Cultured (Cultivated) Meat Production

Modeling and Evaluation using

SuperPro Designer®

by

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This is the ReadMe file of a SuperPro Designer example that analyzes the manufacturing of cultured meat. The process starts with the proliferation of muscle stem cells over multiple culture steps, using bioreactors with microcarriers. After that, the cells are differentiated into muscle fibers by changing the culture medium. Subsequently, the cell suspension is sent to a crossflow filtration system for concentration and diafiltration, and then transferred to a screw press for dewatering. Lastly, the meat product is packaged and refrigerated. Approximately 3,000 MT of cultured meat is produced per year. The flowsheet of the process model is appended to the bottom of this document. You may test-drive the model by downloading the functional trial edition of SuperPro Designer from the downloads page of our website (www.intelligen.com). All the files of this example can be found in the **Examples \ Food Processing \ CulturedMeat** folder. The default installation path of the SuperPro Designer Examples folder follows below.

C:\ Users \ Public \ Public Documents \ Intelligen \ SuperPro Designer \ v# \ Process Library \ Examples

If you have any questions regarding this example or SuperPro Designer in general, please send an email message to dpetrides@intelligen.com

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Introduction

Cultured meat, also known as cultivated meat, in vitro meat, cell-based meat, or lab-grown meat, may be defined as a protein-rich food made mostly of animal muscle cells produced by cell culture that mimics conventional meat (such as beef, pork, chicken, turkey, or fish). The idea of producing meat artificially is not new; in fact, British politician Frederick Edwin Smith suggested it in 1930 [1,2]. Only in the 21st century, however, R&D around cultured meat gained momentum, and in 2013 the first cultured beef burger was created by the group of prof. Mark Post in the University of Maastricht [3]. Since then, many startup companies dedicated to various types of cultured meat have been launched throughout the world, especially in the United States (***Figure 1***).

Figure 1: Geographical distribution of cultured meat companies. Source: [4].

The potential advantages of cultured meat over conventional meat are manyfold. The production of conventional meat has a significant environmental impact, particularly with regard to land use, water consumption, energy consumption and greenhouse gas emissions [2], being considered an inefficient method of protein production in comparison with plant-based protein [5]. Moreover, the environmental impact of meat production is expected to augment as developing countries increase their per capita meat consumption [6]. The large scale production of conventional meat also relies on antibiotics, which are often overused and thereby contribute to the growing issue of antibiotic resistance [7]. In addition, livestock production can be a source of new and dangerous diseases for humans, as demonstrated by the recent

avian flu and swine fever outbreaks [4]. Another major reason for producing cultured meat is to minimize animal suffering [1].

Manufacturing of cultured meat

The production of cultured meat starts with the selection of the animal cells to be cultivated. Meat is mostly composed of muscle fibers, which are essentially mature skeletal muscle cells. Meat also contains significant amounts of fat and connective tissue, which originate from specialized cells (adipocytes, chondrocytes, and fibroblasts) [8] and affect the nutritional and sensorial properties of meat. In addition to these tissues, meat contains a small amount of blood that contributes to its characteristic color [9].

Different types of cells may be used to produce meat:

- Adult Stem Cells
    - Satellite Cells (SCs), a.k.a. Muscle Stem Cells: these are unipotent cells that exist in muscle tissue, being responsible for muscle regeneration upon injury or exercise. When a SC leaves its quiescent (dormant) state and starts to multiply, it becomes a myoblast. Myoblasts can either keep multiplying (proliferate) or differentiate into myotubes, which then mature into muscle fibers. SCs can be isolated from an animal through a muscle biopsy.
    - Mesenchymal Stem Cells (MSCs): these are multipotent stem cells that are present in various tissues such as adipose tissue, skeletal muscle, and the bone marrow. They can differentiate into several cell types that may be useful for cultured meat production, such as adipocytes, chondrocytes, and fibroblasts.
    - Fibro-adipogenic progenitors (FAPs): these cells are in the interstitial space of skeletal muscle and can differentiate into both fibroblasts and adipocytes.
- Pluripotent Stem Cells
    - Embryonic Stem Cells (ESCs): these are pluripotent stem cells isolated from an embryo; under appropriate conditions they can differentiate into any type of adult cell, including muscle cells. However, the differentiation path is longer for ESCs than for SCs, as ESCs must differentiate into mesodermal cells first, and then into muscle progenitor cells (such as myoblasts).
    - Induced Pluripotent Stem Cells (IPSCs): these are functionally like ESCs, being capable to differentiate into any type of adult cell. They are obtained by reprogramming somatic cells (such as blood or skin cells). This is practically achieved by introducing small molecules or transcription factors into the cells [8].

Although it is simpler to terminally differentiate adult stem cells such as SCs than ESCs or IPSCs, pluripotent cells hold a distinct advantage: they have a virtually unlimited proliferative capacity, that is, they can continuously divide as long as proper growth conditions are provided. In contrast, adult stem cells

possess a maximum number of cell divisions [8] situated around 50 (the so-called Hayflick number) [10]. So far, most cultured meat processes have focused on the production of muscle tissue using SCs.

In general, some kind of cell sorting is required to separate the desired cell type from others that are present in the source tissue [8]. From that point on, the manufacturing process may be divided into three major sections: cell proliferation, cell differentiation, and downstream processing [11]. Cell proliferation consists of multiple cell growth steps with the objective of increasing total cell mass, while preventing premature cell differentiation. Appropriate media and culture conditions must be used to sustain cell growth during the proliferation phase. When the target cell number is achieved, the medium and culture conditions are changed to promote cell differentiation [12]. After cell differentiation (i.e., tissue formation), the material is harvested and formulated for consumption (***Figure 2***).

