FEASTS
Fostering European Cellular Agriculture for Sustainable Transition Solutions

Process and infrastructure plan for industrial production of CM/CSF

Process and Infrastructure Engineering Toward Circularity and Minimal Waste

WP no. 3 WP *title*: (Work Package 3 *Sustainability by design*)

Task no: 5

Funded by the European Union

Author(s)	Organization
Katharina Julia Brenner, Marius Henkel, Chantal Treinen, Yasmina Harsy	TUM
Boštjan Vihar, Jernej Vajda, Matej Zadravec	UM
Etienne Duthoit, Claude Rescan	VM
Aleksandra Fuchs	ACIB

Reviewed by	Organization
Carlos Rodrigues	CAP
Affif Grazette	WUR
Kevin Camphuis	Shakeup Factory
Philipp Noll	Future Foods e.V.

Project no. 101136749

Project acronym: FEASTS

Project title: Fostering European Cellular Agriculture for Sustainable Transition Solutions

Call: HORIZON CL6-2023-FARM2FORK-01-13

Start date of project: 1 December 2023

Duration: 36 months

Deliverable title: Process and Infrastructure Engineering Toward Circularity and Minimal Waste in Cultivated Meat Production

Due date of Deliverable/Milestone: 30/11/2025

Date of Deliverable: 04/12/2025

Deliverable Lead Partner: Technical University of Munich, Professorship for Cellular Agriculture

Dissemination level: PUBLIC

The contents of this deliverable will become publicly available only after publication in a peer reviewed journal.

Funded by the European Union's research and innovation program under Grant Agreement no. 101136749.
Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Table of Contents

Table of Abbreviations

Abbreviation	Meaning
CM	Cultured Meat
CSF	Cell-based Seafood
CSF	Cultured Seafood
USP	Upstream Processing
DSP	Downstream Processing
STR	Stirred Tank Reactor
CIP	Cleaning-In-Place
SIP	Sterilization-In-Place
TEA	Techno-Economic Assessment
LCA	Life Cycle Assessment
CAPEX	Capital Expenditure
CQA	Critical Quality Attribute
CQA	Critical Quality Attributes
CQAs	Critical Quality Attributes
CFD	Computational Fluid Dynamics
OUR	Oxygen Uptake Rate
kLa	Volumetric Mass Transfer Coefficient
ρ	Density
FBS	Fetal Bovine Serum
HUVEC	Human Umbilical Vein Endothelial Cells
HUVEC	Human Umbilical Vein Endothelial Cell
VCD	Viable Cell Density
DCW	Dry Cell Weight
ADMEM	Advanced Dulbecco's Modified Eagle Medium
ATF	Alternating Tangential Flow (cell retention)
Ansys CFX	CFD Simulation Software
DS	Disk-Stack Centrifuge

Abbreviation	Meaning
FL	Flask
BR	Bioreactor
WSV	Waste Stream Valorization
WP	Work Package
EU	European Union
bar	pressure unit (1.013 bar reference)
m^3	cubic meter
L	liter
LMT 55	Cell-Culture Polymer Tubing Class
GRAS	Generally Recognized As Safe
FMEA	Failure Mode and Effect Analysis
QbD	Quality by Design
R^2	Coefficient of Determination
SD	Standard Deviation
SDModel	SuperPro-derived Sensitivity & Deployment Model
GF	Growth Factor
Pa	Pascal (shear stress unit)
kW	Kilowatt
SD	Standard Deviation
VCD	Viable Cell Density
VCD	Viable Cell Density
VCD	Viable Cell Density
bar	unit of pressure
SD	Standard deviation
SD	Standard deviation

Acronyms appearing in Tables & Figures

S-145	Process stream leaving cell-expansion STR
S-151	Process stream leaving disk-stack centrifuge
S-155	Process stream entering filling step FL-101
S-163	Solid waste fraction post-centrifugation
S-175	Mixed DSP waste stream post-extrusion
S-255	Utility water stream post-CIP
P-14 / BR-102	STR cell-expansion module
P-17 / DS-101	Disk-stack centrifugation unit
P-23 / FL-101	Filling & packaging unit

Executive Summary

This report outlines the process and infrastructure requirements for scaling cultured meat (CM) and cell-based seafood (CSF) production in Europe, as part of Task 3.5, Process and Infrastructure Engineering towards Circularity and Minimal Waste, within the FEASTS consortium. The objective is to establish technically feasible, resource-efficient, and circular production routes that are compatible with European deployment constraints and provide quantitative engineering inputs for techno-economic assessment (TEA), life cycle assessment (LCA), and strategic pathway evaluation in WP3, WP4, and WP6. The work integrates process modelling, circularity strategies, and facility-level infrastructure assessment, linking biological feasibility with real industrial boundaries. Core areas addressed in this report include:

- assessment of centralized and decentralized production routes for Europe, including reactor scale-up and distributed scale-out
- stainless steel availability, fabrication capacity, and TRL-dependent infrastructure readiness for hygienic industrial reactors
- comparative modelling of batch, intensified, and perfusion modes using SuperPro Designer under equal annual output assumptions
- identification of dominant infrastructure pressure points, including chilled water, steam, gas mixtures, and thermal control ceilings of industrial steel reactors
- system level integration of waste stream valorization through membrane-based media polishing, anaerobic digestion and thermal and CO_2 recovery loops

The work conducted at TUM is built around a unified platform process that integrates upstream, downstream, and utility systems into a coherent quantitative framework. This platform was translated into detailed SuperPro Designer models covering the full seed train, media preparation and sterilization, cell expansion, harvesting and downstream processing, as well as scheduling logic, resource demand, effluent generation and classified waste streams. The models provide a mass and energy balance-based assessment of process performance, allowing for a systematic comparison of expansion routes, reactor sizes, and operational modes.

A second key component is the integration of Waste Stream Valorization (WSV) directly into the process architecture. Waste fractions from media, organic solids, plastics, CO_2, and heat are mapped to technically viable valorization units, including tangential flow filtration, anaerobic digestion, heat recovery, and gas capture. These pathways are evaluated for feasibility, resource savings, and compatibility with industrial CM/CSF operations, forming an essential link between circularity and infrastructure. In parallel, the report outlines the infrastructure limits, including reactor size constraints, stainless steel demand, fabrication timelines, cooling and utility bottlenecks, and the feasibility of retrofitting existing industrial sites. Engineering maturity and deployment risks are positioned along the TRL scale, distinguishing early process concepts (TRL 3–4), pilot learning loops (TRL 5–6), and industrially anchored reactor systems (TRL 7–8+).

