cancer gene 1 (BRCA1) and endothelial nitric oxide synthase (eNOS).



**Figure 7.** Metabolic pathway shown by both pig and human growth factors showing cell proliferation as the outcome.

There are a couple of limitations that need to be looked into. As it turns out, the PDGFB gene has not been annotated in pigs and thus lacks peer-reviewed data in UniProt and KEGG (The UniProt Consortium, 2025; Kanehisa & Goto, 2000). The PDGFA protein is the closest homolog of this protein, and hence for reference in this study, the PDGFA protein of pig was compared with the PDGFB gene of humans. This explains the low sequence similarity and the absence of information regarding the PTM of this growth factor. FGF2 is not a signal peptide, and hence the unknown PTM of this growth factor in pigs makes it hard to compare across species. Regardless of the bioinformatic assays, further wet lab experiments and research on a pilot scale to investigate the attributes of these genes in pigs are necessary before starting with the transfections.

Another GMO recommendation would be to eliminate the Warburg effect with genome editing. A research group eliminated the Warburg effect in CHO and HEK293 cells, with the engineered cells being able to maintain their growth rate. They achieve that by knocking out lactate dehydrogenase as well as regulators that inhibit pyruvate conversion to acetyl-CoA (Hefzi et al.,2024). That is a really important recommendation, especially in the feed and bleed scenario, where toxicity accumulation is a bottleneck.

## Enhanced growth factors

Growth medium for animal cells are the main medium components that drive up the cost of this process, especially in perfusion bioreactor where medium is constantly needed to be replaced. Among them, FGF2 is the potential target for enhancement as it is thermally unstable at 37 °C and loses most of its activity within 24 hours (Chen et al., 2012). One of the most promising approaches to enhance the stability and half-life of FGF2 is stabilization with heparin. Although heparin helps FGF2 to extend its activity (Furue et al., 2008), heparin’s animal origin and high cost limit its suitability. To address this, engineered thermostable variants such as FGF2-G3, which contains nine amino-acid substitutions, have been developed. FGF2-G3 remains active for over 20 days at 37 °C (Dvorak et al., 2018), potentially lowering the need for frequent medium replacement. Even though production costs of this engineered growth factor remain uncertain, it offers a promising route to reduce overall medium expenses regarding the growth factor issue.

# XII. Future considerations

## Medium Recycling

Medium recycling has a potential to reduce medium costs but it is not yet economically viable. That is why it was excluded from the final bioprocess design. Its future feasibility will depend on the development of low-cost filtration membranes, automated nutrient sensing, and closed-loop sterile perfusion systems.

Different recycling bioprocess strategies show 50-70% medium reuse, with 20-80% yield improvement per unit of fresh medium. However, these approaches remain technically complex, with high costs. Dialysis and ultrafiltration cause significant capital and consumable cost, especially where the long-term sterilization is needed. Additionally, the recycled media often requires nutrient and osmolarity rebalancing, which adds a layer of operational complexity (Riese et al., 1994; Madabhushi et al., 2022; Romann et al., 2023; Nath et al., 2017; Nahmias, 2017). Since most results are from pilot-scale studies, there is no certain evidance on the commercial feasibility based on large scale perfusion systems. In fact, Believer Meats reported that dialysis-based media recycling currently increases both the cost and the risk due to membrane expenses and sterility challenges (Quek et al., 2024).

## Growth factor production from yeast

Currently recombinant growth factors are the largest economic bottleneck for producing cultivated fat within price ranges comparable to that of traditional fat cultivation. As a solution, yeast was investigated as an expression system to produce the same growth factors. However, this option is not currently included in the proposed design, due to the complexity of implementing yeast in bioprocess and the need for pilot-scale research before implementation. The complexity of implementing yeast stems from the need of having either a separate production line for growth factor production or a co-culture system of yeast and ADSCs. Also, the objective of this project is to keep the product with limited Genetic Modification (GM), which likely would not be the case if yeast were to be implemented.

Although yeast is currently not implemented in the process there is a future potential if GM regulation changes and more research is done on a larger scale. Yeast being an eukaryote itself, has similar post translation modifications (PTM) with ADSCs and hence would be an ideal expression system to produce the appropriate growth factors. *Pichia pastoris* (P. pastoris) can be cultured to very high cell densities with just minimal media, and previous studies show that these species have been used to produce GFs and EGF or overexpress FGF2 by porcine ADSCs (Liu et al., 2005; Sedlář et al., 2021; Eissazadeh et al., 2017). Additionally, *Pichia pink* (P. pink), a commercially available expression system with 100% genetic similarity to P. pastoris, has been previously utilized to overexpress PDGF-BB (Babavalian et al., 2016; Thermo Fisher Scientific, n.d.).

## Alginate Encapsulation

Alginate encapsulation was investigated as a potential approach to improve cell viability and protect porcine immortalised adipose-derived stem cells from shear stress, but it was ultimately not implemented in the current design as it is currently not feasible. Alginate encapsulation is a technique that disperses cells in a sodium-alginate solution, which are then crosslinked to form hydrogel microcapsules that act as semi-permeable barriers (Ashimova et al., 2019; Hoesli et al., 2017; Kang et al., 2021).