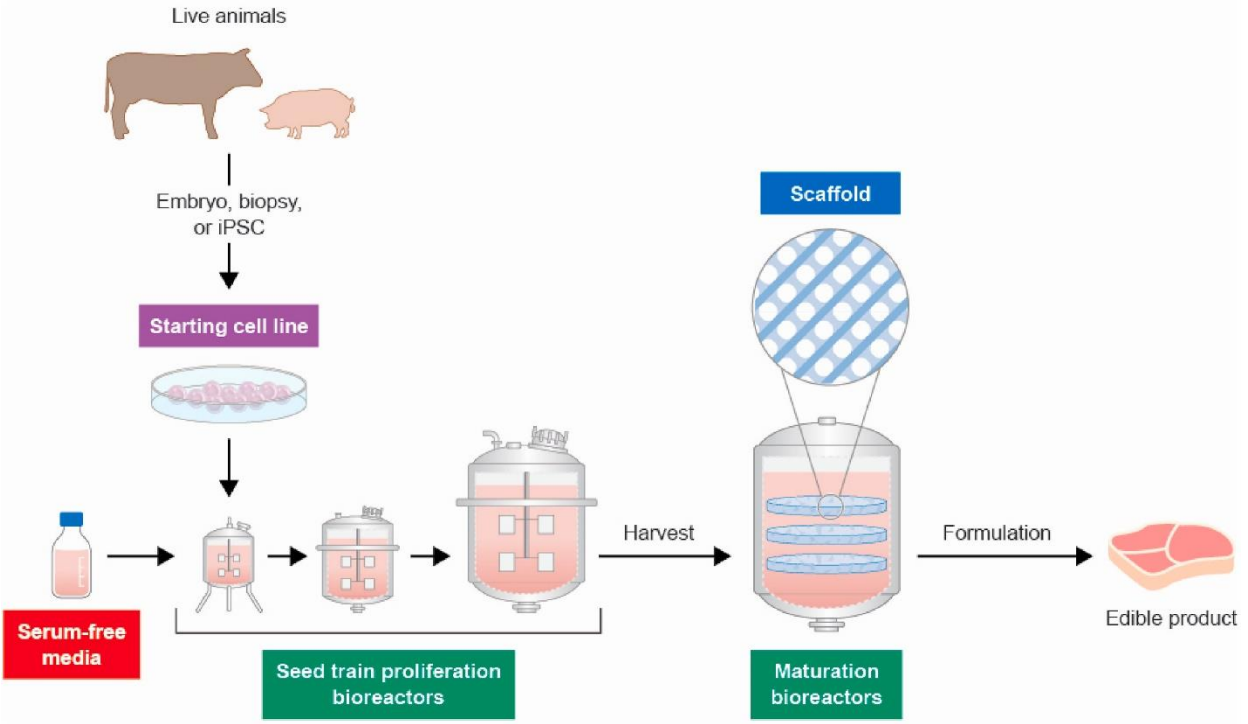

Figure 2: Main steps in the large-scale production of cultured meat. The maturation (differentiation) step may employ 3D scaffolds as shown in the illustration or microcarriers. Source: [7].

Most mammalian cells are anchorage-dependent, and thus require an appropriate surface to grow. Such is the case of SCs, which normally require a surface for both proliferation and differentiation steps. Although bottles and shake flasks may be utilized as vessels for the first cell culture steps (small volumes), bioreactors must be used for later cell culture steps (large volumes) [12,13]. All these culture systems must, at the same time, provide a surface for cell adhesion; ensure adequate mass transfer of oxygen, medium components, and metabolic waste removal; and not damage the cells with excessive shear forces. Various bioreactor types have been used in this field, including rotating wall, hollow fiber, stirred-tank, rocking motion, fluidized bed, and packed bed bioreactors [10,11,13,14], though rocking motion and stirred-tank

bioreactors have been generally preferred [10,13]. Most of these bioreactors are compatible with microcarriers (MCs), which consist of micro-scale porous beads that provide a large surface area suitable for cell growth. Alternatively, fixed 3D scaffolds may be embedded into the vessel to perform a similar function. This type of system mimics more closely the environment of cells in vivo and is particularly interesting for the cell differentiation phase, as it allows the formation of a structured material that resembles a real meat cut [2,4,7]. If MCs are used, on the other hand, the resulting material exhibits little structure, which restricts its use to the preparation of unstructured types of meat such as ground beef or patties. MCs are however much easier to scale-up due to their high surface-to-volume ratio [15]. Yet another possibility is to employ 3D bioprinting to produce complex tissue structures. 3D bioprinting consists of additive manufacturing of biomaterials using living cells, biomolecules or polymers [9]

The material of the MC or 3D scaffold used in the cell proliferation and differentiation steps must also be considered. If the material is not edible, then it must be food-compatible and either a method to separate the meat from the MC/scaffold must be available, or the material must be degradable. The separation of the MC/scaffold from the product can be achieved by various techniques, such as enzymatic, chemical, thermal or mechanical methods. If the scaffold is edible, however, then it may be partially degradable or entirely incorporated into the final product, possibly helping to provide texture to the meat product. MCs and scaffolds can be made of synthetic polymers such as poly (lactic-co-glycolic acid) (PLGA) and polylactic acid (PLA), or of natural polymers such as cellulose, alginates and chitosan [15]. Collagen, which is an animal-derived protein, is unsurprisingly a good substrate for cell growth, but should be avoided since it goes against one of the main objectives of cultured meat (to avoid animal slaughter) [16].

Process scale-up represents one of the greatest technical challenges for cultured meat production. So far, mammalian cell culture-based processes have been mostly used in the biopharmaceutical industry for the production of high-value, low volume products, with a maximum cell culture volume of 20 m^3.[7,11] (in contrast, bacterial and yeast cultures are routinely conducted on the scale of 100s of m^3). Considering a final cell density of 4 x 10^7 cells/mL and a specific wet cell weight of 1.7 x 10^{-6} mg, a volume of 20 m^3 would translate to about 1.3 metric tons (MT) of meat. Further assuming 33 production batches per year leads to an annual production of 43 MT. For comparison, global meat production reached 337 million MT in 2020. One of the reasons why it is difficult to scale-up mammalian cell processes is that mammalian cells are sensitive to shear stress, which limits the mixing rate that can be applied to the culture without damaging the cells. Consequently, it is challenging to ensure adequate mass transfer of oxygen, CO_2, medium components, and waste metabolites on large scale. Another reason is related to sterility: implementing a highly aseptic process is expensive. Sterility is particularly crucial in mammalian cell processes because mammalian cells grow much more slowly than bacterial cells, and therefore a small bacterial contamination can quickly take over the entire culture and lead to batch failure [17].