Together, the platform process modelling, circularity integration, and infrastructure analysis form the engineering foundation for scalable CM/CSF production. These results support TEA and LCA modelling in WP4 and WP6 and interface with the four strategic case studies of Task 3.5, which refine sustainability and circularity strategies across consortium partners. The deliverable focuses on production engineering and infrastructure; food structuring and consumer-facing product evaluation are outside the scope of this report.

Developing a Cultured Meat Production Process and Platform Design

Introduction and Positioning of Task 3.5

The industrial production of cultured meat and cell-based seafood (CM/CSF) requires scalable and resource-efficient processes that integrate biological constraints, engineering feasibility, and principles of circularity. Developing such systems depends on quantitative engineering models that describe mass and energy flows, utility demand, waste generation, and infrastructure requirements across the entire production chain. Task 3.5 of FEASTS addresses this need by establishing process and infrastructure strategies that minimize waste, evaluate valorization routes, and support the sustainable scale-up of CM/CSF. The task links process architecture, circularity considerations, and facility-level requirements, providing essential engineering inputs to WP3, WP4, and WP6. Within this framework, TUM develops a unified modelling approach based on a platform process that integrates all major USP, DSP, and utility operations. Detailed SuperPro Designer models quantify media consumption, thermal loads, CO_2 emissions, organic residues, and effluent streams, enabling a comparison of alternative process modes. Waste Stream Valorization (WSV) pathways are systematically mapped onto these flows, supporting the assessment of recovery potential and the benefits of circularity. Complementary infrastructure analysis encompasses stirred-tank reactor (STR) geometry, cooling and sterilization limits, retrofitting potential, and resource bottlenecks relevant to European deployment. Together, these elements form the engineering foundation for evaluating scalable and circular CM/CSF production routes within FEASTS, providing the quantitative basis for TEA, LCA, and scenario development. This deliverable focuses on circularity-aligned WSV and bioprocess engineering at an industrial scale. It covers cell expansion in 20,000 L seed train systems and downstream process integration. Structuring, texturing, and consumer-facing food-product evaluation are not part of its engineering and infrastructure scope.

Overview of the CM Production Process and Platform Process Design

The platform process developed in Task 3.5 provides a unified engineering description of CM/CSF production based on the Main Model, Seed Train, Media

Preparation, USP STR operation, and DSP configuration implemented in SuperPro Designer. It covers the entire process chain, from thawing to final product, and quantifies all relevant mass flows, utilities, and waste streams required for TEA/LCA and scenario modeling. The process begins with a staged seed expansion from 2 mL at 1×10^7 cells/mL through six scale steps to a 20,000 L working-volume STR. Growth follows 5.64 doubling cycles, resulting in ~1×10^{15} cells and 1,990.76 kg of biomass per batch (Table 1).

Table 1: Composition of stream S-145 leaving the cell expansion stage. S-145 leaves unit procedure P-14 / BR-102 towards downstream processing. The stream's temperature is 37.0 °C at 1.1013 bar.

Component	Yield in kg/batch	Mass Composition in %	Concentration in g/L
Biomass	1990.76	10.52	104.87
Depleted Medium	15926.07	84.21	838.98
Impurities	995.37	5.26	52.43

The STR operation is constrained by oxygen transfer, shear, and heat removal requirements, with an agitation power of 9.69 kW and cooling-water flows of ~748 kg/h. Media preparation is implemented via a two-fraction strategy (heat-sterilisable vs. filter-sterilisable components). Depending on whether a 50:50 or 90:10 sterilization split is applied, heating duties range from 5 to 25 kW, and cooling duties range from 0.48 to 2.41 kW. The total batch medium volume is 22,000 L. Downstream, the broth is cooled and centrifuged to achieve a 95% solids recovery, then washed with 2,000 L of buffer, and conditioned through extrusion. Product output is ~1,891 kg per batch, while DSP generates ~4,839 kg of mixed waste and a significant thermal load due to the cooling and extrusion steps (Table 2; Table 3).

Table 2: Composition of stream S-151 leaving the centrifuge P-17 / DS-101. S-151 contains most of the "Biomass" while the majority is "Depleted Medium". The stream's temperature is 15.0 °C at 1.1013 bar.

Component	Yield in kg/batch	Mass Composition in %	Concentration in g/L
Biomass	1891.22	38.39	390.64
Depleted Medium	2985.38	60.60	616.64
Impurities	49.76	1.01	10.28

Table 3: Composition of stream S-155. S-155 enters the filling unit procedure P-23 / FL-101 and contains the product. The stream's temperature is 4.0 °C at 1.013 bar.

Component	Yield in kg/batch	Mass Composition in %	Concentration in g/L
Biomass	1891.22	90.42	945.06
Buffer Solution	200.47	9.58	100.18