Multiple limitations make alginate encapsulation less suited for BigFat’s industrial process. The main constraint is mass-transfer limitation, since in alginate capsules, oxygen and nutrients move through the gel much more slowly than they do in water (Caliari & Burdick, 2016; Hoesli et al., 2017; Najdahmadi et al., 2018). Another issue is that compounds that have molecular sizes above roughly 100 kDa, such as insulin complexes, move very slowly through the alginate gel (Sahoo & Biswal, 2021). Mechanical stability of alginate capsules also presents challenges during long-term perfusion or agitation, since gels can gradually weaken as calcium ions leach out or react with phosphate (Smidsrød & Skjåk-Bræk, 1990; Ashimova et al., 2019; Breguet et al., 2007). From an economic perspective industrial-grade encapsulation equipment, sterile preparation, washing, and validation steps add significant operational and capital costs. Implementing encapsulation at ton-scale throughput would require high-speed jet-cutting or spray-based encapsulators capable of generating billions of uniform beads, which currently remain expensive and energy-intensive (Caliari & Burdick, 2016; Tan & Takeuchi, 2007; Nebel et al., 2022).

# XIII. Conclusion (Final Consultancy)

BigFat has investigated the technical and non-technical feasibility of the production of 5,000 MT of mature adipocyte using immortalized, GMO-free ADSCs in single cell suspension culture. This process is technically feasible and is more sustainable in terms of water usage than traditional pork livestock cultivation. However, with a break-even selling price of [€](https://nl.wikipedia.org/wiki/Euroteken)675, it is economically not a feasible process and thus we do not recommend pursuing it in its current state.

With the main cost driver being the medium, we recommend further research into this aspect of the process to make it economically feasible at this large scale. Additionally, while the process is more sustainable in terms of water usage, the GHG emissions are high compared to conventional pork production. Because of this renewable energy is strongly recommended for the process to be more sustainable than traditional livestock cultivation.

Since the process from a technical standpoint is feasible we do believe that a pilot scale demonstration is achievable. Stainless steel stirred tank bioreactors in perfusion allow for proliferation and airlift bioreactors in perfusion allow for differentiation, while keeping the shear stress at an acceptable level. Furthermore, the innovative design for passive decantation provides low-shear, efficient recovery of mature adipocytes. Food-grade and plant-based components used in the media maintain robust adipogenesis while being cost efficient. Further research is required to scale the process up to a production rate of 5,000 MT of adipocytes in an economically feasible way.

Genetic modification of ADSCs for autocrine growth factor expression and thermostable engineered FGF2, could offer a more financially realistic process, though this is not allowed under current EU regulations. There are a few additional considerations, that are not economically feasible now but can be implemented in the future. These include medium recycling using automation, alginate encapsulation to decrease shear stress sensitivity and yeast based growth factor co-production. In conclusion, based on the overall assessment, pilot scale validation, further wet-lab studies and investment in renewable energy integration could potentially achieve both economic feasibility and commercial desirability. However, in its current state, the proposed bioprocess is not considered feasible.

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# XV. Product sources

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2. Boyd Biomedical. (2018, June 21). The rise of single-use bioreactors: Why make the switch? Boyd Biomedical. <https://boydbiomedical.com/knowledge-center/articles/the-rise-of-single-use-bioreactors-why-make-the-switch>
3. Magnolol – Natural Magnolia Officinalis Extract (Honokiol, Magnolia Bark Extract) — Wellgreen Technology Co., Ltd., Xi’an, China. https://wellgreenxa.en.made-in-china.com/product/rmzYVJFoaRWv/China-Magnolol-Natural-Magnolia-Officinalis-Extract-Honokiol-Magnolia-Bark-Extract.html
4. MESOkine Porcine FGF basic Growth Factor — ORF Genetics, Reykjavik, Iceland. https://www.orfgenetics.com/product/mesokine-fgfb-porcine
5. MESOkine Porcine EGF Growth Factor — ORF Genetics, Reykjavik, Iceland. https://www.orfgenetics.com/product/mesokine-egf-porcine
6. MESOkine Porcine PDGF-BB Growth Factor — ORF Genetics, Reykjavik, Iceland. https://www.orfgenetics.com/product/mesokine-pdgfbb-porcine
7. Methylcellulose thickener and emulsifier, food grade — Hunan Sentai Biotechnology Co., Ltd., Hunan, China. https://dutch.alibaba.com/product-detail/Pure-Original-Ingredients-Methylcellulose-Thickener-Emulsifier-1601439301376.html
8. Oleic acid – food and industrial grade — Hubei Hongkang Daxin Chemical Co., Ltd., Hubei, China. <https://hkdhchem.en.alibaba.com/search/product?SearchText=oleic%20acid>
9. PichiaPink™ Yeast Expression System — Thermo Fisher Scientific, Waltham, MA, USA. https://www.thermofisher.com/nl/en/home/life-science/protein-biology/protein-expression/yeast-protein-expression/pichiapink-yeast-expression-systems.html
10. Sodium Pyruvate 99% – Food Grade — Zhengzhou Alpha Chemical Co., Ltd., Zhengzhou, China. https://www.alibaba.com/product-detail/Manufacturer-Direct-Sale-Sodium-Pyruvate-99\_1601566810861.html
11. Sunflower lecithin powder (pallet 540 kg, 27 boxes) — Nature Foods, USA. <https://www.naturefoods.us/product-page/sunflower-lecithin-powder-pallet-540-kg-27-boxes-bulk-wholesale>