The development of cheaper culture media for cell proliferation and differentiation is another significant challenge for cultured meat production. Cell culture media are the basic raw materials for cultured meat

and are generally considered to be the largest contributor to its manufacturing cost [18]. To some extent, the large cost of media is due to the complex requirements of mammalian cells, that need various vitamins, minerals, amino acids, lipids, growth factors, attachment factors, hormones, trace elements and other compounds to grow. Traditionally, a key component of mammalian cell culture media is Fetal Bovine Serum (FBS), which contains thousands of molecules including growth factors, attachment factors and hormones that support the growth and adhesion of a broad range of mammalian cells. In the culture of skeletal muscle cells, FBS is usually added at 10-20% in the proliferation phase, and 0.5-2% in the differentiation phase (the reduction of serum concentration helps to trigger differentiation). Nevertheless, the addition of FBS carries the risk of introducing adventitious agents into the process, and, since it is obtained from animal fetuses, its usage goes frontally against one of the main reasons to produce cultured meat in the first place, that is, to minimize animal suffering. Moreover, if cultured meat displaced a significant percentage of conventional meat, the demand for FBS would drastically increase, making it difficult to supply [10]. Serum-free media do exist, however; in addition to basic nutrients such as carbohydrates, amino acids and vitamins, they contain key growth factors, hormones and other proteins that are present in serum (or that perform the same function as serum), such as albumin, transferrin, and insulin [2,10]. This type of medium has been widely employed in biopharmaceutical processes, and the high cost of mammalian cell culture media is partially due to the very fact that they are mostly applied to the biopharmaceutical industry. Indeed, mammalian cell culture media components generally have a very high degree of purity (“pharmaceutical grade”) to comply with biopharmaceutical product regulations. Besides, biopharmaceuticals are high value products that can easily accommodate expensive raw materials, giving suppliers little incentive to find cheaper alternatives. It is worth noting that the cost of serum-free media is mostly due to the proteins (growth factors, hormones, etc.); therefore, a potential strategy for medium cost reduction would be to produce them more efficiently through microbial culture [10,18].

Process Description

The following SuperPro file is included with the present example:

- **CulturedMeat.spf**

This file models the production of cultured meat using bovine satellite cells (SCs). The process starts with the proliferation of SCs over multiple culture steps using microcarriers for cell attachment. The last cell proliferation step is conducted in a bioreactor of approximately 100 m^3, in fed-batch mode, generating a cell suspension of approximately 80 m^3 with a cell density of approximately 4×10^7 cells/mL. This suspension is then concentrated by a factor of 5 through crossflow microfiltration and transferred to another 100-m^3 bioreactor for cell differentiation / tissue formation. Cell differentiation occurs in batch mode using serum-free medium specific for the differentiation of SCs and includes one medium exchange step. After that, the suspension is subjected to crossflow filtration for concentration and diafiltration, and then sent to a screw press for dewatering. The resulting meat product (containing 27% of dry solids) is then packaged and refrigerated. Approximately 3,000 MT of cultured meat is produced per year.

For reporting and analysis purposes, the process has been divided into three sections:

- Cell Proliferation (dark red)
- Cell Differentiation (blue)
- Harvest & Formulation (green)

Flowsheet sections in SuperPro are simply sets of related unit procedures (processing steps). For information on how to specify flowsheet sections and edit their properties, please use the Help tool (**Help➲ Index➲ Section**). Each section is described in greater detail next. The flowsheet of the process model is appended to the bottom of this document.

Cell Proliferation

The process starts with the culture of satellite cells previously extracted from a live animal. A sequence of 6 cell culture steps of increasing size is performed to increase the total mass of SCs. The first step (P-04 / SPR-101) is carried out in a 2-L shake flask with a working volume of 280 mL; the second step (P-05 / SPR-102) is carried out in three 4-L flasks with a total working volume of 2.8 L; the third step is carried out in a rocking bioreactor (P-06 / RM-101) with a working volume of 28 L; the fourth step is carried out in a single-use stirred-tank bioreactor (P-07 / SUB-101) with a working volume of approximately 280 L; and the fifth step (P-11 / SFR-101) is carried out in a stainless steel stirred tank bioreactor with a working volume of approximately 2,800 L. The sixth and last cell proliferation step is carried out in a stainless steel stirred tank bioreactor (P-26 / FR-101) with an initial working volume of approximately 28,000 L and a final working volume of approximately 78,000 L. Every cell culture step from P-05 to P-26 is initiated by adding the entire

suspension from the previous step to the culture medium. The first 5 cell culture steps are entirely conducted in batch mode, whereas the sixth (main) cell proliferation step includes a batch phase and a fed-batch phase. During the fed-batch phase, approximately 52,000 L of medium is added to the vessel. The culture medium is assumed to be the same for all cell proliferation steps, except for the fed-batch phase of the sixth step. The composition of the cultured media used for cell proliferation is shown in ***Table 1***.

Table 1: Composition of the culture media used for cell proliferation.