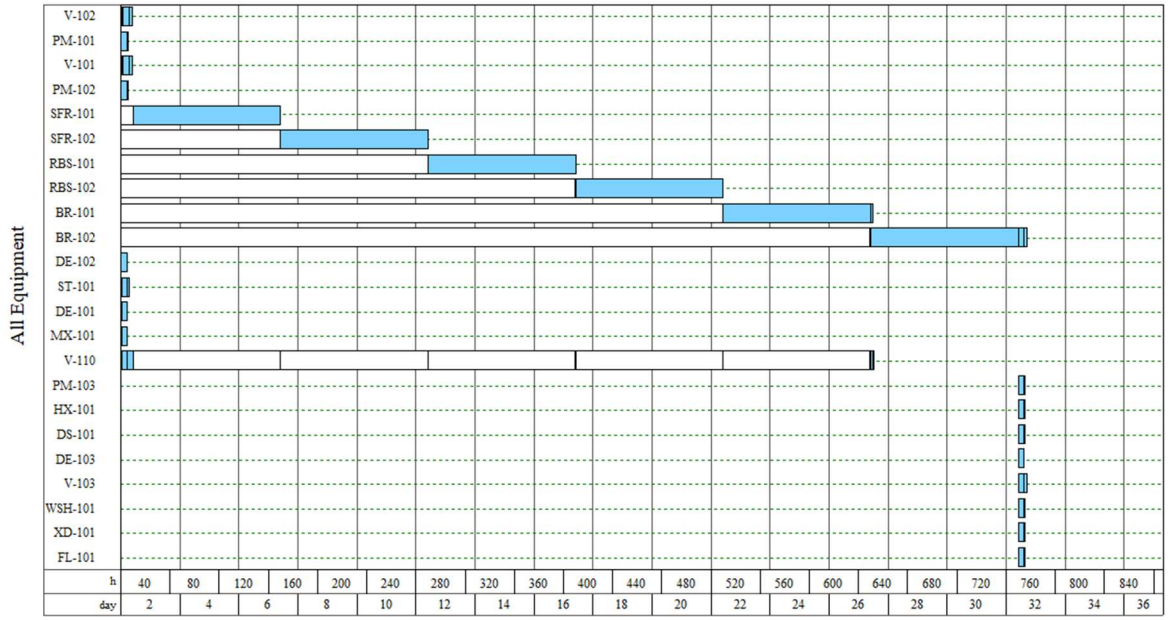

*Figure 1: Equipment Scheduling and Occupancy of a CM production process. Abbreviation key: **V** Media or buffer hold vessels; **PM** Pump modules or fluid actuation units (e.g., peristaltic or motor-driven), **SFR** Stirred tank bioreactors in the seed expansion train, **BR** Stainless steel bioreactor, **DS** Disk-stack separation units (centrifugation or broth clarification), **RBS** Buffer washing and solution conditioning clusters, **HX** Heat exchanger cooling or thermal transfer units, **FL** Filling or extrusion-linked downstream modules, **MX/XD** Mixed or auxiliary downstream equipment not tied to reactors*

Figure 1 illustrates equipment occupancy along the upstream and downstream processes for cultured biomass modeled in SuperPro Designer. Each row represents one unit procedure or bioreactor cluster, with blue blocks marking active time windows for inoculation, 37 °C cultivation, broth cooling, buffer washing, biomass separation, and CIP cycles. It is used to evaluate scalability

across batch and perfusion expansion routes under equivalent reactor geometry and comparable utility constraints.

Across USP and DSP, the model quantifies liquid organic waste (12,940 kg of depleted medium), CO_2 evolution from stoichiometric conversion, CIP water use (exceeding 60,000 kg per batch), and thermal waste from cooling operations. These flows directly map to WSV categories (media, organic, heat, CO_2, plastics) and feed into valorization pathways. The platform process aligns with WP3 by providing engineering boundaries for media optimization, scaffold integration, and reactor strategies. It supports WP6 by delivering mass and energy data, waste profiles, and infrastructure-relevant parameters for scale-out scenarios. Its modular structure allows for the consistent comparison of fed-batch, intensified, and perfusion strategies under uniform modeling assumptions.

Process and Infrastructure for Industrial Production of CM/CSF

Process and Infrastructure Plan for Industrial Production of CM and CSF in Europe until 2050

Infrastructure modelling for cultivated biomass manufacturing in the European Union integrates demand projections, fabrication thresholds, decentralised deployment models and waste-recovery stoichiometry corridors at full industrial scale. The yearly total meat consumption in the EU is 28 Mio kg; derived from population projections of 448 million inhabitants multiplied by an average annual individual meat consumption of 63 kg (Eurostat; Food and Agriculture Organization). The estimated market-capture corridors for cultivated alternatives reach 0.2–9% by 2050, depending on the ambition corridors, mapping to 141–2,540 million kg of consumption per year (Systemiq, 2024; Brennan et al., 2021) (Figure 2; Table 4).

Table 4. Cultured meat market share scenarios for Europe based on assumed annual meat demand.

CM Capture of overall meat market	CM meat consumption per year	Source
0.20%	141,120,000 kg	Systemiq (2024); low ambition
0.50%	423,360,000 kg	McKinsey (Brennan et al. 2021)
3.00%	846,720,000 kg	Systemiq (2024); medium ambition
9.00%	2,540,160,000 kg	Systemiq (2024); high ambition

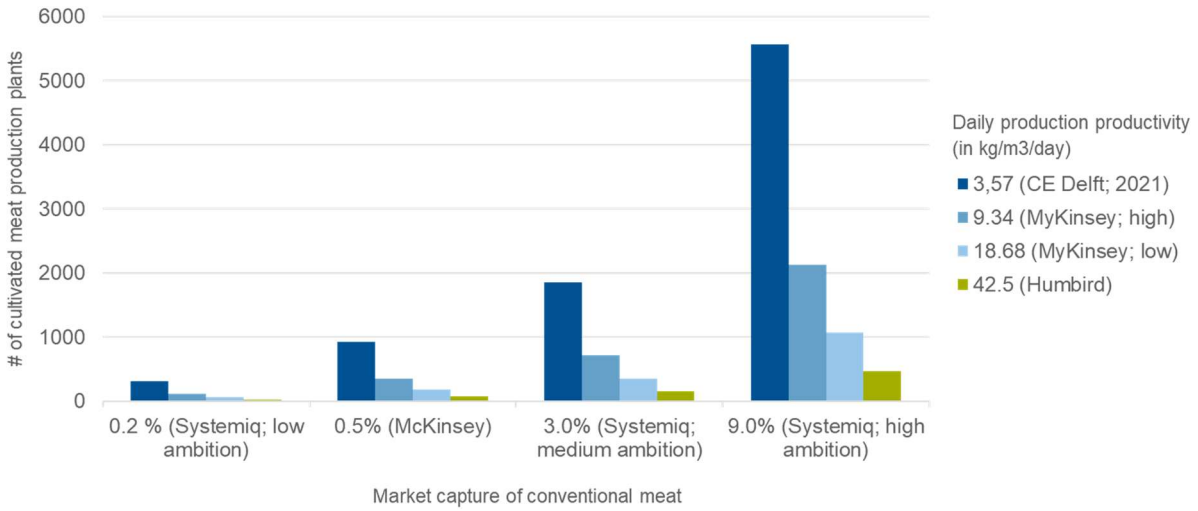

Figure 2: Facility Count Varies by Demand and Productivity Scenario

Rather than assuming linear adoption, scale-out boundaries are derived by explicitly quantifying reactor equivalents, commissioning intensity, and steel supply velocity limits in Europe. At process-scaling unit equivalence, large stirred-tank reactors of 20 m³ form the baseline for industrial deployment modeling. Operated in batch equivalents, one 20 m³ stirred tank reactor yields 2 tonnes of biomass per batch with a 5-day cycle, resulting in 73 batches per year and an annual throughput of 130–150 tonnes per reactor (Humbird, 2021; O'Flaherty et al., 2022; Miller et al., 2020). Moderate adoption corridors between 0.4–2.0 million tonnes annualised cultivated biomass thus map directly to a required fleet of 3,000–15,000 new stainless reactors under identical geometry equivalence commissioning loops for annual throughput ceilings (CE Delft, 2021; McKinsey, 2021; Eurostat).