# XVI. Supplementary Files

## Appendix A: Medium formulation

Table 7. An overview of the concentration, cost and suppliers of the components present in the basal medium. Adapted from Specht (2020).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Category | Component | Final Concentration (g\*m-3) | Cost per kg (€) | Cost per m3 (€) | Supplier/source |
| Inorganic  Salts | Calcium chloride  (CaCl₂) | 116.7 | 0.26 | 0.03 | (Specht. 2020) |
|  | Cupric sulfate  (CuSO₄·5H₂O) | 0.0013 | 2.16 | 0.000003 | (Specht. 2020) |
|  | Ferric nitrate  (Fe(NO₃)₃·9H₂O) | 0.05 | 0.52 | 0.00003 | (Specht. 2020) |
|  | Ferrous sulfate  (FeSO₄·7H₂O) | 0.417 | 0.09 | 0.00004 | (Specht. 2020) |
|  | Potassium  chloride (KCl) | 311.8 | 0.34 | 0.10 | (Specht. 2020) |
|  | Magnesium  chloride  (MgCl₂) | 28.64 | 0.28 | 0.01 | (Specht. 2020) |
|  | Magnesium  sulfate  (MgSO₄) | 48.84 | 0.56 | 0.03 | (Specht. 2020) |
|  | Sodium chloride  (NaCl) | 6995.5 | 0.34 | 2.41 | (Specht. 2020) |
|  | Sodium  bicarbonate  (NaHCO₃) | 1200 | 0.43 | 0.52 | (Specht. 2020) |
|  | Sodium  phosphate  Monohydrate  (NaH₂PO₄·H₂O) | 62.5 | 1.72 | 0.11 | (Specht. 2020) |
|  | Sodium  phosphate  dibasic (Na₂HPO₄) | 71.02 | 1.81 | 0.13 | (Specht. 2020) |
|  | Zinc sulfate  (ZnSO₄·7H₂O) | 0.432 | 0.72 | 0.00 | (Specht. 2020) |
| Other  Compounds | D-Glucose | 3151 | 0.69 | 2.17 | (Specht. 2020) |
|  | Hypoxanthine | 2.05 | 86.20 | 0.18 | (Specht. 2020) |
|  | Linoleic acid | 0.042 | 43.10 | 0.00 | (Specht. 2020) |
|  | Lipoic acid | 0.105 | 58.62 | 0.01 | (Specht. 2020) |
|  | Phenol red | 8.1 | 21.55 | 0.17 | (Specht. 2020) |
|  | Putrescine·2HCl | 0.081 | 2573.07 | 0.21 | (Specht. 2020) |
|  | Sodium pyruvate | 55 | 86.20 | 4.74 | (Specht. 2020) |
|  | HEPES | 3575 | 47.41 | 169.49 | (Specht. 2020) |
|  | Thymidine | 0.365 | 258.60 | 0.09 | (Specht. 2020) |
| Amino Acids | L-Alanine | 4.45 | 25.86 | 0.12 | (Specht. 2020) |
|  | L-Arginine HCl | 147.5 | 25.86 | 3.81 | (Specht. 2020) |
|  | L-Asparagine·H₂O | 7.5 | 25.86 | 0.19 | (Specht. 2020) |
|  | L-Aspartic acid | 6.65 | 2.59 | 0.02 | (Specht. 2020) |
|  | L-Cysteine·HCl·H₂O | 17.56 | 21.55 | 0.38 | (Specht. 2020) |
|  | L-Cystine | 24 | 21.55 | 0.52 | (Specht. 2020) |
|  | L-Glutamic acid | 7.35 | 25.86 | 0.19 | (Specht. 2020) |
|  | Glycine | 18.75 | 1.72 | 0.03 | (Specht. 2020) |
|  | L-Histidine HCl H₂O | 31.48 | 43.10 | 1.36 | (Specht. 2020) |
|  | L-Isoleucine | 54.47 | 43.10 | 2.35 | (Specht. 2020) |
|  | L-Leucine | 59.05 | 12.93 | 0.76 | (Specht. 2020) |
|  | L-Lysine HCl | 91.25 | 25.86 | 2.36 | (Specht. 2020) |
|  | L-Methionine | 17.24 | 12.93 | 0.22 | (Specht. 2020) |
|  | L-Phenylalanine | 35.48 | 24.14 | 0.86 | (Specht. 2020) |
|  | L-Proline | 17.25 | 17.24 | 0.30 | (Specht. 2020) |
|  | L-Serine | 26.25 | 34.48 | 0.91 | (Specht. 2020) |
|  | L-Threonine | 53.45 | 2.16 | 0.12 | (Specht. 2020) |
|  | L-Tryptophan | 9.02 | 12.93 | 0.12 | (Specht. 2020) |
|  | L-Tyrosine | 38.7 | 30.17 | 1.17 | (Specht. 2020) |
|  | L-Valine | 52.85 | 25.86 | 1.37 | (Specht. 2020) |
| Vitamins | Biotin | 0.0035 | 43.10 | 0.00 | (Specht. 2020) |
|  | D-Calcium pantothenate | 2.24 | 12.93 | 0.03 | (Specht. 2020) |
|  | Choline chloride | 8.98 | 30.17 | 0.27 | (Specht. 2020) |
|  | Folic acid | 2.65 | 51.72 | 0.14 | (Specht. 2020) |
|  | i-Inositol | 12.6 | 12.93 | 0.16 | (Specht. 2020) |
|  | Niacinamide | 2.02 | 6.03 | 0.01 | (Specht. 2020) |
|  | Pyridoxal HCl | 2 | 21.55 | 0.04 | (Specht. 2020) |
|  | Pyridoxine HCl | 0.031 | 25.86 | 0.00 | (Specht. 2020) |
|  | Riboflavin | 0.219 | 21.55 | 0.00 | (Specht. 2020) |
|  | Thiamine HCl | 2.17 | 34.48 | 0.07 | (Specht. 2020) |
|  | Vitamin B₁₂ | 0.68 | 12.93 | 0.01 | (Specht. 2020) |
| Added  components | Methylcellulose | 300 | 0.86 | 0.26 | Methylcellulose food grade;  Hunan Sentai  Biotechnology Co. China) |
|  | Sodium-pyruvate | 1100 | 0.22 | 0.24 | Sodium  pyruvate 99%  food grade;  Alibaba.  China |