Component	**Batch Medium**	**Fed-Batch Medium**
Glucose	7.0 g/L	182 g/L
Amino Acids	1.5 g/L	39 g/L
HEPES	3.6 g/L	9 g/L
Salts	8.8 g/L	23 g/L
rProteins (growth factors, hormones, etc.)	30 mg/L	30 mg/L

Medium preparation procedures have been included only for the larger cell culture steps. For culture step P-07, all the medium components are mixed in a single vessel and filter-sterilized. For culture steps P-11 and P-26, the heat tolerant components (glucose, amino acids, HEPES and salts) are mixed and heat-sterilized while the heat-sensitive components (rProteins) are filter-sterilized (for P-11, this filter-sterilization has been omitted).

In all cell proliferation steps, cell growth is assumed to be exponential with a specific growth rate of 0.025 h^{-1} and an initial cell density of 2×10^5 cell/mL = 0.18 g /L (a conversion factor of 1.11×10^6 cell/mg of dry cell weight was calculated considering a cell diameter of 17.6 µm, a specific cell weight of 1.05 g/cm^3, and a cell water content of 70%; more information on this kind of conversion are provided in the Viral Vaccine example located in the **…Process Library / Examples / Pharmaceuticals** folder). Moreover, in all *batch* steps, cell growth was modeled by a Batch Kinetic Fermentation operation using the specific growth rate mentioned above and the following mass stoichiometry:

$$100\ \text{Glucose} + 13\ \text{Amino Acids} + 85\ O_2 \rightarrow 30\ \text{Satellite Cells} + 116\ CO_2 + 52\ H_2O \quad (1)$$

The expansion factor between consecutive batch culture steps is 10×, so that the duration of each one of the first 5 steps must be 92 h. On the other hand, the sixth (main) cell proliferation step has a batch phase that takes 96 h, and a fed-batch phase that lasts 112 h. Each phase is modeled by a Batch Stoichiometric

Fermentation operation. In both operations, cell growth is represented by the same stoichiometry applied to the other steps (Eq. (1)), along with a conversion rate of 90%. The cell density by the end of this step is 3.7×10^7 cell/mL (33 g/L on a dry basis).

Sterile and edible microcarriers are also added to each cell proliferation step to support the growth of SCs (which are anchorage-dependent cells). The concentration of microcarriers is 2 g/L in the batch steps, and 10 g/L at the beginning of the fed-batch step.

After the target cell density is achieved in the main cell proliferation step (P-26 / FR-101), the suspension is sent to a crossflow microfiltration procedure (P-27 / MF-101). This step is carried out using three filters operating in parallel, with a membrane area of 300 m^2 each. The cell suspension is concentrated by a factor of 5, with an average filtrate flux of 30 $L/m^2/h$. The membrane rejection coefficient is assumed to be 0.99 for both cells and microcarriers. The retentate is returned to the bioreactor (P-26 / FR-101), having a volume of approximately 16,000 L and a concentration of dry solids close to 180 g/L (164 g/L of dry cell weight and 16 g/L of microcarriers).

All cell proliferation steps are conducted at 37 °C with an average aeration rate of 0.05 VVM, except for the fed-batch phase of the main proliferation step, which has an average aeration rate of 0.10 VVM. Sterile air is supplied by a set of air filtration (P-23 / AF-101), gas compression (P-24 / G-101) and flow distribution (P-25 / FDIS-101) procedures.

Cell Differentiation

In this section, the SCs are differentiated into muscle fibers, primarily by modifying the culture medium. Firstly, the concentrated cell suspension produced in the previous section is mixed with 62,800 L of cell differentiation medium in a stirred tank bioreactor (P-38 / FR-201). For the sake of simplicity, the differentiation medium is represented by the same composition as the batch cell proliferation medium (in reality, the key difference is the lower concentration of growth factors, which are but a fraction of the rProteins component). As in the main cell proliferation step, the heat tolerant components of the medium are heat-sterilized (P-29 / PZ-201 and P-32 / PZ-202), while heat-sensitive components (hormones, transferrin, etc.) are filter-sterilized (P-35 / DE-201 and P-37 / DE-202). Differentiation is carried out at 37 °C, with an average aeration rate of 0.05 VVM; sterile air is supplied by the same procedures used for cell proliferation. Cell culture is conducted in batch mode but includes one medium exchange half-way through. Each culture stage takes 2.5 days and is modeled by a Batch Stoichiometric Fermentation operation containing three reactions, as indicated in ***Table 2***.

Table 2: Sequence of reactions representing cell differentiation.

Reaction	Conversion Rate	
	Stage 1	Stage 2
Cell Differentiation		
Satellite Cells → 30 Muscle Fibers	50%	100%
Cell Growth		
100 Glucose + 13 Amino Acids + 85 O_2 → 30 Muscle Fibers + 116 CO_2 + 52 H_2O	50%	50%
Cell Maintenance		
180 Glucose + 192 O_2 → 264 CO_2 + 108 H_2O	90%	90%

In both cell differentiation operations, the cell water content was specified as 70% (for more details on how to set the cell water content, please consult the Industrial Enzymes example in the **…Process Library / Examples / Bio-Materials** folder).

The medium exchange step was modeled by a Batch Component Splitting operation (to remove the spent medium) followed by two Pull-In operations (to introduce fresh differentiation medium and rProteins separately). In practice, at first the cells are separated from the medium by sedimentation, which is simple given that the cells are attached to microcarriers; the supernatant is then removed; and finally fresh medium is added to the vessel. In the Batch Component Splitting operation, 80% of the liquid-phase components – glucose, amino acids, HEPES, salts, and water – are removed, while 100% of the cells and microcarriers remain in the vessel. In the subsequent Pull-In operations, fresh differentiation medium is added so that the total volume becomes 80,000 L, and rProteins are added so that their concentration is restored to 30 mg/L. By the end of cell differentiation, the culture volume is 80,000 L, the concentration of muscle fibers is 34 g/L on a dry basis (approximately 113 g/L on a wet basis), and the concentration of microcarriers is approximately 3 g/L.