Stainless Mass Estimation for Industrial Vessel Fabrication

Reactor geometry literals were converted into stainless steel volumes and mass equivalents using a bottom-up cylinder with dished heads approach for industrial pressure-thermal zoning. Given reactor volume V = 20 m³ and mean vessel diameter D = 2.17 m, radius r = 1.085 m and cylinder height equivalence h = 5.42 m, the wetted shell area is 36.91 m², while dished-head arrays add approximately 5.59 m², yielding A_{total} = 42.50 m² surface area per reactor cluster equivalent (Humbird, 2021; CE Delft, 2021). For hygienic 316L stainless shell thickness zoning t = 5–8 mm (0.005–0.008 m) and density ρ = 8,000 kg/m³, steel volume maps to V_{steel} = 0.212–0.340 m³ per shell-plus-head surface depending on thickness ceilings and welding drainage zoning, which yields 1,700–2,720 kg shell mass for polished large-vessel cylinder equivalents excluding built-up internals (Humbird, 2021; Miller et al., 2020). Including published stainless mass buildups for internals, ports, stiffeners, agitator shafts, local supports, frame zoning, and safety fabrication margin equivalents yields a plant-equivalent stainless mass footprint of 15–28 tonnes per entire 20 m³ vessel propagated into commissioning zoning corridors for industrial aseptic deployments (Humbird, 2021; O'Flaherty et al., 2022).

For a typical industrial CM/CSF facility containing 18–24 reactors, the per-plant stainless mass maps to 63–144 tonnes at reactor-cluster equivalence, depending on productivity assumptions and welding-drainage zone validation in ports, agitation-shear zone ceilings, thermal jackets, and safety fabrication margin zoning. Gregorian vessel fabrication velocity surveys for pressure-thermal hygienic stainless indicate that 5,000–6,000 large vessels are manufactured annually within Europe across all sectors, but only a small validated bioprocess-share meets Ra < 0.6–0.8 μm mechanical polishing and SIP heights, causing longer lead-times when defaulting to legacy food-fabrication assumptions without major over-engineering loops in hygiene-grade zoning corridors (CE Delft, 2021; McKinsey, 2021; Miller et al., 2020; Allan et al., 2018). Multiplying the mass footprint per reactor-plus-plant equivalent by reactor-fleet counts (3,000–15,000 reactors) yields European regional stainless-fabrication demand between 50,000–400,000 tonnes for moderate adoption, surpassing >1 million tonnes stainless under high adoption corridors, which cannot be met without parallel hygiene-zoned fabrication corridors over the remaining industrialisation timeline until 2050 (CE Delft, 2021; McKinsey, 2021; Humbird, 2021; Stephens et al., 2018).

Capital equivalence modelling for Greenfield, Brewery Reuse, Dairy Reuse

Retrofit assessments of breweries and dairies show that only a small fraction of infrastructure, typically 6–15 percent, can be reused for CM production (Stephens et al., 2018). While building shells, utilities corridors, and select piping can be retained, core process systems such as sterile bioreactors, media kitchens, filtration lines, CIP/SIP loops, high-grade HVAC, and hygienic drainage must be replaced entirely due to insufficient cleanability, surface finish, material compatibility, and pressure rating (O'Flaherty et al., 2022). As a result, retrofits reduce civil and structural CAPEX but do not alter the requirement for CM-grade equipment (Figure 3).

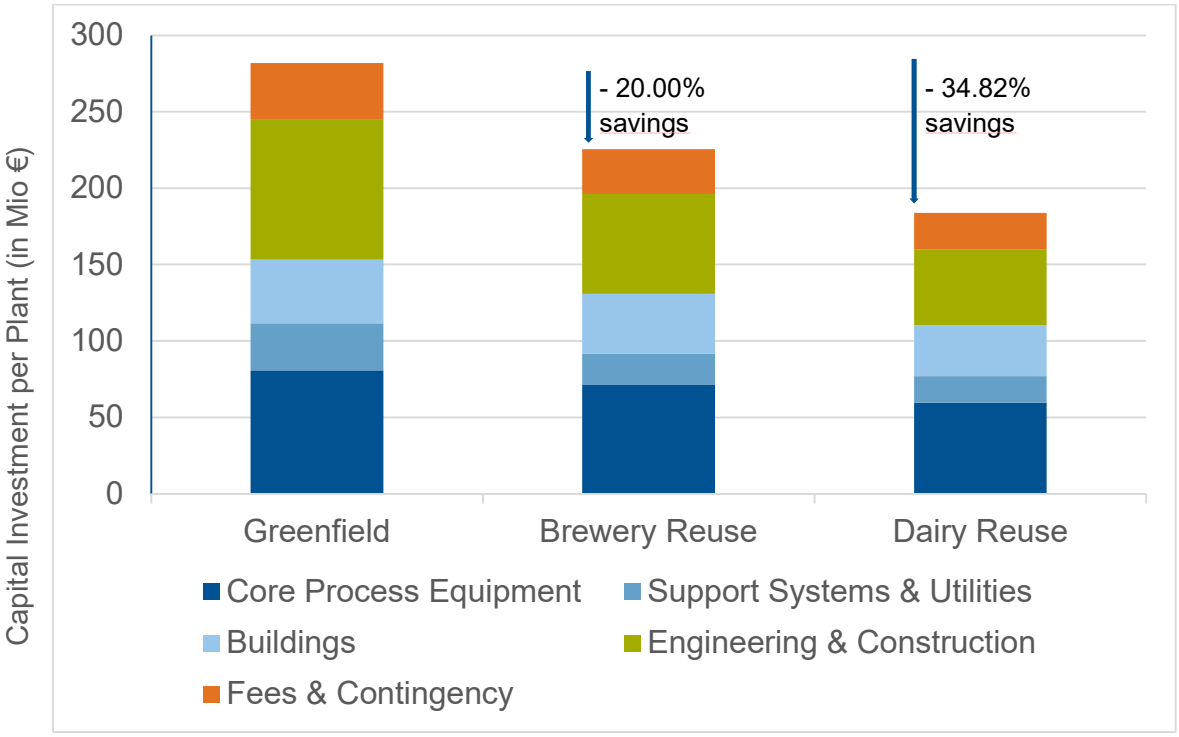

Figure 3: Capital Expenditures of Greenfield, Brewery and Diary Reuse Plants

Capital expenditure implications follow directly from these constraints. Greenfield CM facilities are estimated to cost approximately 200–300 million euros per plant, depending on reactor count, utility density, automation level, and hygienic zoning (CE Delft, 2021; McKinsey, 2021). Retrofits of dairy or brewery facilities reduce this to around 70–120 million euros, primarily by reusing building and utility infrastructure. However, the majority of the cost remains tied to bioreactors, media systems, and downstream equipment, which cannot be repurposed (Humbird,