## Appendix B: Parameters

Table 8. overview of the parameters used for calculations and the assumptions made.

|  |  |  |  |
| --- | --- | --- | --- |
| Symbol | Value | Unit | Source/reason |
| νb | 0.25 | m\*s-1 | (Park et al., 2017)1 |
| db | 10-3 | m | (Park et al., 2017)1 |
| KM | 6\*10-3 | mol\*m-3 | (Ludvigsen et all., 1979) |
| cS | 6\*10-2 | mol\*m-3 | 10\*KM 2 |
| cSIN | 17.49\* | mol\*m-3 | 3 |
| qO.UNDIF | 3.6\*10-8 | mol\*cell-1\*h-1 | (Von Heimburg et al., 2005) |
| qO.DIF | 1.8\*10-7 | mol\*cell-1\*h-1 | (Von Heimburg et al., 2005) |
| qS.UNDIF | 1.915\*10-12 | mol\*cell-1\*h-1 | (Schop, D. 2010)4 |
| qS.DIF | 1.915\*10-10 | mol\*cell-1\*h-1 | (Schop, D. 2010)4 |
| dC.UNDIF | 3\*10-5 | m | (Ge et al., 2014) |
| dC.DIF | 2.5\*10-4 | m | (Stenkula et al., 2018) |
| VK | 10-13 | M3 | (Martens 2025) |
| KOL | 4\*10-3 | m\*s-1 | (Martens 2025) |
| cOL | 0.052 | mol\*m3 | 5/ |
| cOL\* | 1.25 | mol\*m3 | 6 |
| RHD | 5 |  | 7 |
| tDIF | 240 | h | (Cheng et al., 2023) |
| η | 0.001 | N\*s\*m-2 | Viscosity of water |
| ρ | 1000 | Kg\*m-3 | Density of water |
| Np | 1.5 |  | Power number for impeller |
| Rimpeller | 1/3 |  | 8 |
| µ | 0.035 | h-1 | (Porcine Immortalised Adipose-derived Stem Cells | Dragon Biotechnologies, n.d.) |
| kd | 0.0035 | h-1 | 0.1\*µ |
| Cell weight | 1.414\*10-11 | Kg\*cell-1 | 9 |
| Cell weight | 1.59\*10-9 | Kg\*cell-1 | 9 |
| YXS | 1/3 |  | 10 |

1Bubbles rise roughly at the same speed from a diameter of 1 mm or larger. 1 mm has relative to the volume the biggest surface area. so 1 mm was chosen.

2Chosen so the µ is close to the maximum possible µ

3Apendix xxx, sugar converted to moles. For the fed-batch it is concentrated 3 times.

4Increased from source based on relative increase in surface area from human stem cells to undifferentiated cells (times 5) and differentiated cells (500 times)

5Chosen for 20% of the maximum amount of dissolved oxygen possible

6Maximum amount of dissolved oxygen possible in water

7Chosen ratio between height and diameter

8Chosen ratio between diameter of the reactor and the impeller

9Cell weight calculated. assuming cell is a sphere. Undifferentiated cells have a density of water (1000 kg\*m-3). and differentiated cells of fat (900 kg\*m-3).

10Estimated yield of biomass on glucose

## Appendix C: Agitation speed

The agitation speed was estimated using the Kolmogorov length scale too find the size of the eddy currents. If these currents are smaller than the cells. a shear stress though to cells created and that could rip open the cell. The smallest the current can be is the cell size. so the eddy currents will be set at the cell size.

A math equation with numbers and symbols

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*Formula to calculate the size of the eddy currents (λK). Depended on Kinematic viscosity (ν) and Energy dissipation (εT) (Croughan et al., 1987).*

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*Kinematic viscosity (ν) depended on Viscosity (η) and density (ρ) (Croughan et al., 1987).*

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*Energy dissipation (εT) depended on the power number (NP). agitation speed (N). impeller diameter (Di) and volume of the reactor (VL) (Croughan et al., 1987).*

Combining the formulas. the agitation speed can be made dependent on the reactor volume. this results in a maximum agitation speed for each volume.

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*Figure 8. Graph of agitation speed (y-axis in rpm) for undifferentiated cells (blue) and differentiated cells (black) depending on volume (x-axis in m3)*

For differentiated cell the agitation speed is too low to mix the reactor properly (1-3 rpm). a different type of reactor is needed to differentiate the cells.

## Appendix D: Maximum cell density

As animal cells are quite fragile. the bursting of air bubbles can create pressure differences which can burst cell. To minimize this. the correct airflow should be chosen that allows for a high amount of cells in the reactor but minimizes the decaying cells.

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*Decay rate (kd) depends on the killing volume (Vk). gas flow rate (FG). bubble diameter (db). and diameter (D) and height of the reactor (H).*

As the diameter and the height have a ratio of 5. they both can be made depending on the volume. The formula was rewritten to make the gas flow rate depend on the volume.