Harvest & Formulation

After cell differentiation, the spent medium must be removed, as it contains buffer components, hormones, growth factors, and other molecules that should not be present in an edible product. For that purpose, the suspension is sent to a microfiltration system composed of a retentate tank (P-40 / TDF-301) and three crossflow filters operating in parallel (P-41 / DF-301). Each filter has a membrane area of 300 m^2. Firstly,

the insoluble components (muscle fibers and microcarriers) are concentrated by a factor of 2×, and then the concentrated suspension is diafiltered with 5 volumes of water. The average filtrate flux is 30 L/m^2/h during both concentration and diafiltration operations. The membrane rejection coefficient is assumed to be 1.0 for both muscle fibers and microcarriers in the concentration operation, and 0.995 for both muscle fibers and microcarriers in the diafiltration operation. The resulting suspension has a volume of approximately 40,000 L, a concentration of muscle fibers near 66.5 g/L (on a dry basis), and a concentration of microcarriers near 6.3 g/L.

Next, the suspension is dewatered by means of a screw press (P-42 / SP-301). In the Screw Pressing operation, muscle fibers and microcarriers are considered as particulate solids with a retention rate of 97%. The total amount of (dry) particulate solids in the cake is set to 27% (w/w); considering that muscle tissue contains approximately 70% of water, the amount of wet solids in the cake is approximately 85%. Given that this cake is essentially composed of unstructured muscle fibers, it would be suitable for manufacturing burgers, for example. It is worth noting that about 2% of the cake mass corresponds to microcarriers, which were assumed to be edible and therefore may be present in the product.

Lastly, the meat product is packaged in plastic bags (P-43 / DCS-301) and refrigerated (P-44 / FT-301).

Process Scheduling and Cycle Time Analysis

The overall Batch Time for the production of cultured meat is 34 days. This is the time elapsed from the start of a given batch (cell culture in shake flasks) to the end of that batch (refrigeration). On the other hand, the Recipe Cycle Time (RCT), defined as the time between consecutive batches, is only 24 h. This is possible because many unit procedures in the process are shorter than one day, and multiple (staggered) units are used for those that are longer. The user can find the overall Batch Time and the minimum RCT for the process in the Recipe Scheduling Information dialog (**Tasks➲ Recipe Scheduling Information…**). In this dialog, the user may also specify the RCT that he or she desires, as long as it is larger than or equal to the minimum RCT, which is 23.76 h for the present process.

To visualize the process schedule, the user may select **Charts➲ Equipment Occupancy➲ Multiple Batches**. This will generate the Equipment Occupancy Chart (EOC), presented in Figure 3. The EOC displays the utilization of each equipment item over time. A total of 20 batches are shown, with each batch marked by a different color. The process sections are also indicated in Figure 3.

It is easy to see in the EOC that each cell culture step is carried out in multiple equipment units that rotate between batches; for example, six bioreactors are employed for Cell Differentiation (FR-201, FR-201b, FR-201c, etc.). These equipment units are said to operate in **Stagger Mode**; this configuration enables the plant to initiate a new batch every 24 h, even though each cell culture step takes longer than one day. More information on how to specify equipment in Stagger Mode can be found in the **Farnesene** example located in the **…Process Library / Examples / Bio-Materials** folder.

Another view of the process schedule is provided by the Operations Gantt chart (in the MS Project style). The Gantt chart displays detailed scheduling information for one or multiple batches. The Gantt chart for a single batch is generated by selecting **Charts➲ Gantt Charts➲ Operations GC**. Figure 4 displays a portion of the Gantt chart for the present process, showing the scheduling of the first operations in the Cell Proliferation section. The golden bar indicates the duration of the entire recipe, while the dark blue and cyan bars represent the durations of procedures and operations, respectively.

The Gantt chart enables users to visualize the execution of a batch process in detail. It also facilitates editing of batch recipes. Double-clicking on any of its bars brings up the dialog of the corresponding entity (e.g., operation, procedure, recipe, etc.). The simulation calculations can then be redone, and the chart can be updated by clicking on the refresh button of the chart ().

Furthermore, SuperPro can export its scheduling data to **MS Project** by selecting **File➲ Export to MS Project XML File.** Likewise, SuperPro can export its recipe data to SchedulePro by selecting **File➲ Export to SchedulePro's Recipe DB.** SchedulePro is a resource management, production planning and scheduling tool available from Intelligen. Please consult the Help facility for information on these two exporting options (**Help➲ Index➲ Exporting**).