2021). Across all modelling approaches, the relevance for CM-grade facilities becomes clear. Cooling loads per 20 m³ reactor frequently exceed 700–900 kg chilled water per hour, aeration requirements surpass 10 tonnes of filtered gas mixture per batch, water consumption exceeds 100 tonnes per batch, and steam loads remain high due to sterilisation and CIP requirements (Humbird, 2021; O'Flaherty et al., 2022; Miller et al., 2020). Brewery or dairy reuse pathways offer partial civil CAPEX reductions of 20–35% by retaining buildings or spatial-utility corridors, yet only 6–15% of legacy process equipment can be reused due to cleanability surface ceilings, pressure thermal incompatibility, Ra finish zoning, lack of SIP port equivalence corridors and welding-drainage zoning that meets sterile-drip loop validation thresholds (Stephens et al., 2018; O'Flaherty et al., 2022; CE Delft, 2021). Thus, the predicted CAPEX falls to 68–125 million € for reuse corridors, versus 200–300 million € for Greenfield facility equivalents, and does not change de novo core equipment demand for 20 m³ reactor clusters or DSP and media-preparation pipelines. Without multi-reactor clustering equivalence and CM-grade steel design, civil reuse corridors cannot close reactor-commissioning or DSP utility intensity thresholds for the European regional stainless fleet, making parallel hygienic fabrication pipelines, multi-site deployment velocity modelling, and waste-recovery stoichiometry corridors the key cost-efficiency backbone for EU submissions by 2050 (Stephens et al., 2018; CE Delft, 2021; McKinsey, 2021). The full model of needed infrastructure requirements can be found in Figure 4.

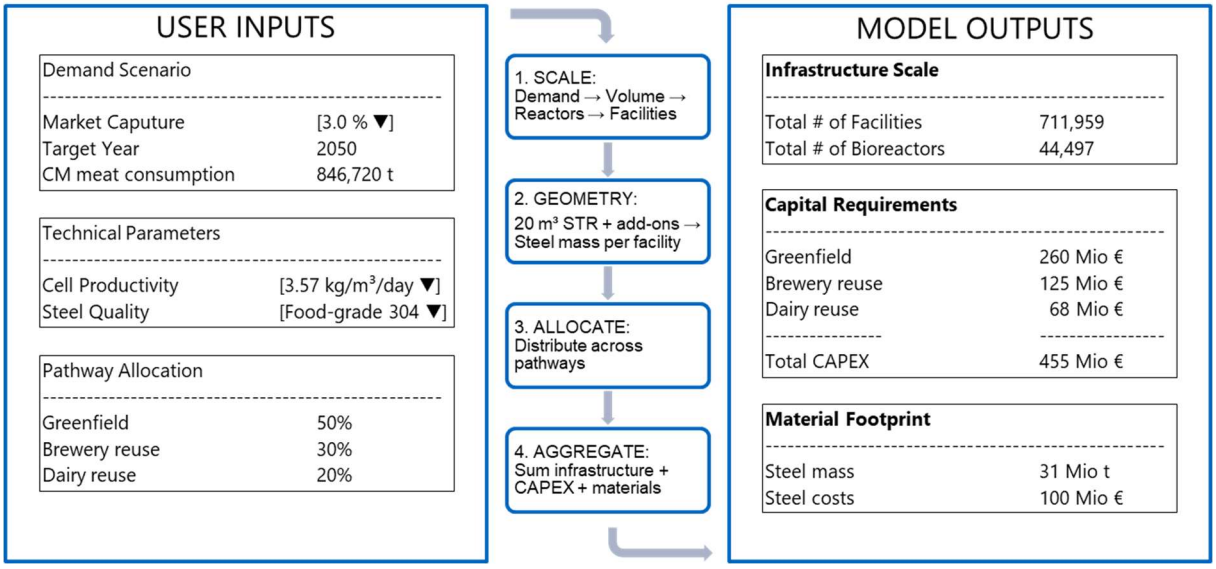

Figure 4: User input and Model Outputs for Infrastructure Modelling

Centralization vs. Decentralization in Production Planning

Centralization and decentralization are two complementary planning logics for industrial reactor fleet expansion, describing different pathways of increasing capacity and replicating it across manufacturing sites in Europe. Centralized industrial production relies on scale up, meaning a stepwise increase in reactor size from very small laboratory systems to 20,000 L (20 m^3) stainless steel stirred tank equivalents, typically clustered at 18 to 24 reactors per facility, enabling high throughput per site, tight process timing, automation learning effects, and plant-level CAPEX bundling (Héder, 2017; Mankins, 2009; Humbird, 2021; Stephens et al., 2018; CE Delft; McKinsey & Company; Miller et al., 2020). However, centralized scale-out ceilings do not refer to smaller reactors, but to replicating full industrial plant modules, which is described as scale-out, meaning the multiplication of identical facilities across regions rather than increasing vessel size within one facility. Decentralized local planning follows the same bioprocess logic but operates in smaller modules, where 2,000 L (2 m^3) stainless pilot reactors are clustered in 2 to 6 units per local site. This approach supports earlier learning, faster commissioning per module, reduced transport complexity, and flexible deployment near demand or resource hotspots. Both pathways share the same bottleneck principle: stainless large vessel fabrication capacity bounds, realistic utility loads and reactor commissioning limits define the outer scaling corridor for industrialisation, so facility replication (scale out) can only happen meaningfully after the reactor size has reached relevant scaling corridors via scale up, while local 2,000 L deployment accelerates learning loops but cannot replace centralised 20,000 L industrial scale up reactors for high throughput applications. The combined logic highlights that scale up describes reactor size evolution, while scale out describes facility-level replication across sites, both driven by reactor counts, materials, and utility corridors until 2050 in Europe (Héder, 2017; Mankins, 2009; Humbird, 2021; Stephens et al., 2018; CE Delft; McKinsey & Company; Miller et al., 2020).