A math equations on a graph paper

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Specific surface area (a) depends on the bubble diameter (db). bubble rising velocity (vb). diameter of the reactor (D) and the gas flow rate (FG).

The diameter and the flow rate are both depend on the volume of the reactor. the specific surface area is then only dependent on the volume of the reactor.

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*Oxygen transfer rate (OTR) depends on the transfer coefficient (kol). Specific surface area (a). maximum oxygen concentration possible (col100) and the set oxygen concentration (col).*

As the specific surface area is dependent on the reactor volume. the OTR is then also dependent on the volume. as the other symbols are known or chosen and constant.

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*Specific oxygen uptake rate (qO) times the maximum cell concentration (cX.MAX) is equal to the oxygen uptake rate (OUR).*  
The OUR should be equal to the OTR and the qO is known for undifferentiated cells and differentiated cells). The maximum cell concentration could be calculated for the desired volume. If all the cells are transferred from the proliferation reactor to the differentiated reactor. to accumulate the increase of oxygen consumption. either multiple reactors would be needed or a larger reactor for the differentiated would be needed. Resulting in a maximum cell concentration for the proliferation of 4.485 kg\*m-3 for a 20 m3 reactor and 151 kg\*m-3 for a 67 m3 reactor for differentiation.

A graph of a function

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*Figure 9. Concentration of cells (kg\*m-3 on the y-axis)in a stirred tank, volume of the reactor (m3 on the x-axis) of 1-20 m3. cell concentration of undifferentiated cells (blue) and differentiated cells divided by 15 for better readability (red)*

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*Figure 10. Concentration of cells (kg\*m-3 on the y-axis) in an airlift. volume of the reactor (m3 on the x-axis) of 1-100 m3. and differentiated cells (red)*

## Appendix E: Cell proliferation

The proliferation of undifferentiated cells in a prefusion reactor has no incoming or outgoing cells. only an accumulation of the proliferation of the cells.

A math equation on a graph paper

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*Difference in cell concentration (cX) divided by the difference in time (t) equals the growth rate (µ) times the cell concentration (cX).*

When solving this equation. the cell concentration. the starting cell concentration and the time can be used to find the cell concentration.

The differentiated cells do not increase in numbers but only in size. They grow linearly from the undifferentiated to the differentiated size:

A math equation with a plus and a line

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*The current cell size (dc). the undifferentiated cell size (dc.undif). the differentiated cell size (dc.dif). the time of differentiation (tdif) and the time passed (t).*

A graph of a function

AI-generated content may be incorrect.

*Figure 11. Cell mass concentration over time for perfusion reactors. Concentration (kg\*m-3 on the y-axis), time (h on the x-axis), cell concentration of proliferation (blue) and differentiated cell concentration divided by 15 for better readability (black).*

The cell balance for a fed-batch reactor is almost the same as for the prefusion. but the volume increases which impacts the concentration of cells. To mitigate this. the total amount of cells (MX) are used.

A math equation on a graph paper

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*Difference in cell concentration (MX) divided by the difference in time (t) equals the growth rate (µ) times the cell concentration (MX).*

A graph of a function

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*Figure 12. Cell mass concentration over time for fed-batch reactor. Concentration (kg\*m-3 on the y-axis), time (h on the x-axis) and cell concentration of the fed-batch over time (blue).*

## Appendix F: Sugar consumption

The sugar concentration in the reactors should be kept at the same concentration.

**Perfusion**

In perfusion for proliferation and differentiation there is no accumulation of sugar as the volume is kept constant. The sugar is added and removed in the liquid stream and consumed by the cells based on the specific cell consumption and the amount of cells.

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*There is no accumulation of sugar. so the incoming stream consists of the incoming sugar concentration (cSIN) and the liquid flow (FL) minus the outgoing stream of sugar (cS) and minus the consumed sugar. The consumption is the specific sugar consumption (qs). divided by the yield of biomass on sugar (YXS) times the cell concentration (cX) and the volume (VL).*

The formula can be rewritten so the liquid flow rate (FL) is dependent on the cX for proliferation and the specific sugar consumption rate (qS) for differentiation as both are the variable depended on time. For a total liquid flow rate of 57.7 m3\*batch-1 for proliferation and 7684 3\*batch-1 for differentiation and a total amount of sugar of 181.7 kg\*batch-1 for proliferation and 2.42\*104 kg\*batch-1 for differentiation.

A graph of a function

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*Figure 13. Flow rate (m3\*h-1 on the y-axis), time (hon the x-axis), flow rate proliferation perfusion (blue) and flow rate differentiation divide by 15 for better readability (black).*

**Fed-batch**

In a fed-batch the concentration of the sugar does not change, but the volume does. This has the effect of having an increase in total amount of sugar in the reactor.

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*dMS (the change in sugar in the system of time), dt (passed time), FL (flow rate), cSIN (sugar concentration in), qS (specific sugar consumption), YSX (yield of biomass on sugar), MX (total cells in system)*

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The total amount of sugar in the system equals volume of the system time the sugar concentration. This can replace the MS and as there is no changes in the sugar concentration, dcS is equal to zero and can be removed. Combining this with the fed-batch sugar balance would remove an unknown variable.

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*Total mass balance for fed-batch. dMTOT (total mass change in system). dt (passed time). FL (flow rate). ρin (density of the incoming liquid)*

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The total mass of the system depends on the volume of the system and density of the system. The assumption is that the density of the system does significantly change over time so it can be assumed that equals to zero. Combining this with the fed-batch total mass balance would remove an unknown variable.