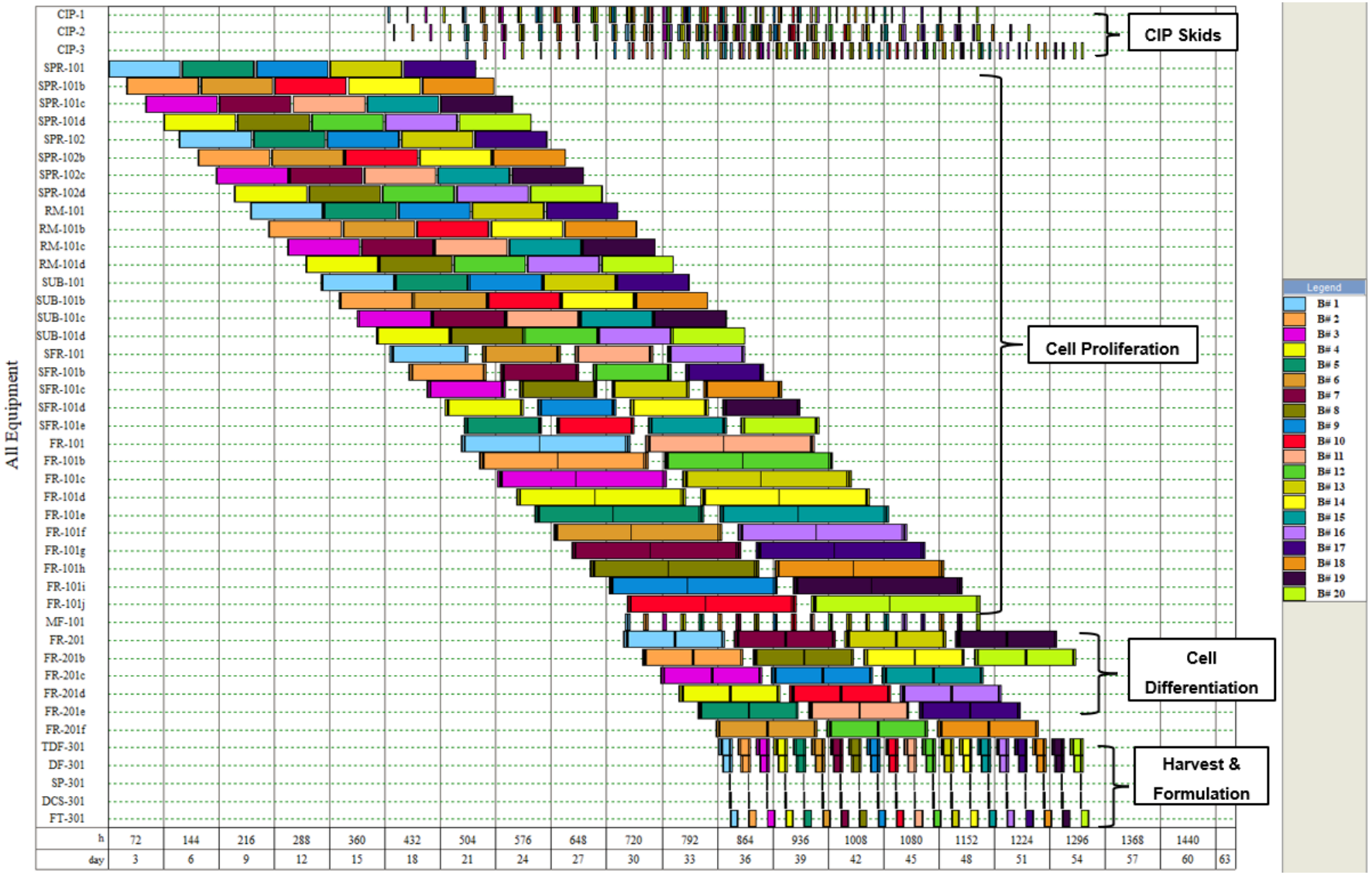

Figure 3: Equipment Occupancy Chart (EOC) for 20 batches (medium preparation equipment omitted for the sake of clarity).

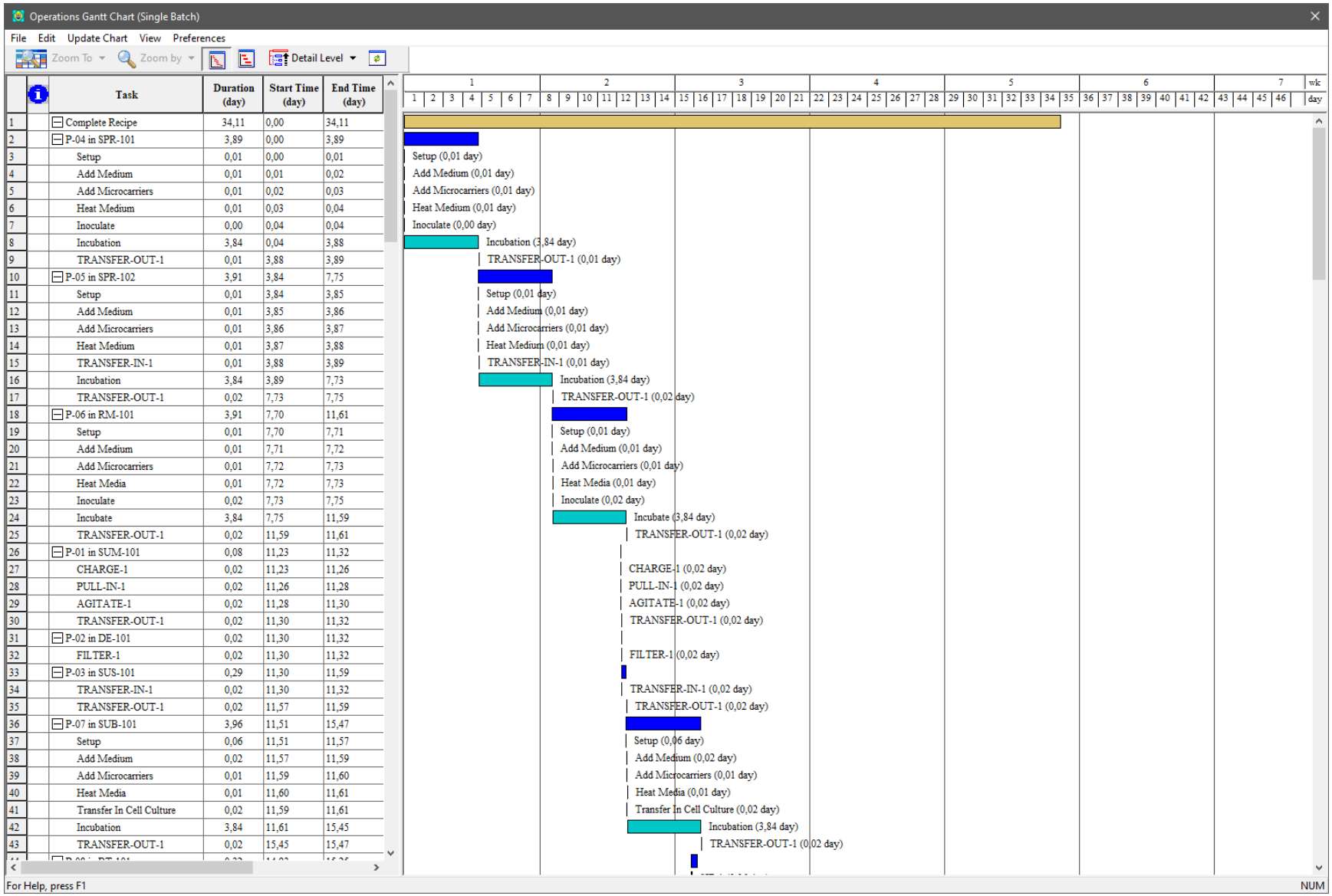

Figure 4: Operations Gantt Chart (portion of a single batch).