Technology Readiness Level Integration into Infrastructure Modelling

Technology maturity is a decisive criterion in European innovation assessment, ensuring that infrastructure models for cultivated meat (CM) and cell-based seafood (CSF) rely on validated engineering scale equivalents rather than

extrapolated laboratory assumptions. The TRL framework (Technology Readiness Levels) defines technical maturity across nine levels, and is applied by the European Commission to characterise the development and deployment readiness of emerging technologies in large-scale biomanufacturing ecosystems. Early academic work on cell lines, media design and oxygen-transfer observation is assigned to TRL 1 to 2, representing fundamental biological principle observation and conceptual application framing without prototype operation (Héder, 2017; Mankins, 2009). Controlled lab validation of biological correlations (e.g., growth envelopes or preliminary kLa observation) is conducted in very small bioreactors, typically 1 to 10 L working volume, and corresponds to TRL 3 to 5, where components are analytically verified but remain too small to reflect real infrastructure demand (Mankins, 2009; Héder, 2017). The first industrially relevant prototype reactors in cellular agriculture correspond to TRL 6, where biological performance is validated in a relevant environment, typically using 2,000 L stainless pilot reactors operated as single units or very small clusters (Stephens et al., 2018; CE Delft, 2021). True reactor commissioning and pilot plant reliability is only captured at TRL 7 to 9, where prototypes operate at either 20,000 L (20 m^3) centralised industrial pilot reactors, or 2,000 L decentralised units under parallel modules, enabling realistic observation of material, utility, deployment velocity and downstream performance at representative scales (Humbird, 2021; CE Delft; McKinsey & Company). TRL 8 confirms that systems are fully qualified for industrial deployment, once both CAPEX ranges and reactor footprints are proven realistic, while TRL 9 describes commercially operational scale-out, where multi-facility manufacturing is fully reproducible, uncertainties stabilise, and infrastructure timing and deployment velocity boundaries are reliably observed. Local decentralised production in Europe is thus connected to TRL 6 to 8 at 2,000 L pilots, whereas centralised industrial reactor pilots align with TRL 7 to 9 at 20,000 L validation equivalents, while very small lab reactors remain at TRL 3 to 5 and cannot represent infrastructure scale-out boundaries. This ensures a robust differentiation of what scale and maturity claims are scientifically supported in reactor geometry, material footprints and plant-level replicability, confirming that only 2,000 L and 20,000 L pilot reactors are considered sufficient for scale-out reliability calculations, while lab systems are used exclusively for early biological parameter discovery (Stephens et al., 2018; Héder, 2017; Humbird, 2021; CE Delft, 2021; McKinsey, 2021). The following sections outline the conducted bioprocess modelling part, following the introduction to the CM process and infrastructure plan.

Key Process Parameters and Constraints for Industrial CM/CSF Production

The main engineering constraints of the CM process at large scale are defined by oxygen transfer, mixing performance, heat removal capacity, media demand, metabolite accumulation, and achievable cell densities. Bioprocess modelling results within this study show that the 20 m^3 STR is limited primarily by oxygen transfer, consistent with published CM scale-up analyses, which indicate that OUR exceeds the practical kLa range once cell densities rise beyond approximately 20–60 × 10^6 cells/mL (Humbird, 2021; Allan et al., 2019). The requirement for synthetic air with elevated CO_2 content and the high agitation power needed at large scale lead to oxygen transfer conditions where further biomass increase becomes infeasible without exceeding shear or thermal limits. Mixing constraints arise because increasing biomass fraction elevates effective viscosity and reduces mixing homogeneity. This behaviour mirrors observations from CM process characterisations, where large STRs exhibit long mixing times, poor micro-environment uniformity, and shear-limited impeller velocities (Stephens et al., 2018; MacQueen et al., 2019). These constraints restrict feasible agitation power density and limit intensified or perfusion-type operations. Heat removal is the secondary limiting parameter. Both metabolic heat generation and agitation-derived mechanical heat scale with cell density. CM process analyses indicate that industrial STRs require large chilled-water capacities for maintaining 37 °C, and that cooling becomes a dominant utility burden at high densities (O'Flaherty et al., 2022; Risner et al., 2023). Thermal load from media sterilisation and chilled storage further increases the overall cooling demand. Media consumption significantly influences resource use and waste generation. CM TEAs and process studies consistently identify media as the most significant contributor to operating cost, water demand, and liquid waste generation (Specht, 2020; Miller et al., 2020). The high nutrient content of CM media results in large volumes of depleted medium and correspondingly high CIP and sterilization requirements. Metabolite accumulation is also a growth-limiting factor. Lactate and ammonia build up in batch and intensified systems, reducing viability and shortening the productive residence time (Risner et al., 2020; Stout et al., 2023). Growth kinetics in current CM cell lines typically support doubling times of 18–24 hours under optimal conditions, enabling densities of up to 20–60×10^6 cells/mL, but engineering constraints, rather than biological potential, set the upper boundary for large-scale operation (Humbird,

2021; O'Flaherty et al., 2022). As a result, oxygen transfer, thermal control, mixing performance, media usage, and metabolite inhibition collectively define the feasible operational window for industrial CM production.

Modelling Framework and Methodology

The modelling framework integrates all USP, DSP, and utility operations into mass- and energy-balanced flowsheets, following the structure of CM process models published in recent scale-up and TEA studies (Humbird, 2021; Miller et al., 2020; Risner et al., 2020). The platform process models include seed expansion, STR scale-up, fermentation, harvesting, washing, concentration, formulation, media preparation, sterilization, chilling, aeration, CIP, and packaging. Each operation is parameterized with batch times, volumetric flows, mass balances, and utility demands consistent with CM modeling practice. USP modelling employs multi-stage STR expansion and encompasses batch, intensified, and perfusion configurations. Parameter selection follows the ranges reported for mammalian suspension cells used in CM studies, including achievable cell densities, oxygen uptake rates, heat generation, shear limits, and viable agitation power densities (Allan et al., 2019; MacQueen et al., 2019). DSP modelling encompasses cooling, centrifugation, washing, and biomass conditioning steps, analogous to those proposed for CM biomass recovery (Stout et al., 2023; Zhang et al., 2022). Media preparation and sterilisation are modelled by integrating thermal and filtration-based routes. CM analyses emphasise that sterilisation configuration strongly influences energy and water demand (Risner et al., 2023; Specht, 2020). Utilities such as chilled water, steam, aeration, electricity, and CIP are included as explicit unit operations to quantify total thermal waste, CO_2 emissions, and effluent generation. All mass and waste streams are mapped to the WSV categories defined for FEASTS, including media waste, organic waste, heat waste, CO_2 off-gas, plastics/filters, and mixed equipment residues. This enables the integration of recovery routes, such as media recycling, heat integration, anaerobic digestion, and CO_2 reuse, in line with published CM sustainability frameworks (Ellis et al., 2022; Risner et al., 2023).