For fed-batch. the volume changes and the total sugar (Ms). Combining the sugar balance with the total mass balance, a formula for the liquid flow rate could be created:

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Flow rate based on time. MX.FB (mass at the start of the fed-batch)

Total flow rate 16 m3 and total amount sugar added of 840 kg.

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*Figure 14. Fed-batch feed rate over time. Flow rate (m3\*h-1 on the y-axis), time (h on the x-axis) and the flow of the medium (blue).*

## Appendix G: Gas flow

To calculate the gas flow needed at each moment. the formulas to calculate the maximum cell concentration could be reversed. Working from an increasing cell concentration (cX) for proliferation or the oxygen consumption rate (qO) for differentiation over time to the OUT and the OTR to calculate the specific surface area (a) followed by the gas flow rate (FL) depended on the time.

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*Specific surface area (a) can be made dependent on the specific oxygen consumption (qO), cell concentration (cX), transfer coefficient (kol), maximum oxygen concentration possible (col100) and the set oxygen concentration (col). For fed-batch the cell concentration is also dependent on the volume over time, as the volume changes over time.*

For the proliferation the cell concentration will change over time and for differentiation the specific oxygen consumption will change over time.

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*The gas flow rate can be calculated now based on the required specific surface area.*

The total gas flow rate over the whole batch is 2527 m3 for perfusion proliferation, 7426 m3 perfusion differentiation and 4679 m3 for fed-batch proliferation.

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*Figure 15. Gas flow (m3 on y-axis) based on time (h on the x-axis), gas flow of the proliferation perfusion reactor (blue), differentiation perfusion reactor (black) and proliferation fed-batch reactor (red).*

## Appendix H: Biomass yields

Table 9. A comparison of the yield of biomass on sugar and oxygen, the biomass concentration and the number of cells per volume.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Sugar (kgbiomass/kgsugar) | Oxygen (kgbiomass/kgo2) | Biomass (Kg\*m-3) | cells\*m-3 |
| Prefusion proliferation (20 m3) | 0.089 | 0.169 | 4.485 | 3.2\*1011 |
| Prefusion differentiation (67 m3) | 0.075 | 6.487 | 151 | 9.5\*1010 |
| Fed-batch proliferation (20 m3) | 0.06 | 0.078 | 3.835 | 2.7\*1011 |

Table 10. A comparison of liquid and gas flow rates, the total biomass produced each run and the run time.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | FL.TOTAL (m3) | FG.TOTAL (m3) | Total biomass produced (kg) | Run time (h) |
| Prefusion proliferation (20 m3) | 57.7 | 2527 | 89.7 | 96 |
| Prefusion differentiation (67 m3) | 7684 | 7426 | 10120 | 130 |
| Fed-batch proliferation (20 m3) | 15 | 4677 | 76.7 | 74 |

## Appendix I: Upwards velocity

Using the following excel sheet. the velocity of the (pre)adipocytes is calculated according to stokes’ law. The Reynolds number is checked to verify that it is below 1, because stokes’ law is only valid if this is the case. Preadipocytes are assumed to have a cell radius of 7.5 μm (Merck Millipore, 2019), and a cell density of 1052.5 kg\*m-3, which is the upper limit for human adiposed derieved mesenchymal stem cells (Drobek et al., 2023). Mature adipocytes are assumed to have a cell radius of about 100 μm (Yanina et al., 2023), and the density of adipocytes was assumed to be 900 kg\*m-3 (Farvid et al., 2005). The fluid is assumed to behave the same as water.