Material Balances

Table 3 displays overall process data such as batch size, annual production rate and number of batches per year. This table was extracted from the RTF version of the *Materials & Streams report (SR)*, which can be generated by selecting **Reports➲ Materials & Streams** from the main menu bar of SuperPro. The format of the report can be specified through the dialog that is displayed when you select **Reports➲ Options** from the main menu bar.

The table indicates that the batch size is 9,882 kg, which is a little lower than the total mass of the product stream S-111 (10,403 kg). This is because a production failure rate of 5% was assumed for this process (to specify a production failure rate, the user must right-click on an empty space of the flowsheet, select Economic Evaluation Parameters…, and switch to the Production Level tab). The calculated number of batches per year is 309 based on a recipe cycle time of 24 h, as discussed in the previous section. Consequently, the annual production rate is approximately 3,000 MT/year.

Table 3: Overall process data

OVERALL PROCESS DATA

Annual Operating Time	48.87 wk
Unit Production Ref. Rate	3,053,691.71 kg MP/yr
Batch Size	9,882.50 kg MP
Recipe Batch Time	818.63 h
Recipe Cycle Time	24.00 h
Number of Batches per Year	309.00

MP = Total Flow of Stream 'S-111'

Table 4, which was also extracted from the Materials & Streams report, lists all the raw material requirements for the process in kg/yr, kg/batch, and kg/kg of MP (MP stands for main product). It is clear that air, water and medium solids are the materials used in the largest quantities in this process. Medium solids include batch cell proliferation medium with rProteins and without rProteins (Growth Med (+) and Growth Med (-), respectively); fed-batch cell proliferation medium (FB Med (-)); and cell differentiation medium (Diff Med (-)); together, they amount to approximately 17 MT per batch. The consumption of water reaches approximately 554 MT/batch (excluding the water in the composition of the CIP solutions, which are 98% water).

Table 4: Material requirements

BULK MATERIALS (Entire Process)

Material	kg/yr	kg/batch	kg/kg MP
Air	29,038,573	93,976	9.51
CIP-Acid	2,218,952	7,181	0.73
CIP-Caustic	3,701,879	11,980	1.21
Diff Med (-)	997,994	3,230	0.33
FB Med (-)	4,049,224	13,104	1.33
Growth Med (+)	1,812	6	0.00
Growth Med (-)	178,350	577	0.06
Microcarriers	80,413	260	0.03
rProteins	1,907	6	0.00
Satellite Cells	0	0	0.00
Water	171,129,845	553,818	56.04
TOTAL	211,398,949	684,139	69.23

Cost Analysis

SuperPro Designer performs thorough cost analysis, estimating capital costs (CAPEX), operating costs (OPEX) and revenues. Equipment purchase costs can be estimated with built-in cost models, while other costs such as labor and utilities have default values that the user can modify to accurately represent the type of process, location, etc. In the present example, various costs were introduced / modified under rather optimistic assumptions, considering that cultured meat technology is very new and, as the field matures, costs will likely decrease:

- Cell culture is conducted in fermentors instead of bioreactors. Although fermentors and bioreactors are functionally identical in SuperPro Designer, fermentors are less expensive than bioreactors as the latter are applied to biopharmaceutical processes.
- Low capital cost multipliers were applied. Capital costs are estimated through multipliers in SuperPro Designer, and small values were used in this example to represent a facility that manufactures a low-value biotech product. However, multipliers for mammalian cell-based biopharmaceutical processes are typically large. For more information on capital cost estimations, please refer to the **Industrial Enzymes** example in the **…Process Library / Examples / Bio-Materials** folder or consult the Help tool (**Help➲ Search ➲ Capital Investment Dialog: DFC Tab**).
- Low raw material costs were assumed. In particular, the cost of rProteins was assumed to be just $4/g, and the cost of microcarriers was assumed to be only $20/kg. Currently, growth factors and other proteins used in mammalian cell culture media are produced on relatively small scale and have much higher unit prices, as they are mainly used for biopharmaceutical manufacturing. A detailed discussion on this topic can be found in the literature [18]. Likewise, microcarriers currently used in biopharmaceutical processes are much more expensive. It was assumed that new edible and

inexpensive microcarriers based on natural materials such as cellulose, starch, chitosan, etc. will be developed in the future.

SuperPro generates three reports related to process economics: the *Economic Evaluation Report* (*EER*), the *Cash Flow Analysis Report* (*CFR*), and the *Itemized Cost Report* (*ICR*). Table 5 displays the **Executive Summary** extracted from the EER for the cultured meat process.

Table 5: Executive summary.

EXECUTIVE SUMMARY (2022 prices)

Total Capital Investment	288,593,000.00 $
Capital Investment Charged to This Project	288,593,000.00 $
Operating Cost	109,409,000.00 $/yr
Revenues	152,685,000.00 $/yr
Batch Size	9,882.50 kg MP
Cost Basis Annual Rate	3,053,692.00 kg MP/yr
Unit Production Cost	35.83 $/kg MP
Net Unit Production Cost	35.83 $/kg MP
Unit Production Revenue	50.00 $/kg MP
Gross Margin	28.34 %
Return On Investment	20.18 %
Payback Time	4.96 years
IRR (After Taxes)	16.54 %
NPV (at 10,0% Interest)	128,736,000.00 $

MP = Total Flow of Stream 'S-111'

The summary indicates a total capital investment (TCI) of $289 million, an annual operating cost (AOC) of $109 million/year and a unit production cost of $36/kg. This unit production cost is significantly higher than the current retail price of ground beef in the United States, which is around $11/kg (in December 2022, according to the United States Department of Agriculture [19]), but very close to the lower estimation of Humbird [20] at $37/kg. On the other hand, Israeli company Believer Meats (former Future Meat Technologies) announced in 2021 that it had achieved a production cost of just $7.7/lb (equivalent to $17/kg) for cultured chicken meat. The company credits this breakthrough to its proprietary medium recycle system [21].