Results

Platform Process Models

The platform process consists of four interconnected SuperPro models representing the complete cultured-meat production chain. Each model captures specific sections of USP, DSP, utilities, and auxiliary operations, and together they form the quantitative basis for resource assessment, circularity evaluation, and process-mode comparison. The main model provides the complete flowsheet, from medium preparation to final product, including complete seed expansion, the 20 m³ STR, centrifugation, washing, extrusion, and packaging. The graphical layout (Figure 5) defines all unit operations, including explicit mass and energy flows, reactor scheduling blocks, sterilization steps, CIP cycles, and cooling requirements.

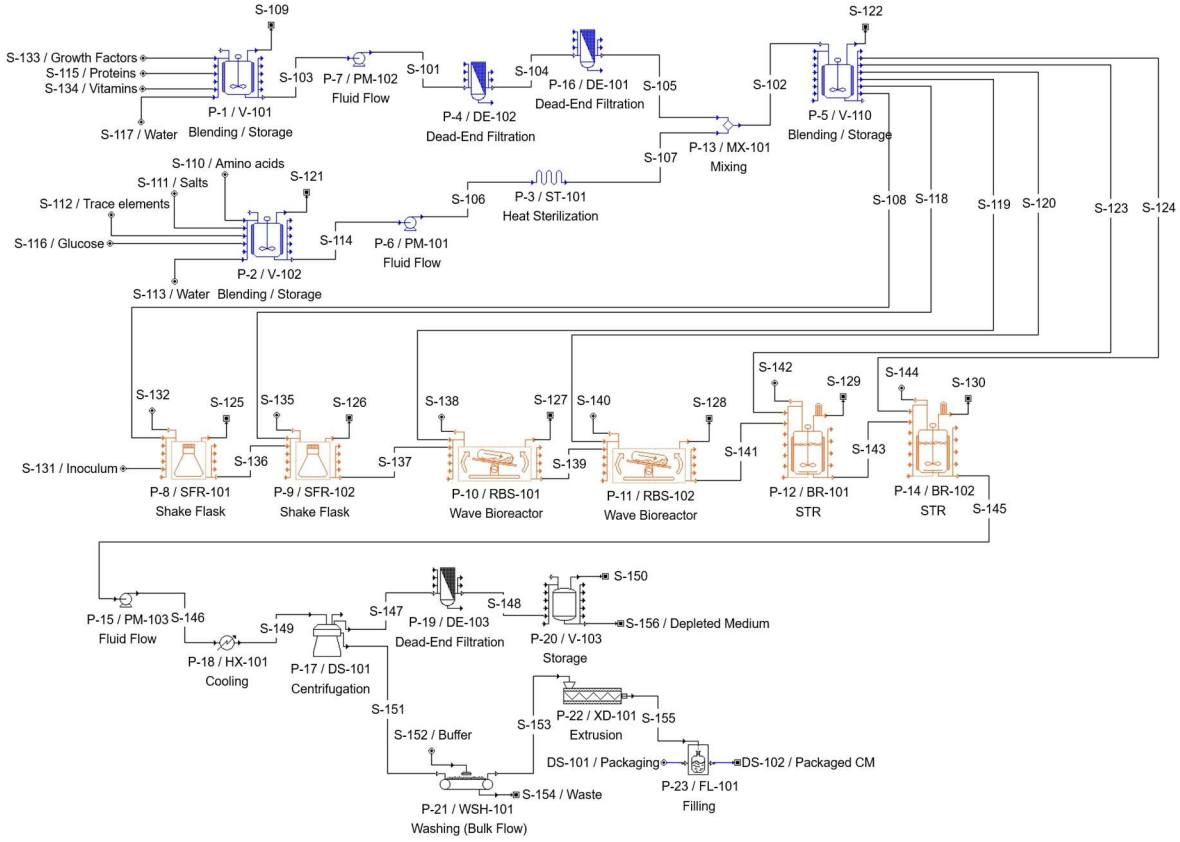

Figure 5: Modeling of an exemplary complete CM production process (Main Model).

The Cell Expansion Variation represents a modified seed train configuration in which early flask or wave bag steps are replaced by STR-based expansion. This configuration reduces the total process time from just below 740 hours (30.8 days) to approximately 620 hours (25.8 days) for the same batch output, while increasing CIP washing solution use and overall chilled water demand, as all seed train vessels require full CIP cycles instead of single-use handling.

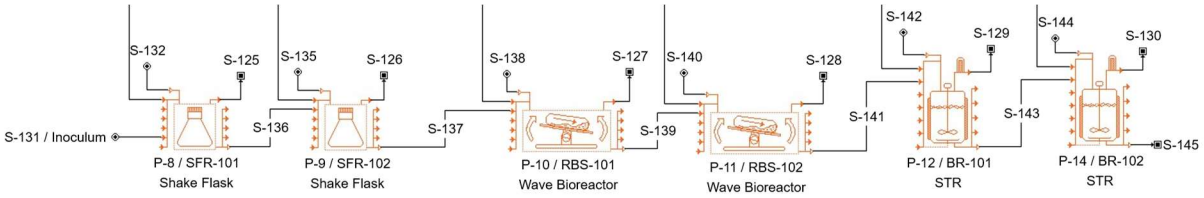

Figure 6: Cell Expansion Variation Diagram of an exemplary CM production process.

The DSP Model focuses on harvest, separation, and conditioning of biomass. It contains the broth cooling stage, the disk-stack centrifuge, wash loops, intermediate storage, and the formulation units. Its graphical structure (Figure 7) illustrates the wash-buffer circuit, the clarified-liquid pathways, and the product line that feeds the extrusion step.