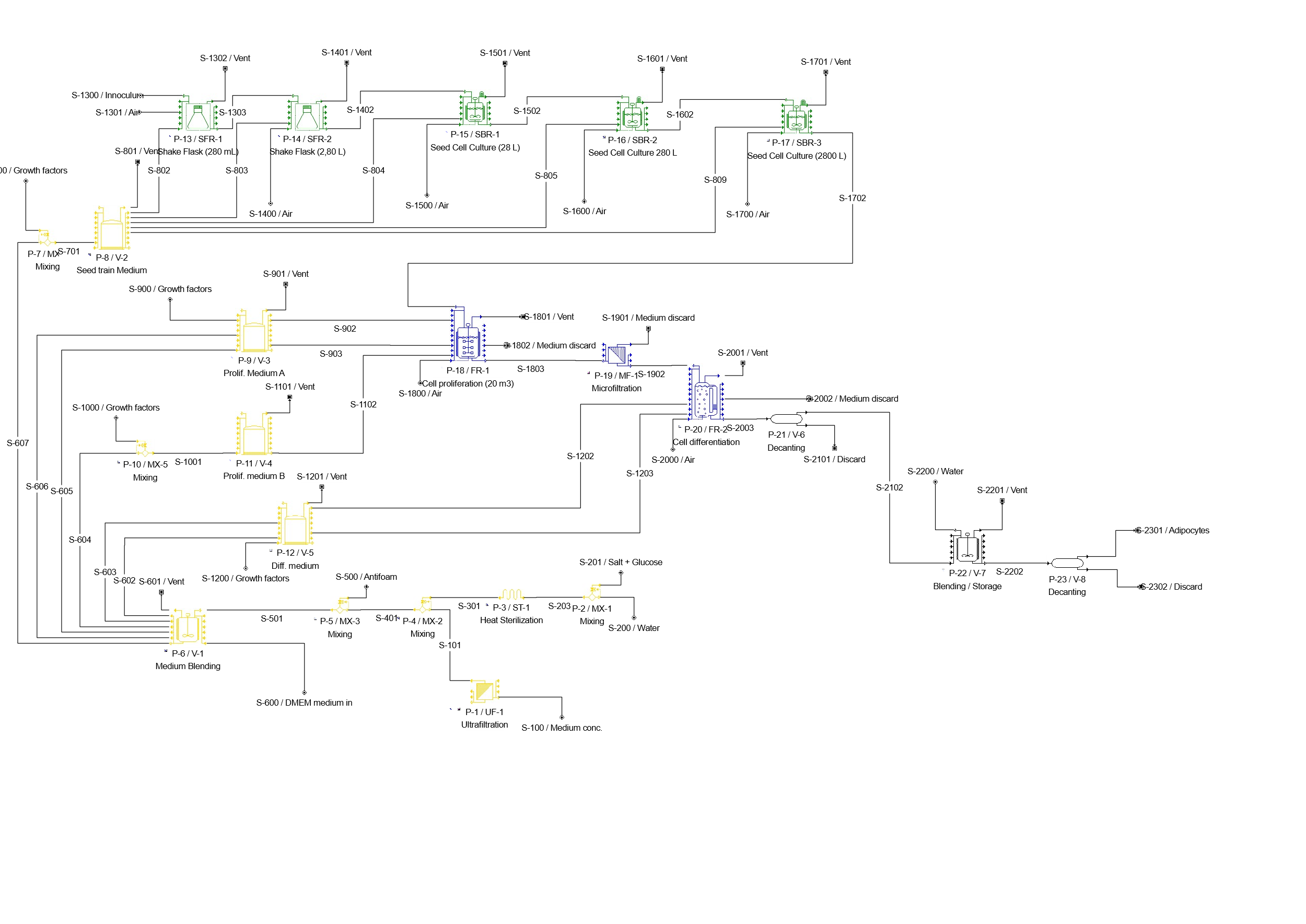
## Appendix J: Downwards velocity

Using the following excel sheet. the downward velocity created by the impellers is used. This is calculated by first calculating the total liquid flow using the flow number. then assuming that about half of the flow contributes to the downward flow. and then calculating the downward velocity based on the tank area. The flow number of the retreat curve impeller was obtained from PECO’s technical data for industrial impellers (Peco, n.d.). The other numbers are based on the MathCad calculations.