Assuming that cultured meat can be sold as a premium product for environmentally conscious consumers at $50/kg, the gross margin would be 28%, and the payback time would be 5 years.

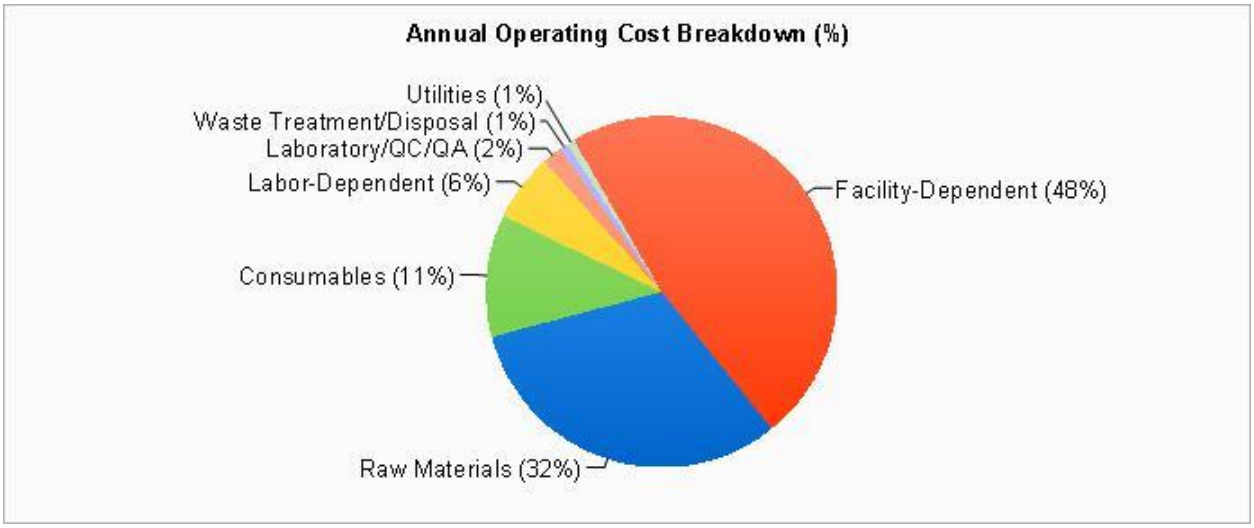

Figure 5: Annual operating cost breakdown

Figure 5 displays a breakdown of the AOC in the form of a pie chart, which was also taken from the EER. This chart shows that the facility-dependent cost accounts for 48% of the total operating cost, while raw materials and consumables represent 32% and 11%, respectively. The facility-dependent cost comprises equipment maintenance, depreciation, and overhead costs, and therefore is strongly related to capital expenses. Given that the large-scale culture of mammalian cells is a sophisticated process, requiring highly aseptic conditions, multiple medium preparation steps, and a large number of bioreactors, the substantial contribution of the facility-dependent cost is unsurprising.

To better understand the contribution of raw materials to manufacturing costs, Table 6 was also extracted from the EER; it provides a breakdown of raw material costs. Medium solids without rProteins represent approximately 62% of the total raw material cost (summing up Diff Med (-), Growth Med (-) and FB Med (-)). The average unit price assumed for these media ranges from $3.5/kg to $6.6/kg. On the other hand, rProteins account for 22% of the raw material cost, despite the small concentrations in which they are used. This is because the unit price of rProteins is assumed to be $4/g, i.e., about three orders of magnitude higher than other medium components (which is actually an optimistic assumption, as mentioned earlier).

Table 6: Breakdown of raw material costs

MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0.00	29,038,573	kg	0	0.00
CIP-Acid	0.06	2,218,952	kg	132,250	0.38
CIP-Caustic	0.03	3,701,879	kg	108,865	0.31
Diff Med (-)	6.58	997,994	kg	6,571,489	18.98
FB Med (-)	3.45	4,049,224	kg	13,954,032	40.30
Growth Med (+)	12.17	1,812	kg	22,056	0.06
Growth Med (-)	6.58	178,350	kg	1,174,381	3.39
Microcarriers	20.00	80,413	kg	1,608,261	4.65
rProteins	4.00	1,907,248	g	7,628,990	22.03
Satellite Cells	0.00	0	kg	0	0.00
Water	20.00	171,130	MT	3,422,597	9.89
TOTAL				34,622,920	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

The results of the present example suggest that the proposed process for production of cultured meat would be viable as long as a niche of consumers is willing to pay a premium for the environmental benefits of the product. It should be noted, however, that many variables affect this kind of analysis, such as: the purchasing price of medium components (particularly rProteins), cell growth yields, specific growth rates, the development of cheap edible microcarriers, etc. As a result, the actual economics of such an investment may be substantially better or worse than the current projections. A useful exercise is to perform sensitivity analyses with SuperPro to determine the impact of these changes; this allows the user to understand the potential risks and rewards of a project under different sets of assumptions. Different scenarios can be evaluated individually (by simply changing parameter values manually and re-running the simulation to see the results), or they can be automated through MS Excel. For information on how to drive SuperPro Designer through MS Excel and automate sensitivity analysis, please consult the examples in the **COM** folder (**…Process Library / Examples / COM**). Related information is available in the Help facility of the tool, which can be accessed by selecting **Help ➲ COM Interface and Library**. Math optimization and Monte Carlo simulation can be performed in a similar manner.

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Cultured (Cultivated) Meat Production