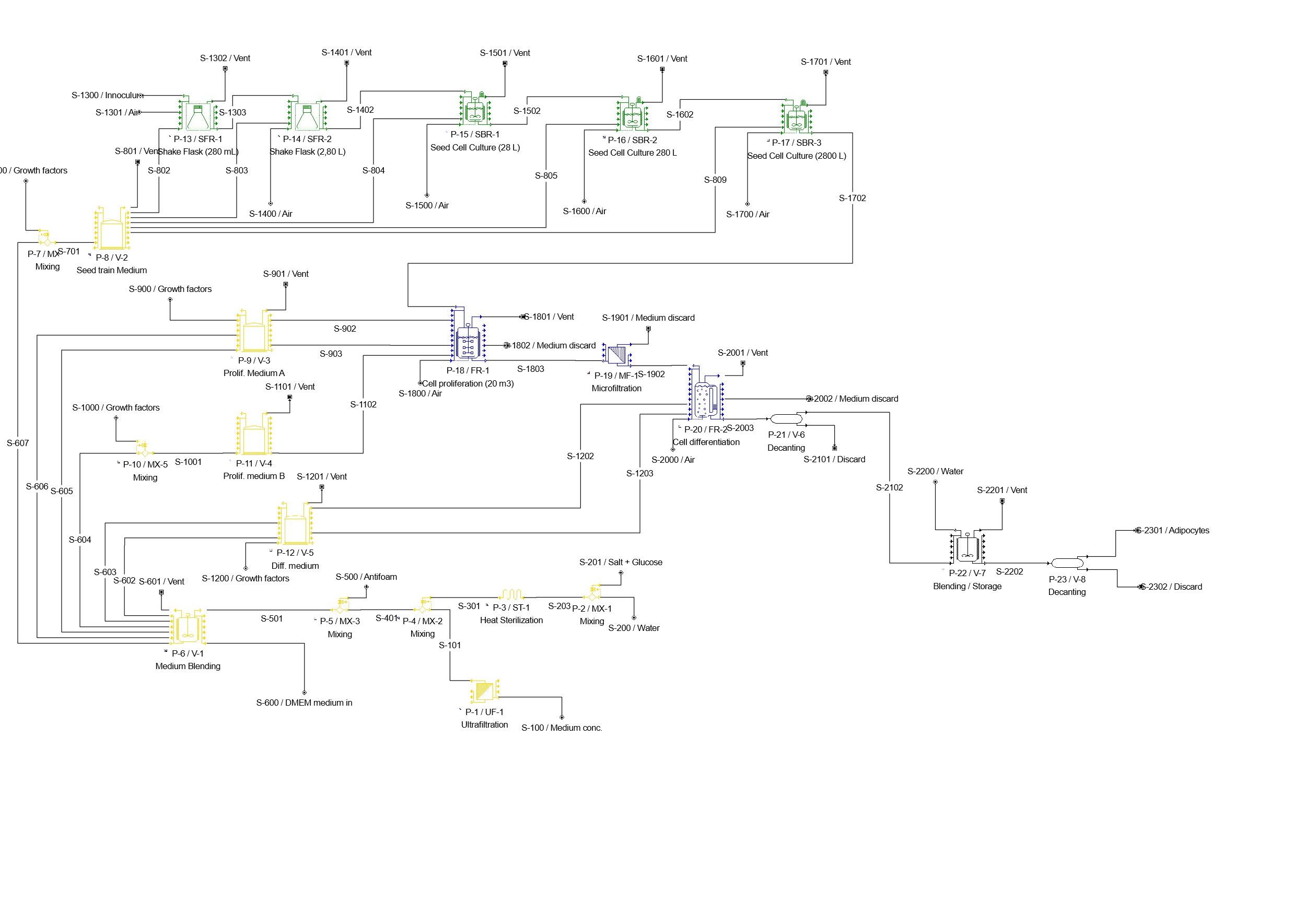


## Appendix K: Superpro design

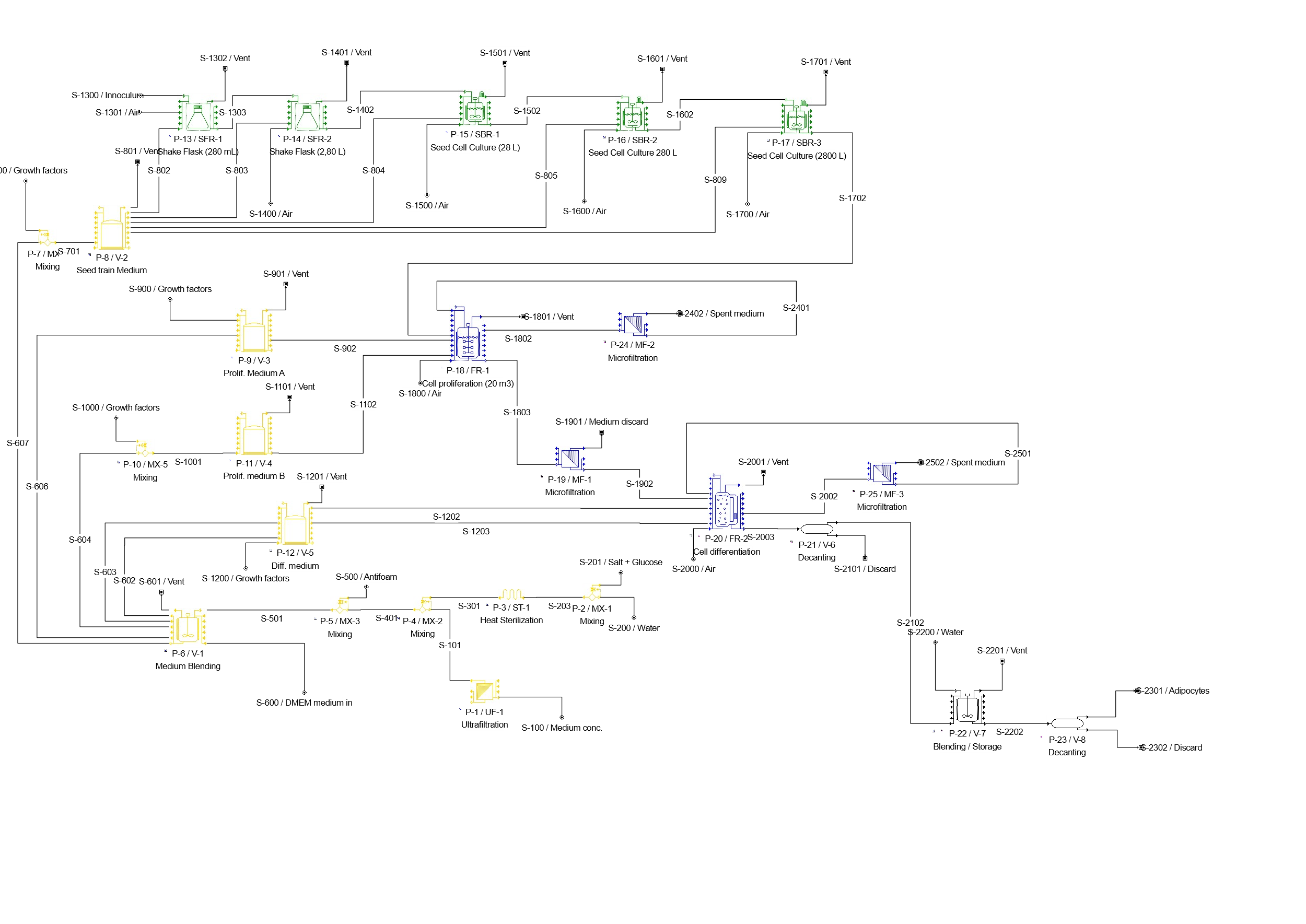
Model 1, perfusion with 3.1 g\*L-1 glucose in medium



Model 2 perfusion with 31 g\*L-1 glucose in medium



Model 3, Feed and Bleed operating system



## Appendix L: Bioinformatic comparison of human and pig growth factors

Table 11.Comparison of human growth factor genes with corresponding pig growth factor genes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Growth factors | KEGG IDs | Sequence similarity in (%) | Structural similarity in (%) | Presence of signal peptide | Post translational modification |
| EGF | 397083 – Pig, 1950 - Human | 80.3 | 70 | Present and has cleavage site at the exact position | Similar glycoslyation and disulfide bond positions |
| PDGF-BB | 100519764 - Pig  5155 - Human | 44.8 | 73 | Present and has cleavage site at the exact same position | Yet to be discovered |
| FGF2 | 397643 – Pig 2247 - Human | 70 | 70 | Does not have a signal peptide | Yet to be discovered |