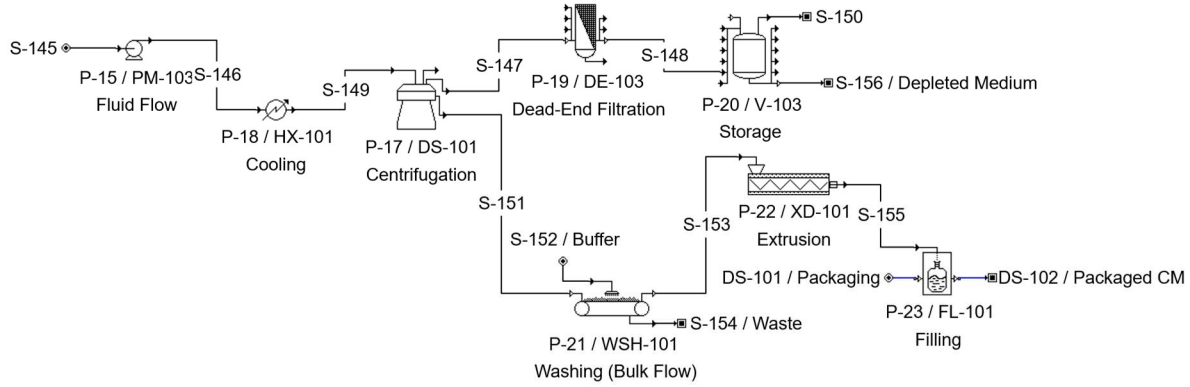

Figure 7: Downstream Processing Diagram of an exemplary CM production process

The Media-Preparation Model encompasses hydration, dissolution, thermal or filtration-based sterilization, cooling to 4 °C, and intermediate storage. The flowsheet (Figure 8) reflects the two sterilization approaches (50:50 and 90:10 heat–filtration splits) and their respective impacts on heating, cooling, and filtration loads.

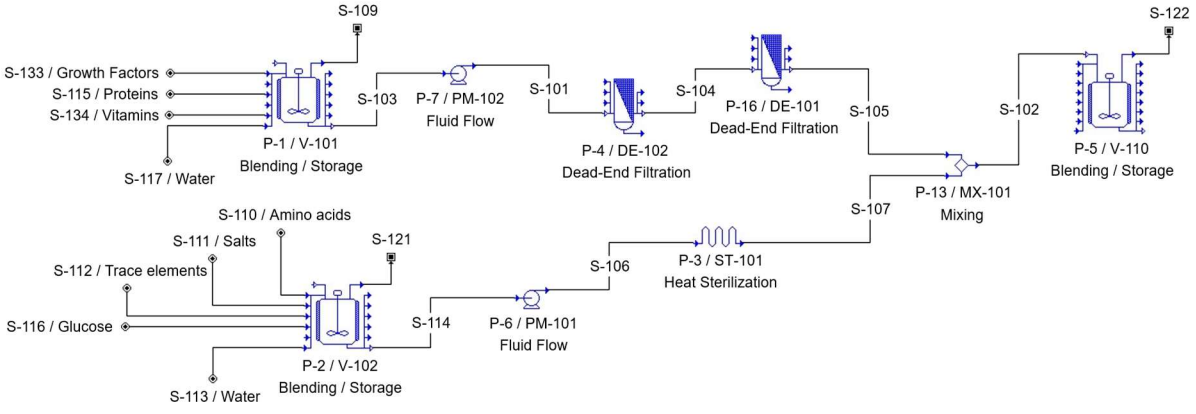

Figure 8: Media-Preparation Model of an exemplary CM production process

Across all models, the process modes fed-batch, intensified batch, and perfusion were compared using common boundaries and equivalent reactor geometry. Perfusion incorporates a continuous feed and bleed loop, extended residence time, and a constant biomass zone, while intensified batch increases feeding intensity within the same STR. Their relative differences are visualised in the Process Mode Comparison Figure, which contrasts cycle times, media turnover, unit-operation load, oxygen demand, and waste-stream generation.

Mass and Energy Balances

The mass and energy balances of the platform process are derived from the full SuperPro simulations and the associated SDModel (2025). They quantify all material flows, utility demands, and effluent streams per batch, as well as the peak load conditions that determine infrastructure requirements. Each batch processes approximately 20,000 kg of culture medium. During the five-day STR cultivation, this results in 15,926 kg of depleted medium, 995 kg of impurities, and approximately 1,990.76 kg of biomass, corresponding to about 1×10^{15} cells. These values define the primary material outputs of USP and inform the sizing of DSP operations. Utility demand is dominated by cooling, steam, and aeration. The 20 m^3 STR requires approximately 748.58 kg/h of chilled water to maintain a temperature of 37 °C, resulting in a cumulative cooling-water demand of roughly 89,830 kg per batch. When including media sterilisation, broth cooling, extrusion cooling, and intermediate storage, the total chilled-water consumption exceeds 190,000 kg per batch. Steam and heating loads originate primarily from media sterilisation and CIP. Depending on the sterilization strategy, the heating duty is 25.4 kW for the 50:50 heat–filtration split and 5.0 kW for the 90:10 split, while the cooling duty is 2.41 kW and 0.48 kW, respectively. These differences result in variations in steam consumption, which in turn impact facility-level thermal integration. CIP contributes additional steam and hot-water demand, depending on the sequence of full and reduced cleaning cycles. Aeration represents the third primary utility flow. The STR uses a synthetic gas mixture composed of 95% air and 5% CO_2. Across one complete batch, the total aeration-gas throughput is 11,506.8 kg, generating a corresponding CO_2-containing off-gas stream that falls within the gaseous WSV category. Medium preparation, washing buffers, and CIP drive water usage. The CIP balances indicate a per-batch usage of 58,778 kg of process water,

3,842 kg of cold water, 2,001 kg of ambient water, and 1,000 kg of sterile water. Combined with media preparation, the total water consumption for utilities exceeds 65,000 kg per batch, excluding the medium itself. Peak loads in the process are associated with STR cooling during exponential growth, DSP broth cooling, and thermal sterilisation. The highest chilled-water peak exceeds 8,900 kg/h during DSP cooling operations. The highest electrical load corresponds to STR agitation at 9.69 kW. The peak heating load occurs during thermal media sterilization in the 50:50 configuration, while the peak gas flow is defined by the aeration requirement of the 20 m^3 STR. Streams consist of depleted medium, wash solutions, impurities, mixed DSP waste, CIP discharge, and off-gas. Per batch, these include approximately 15,926 kg of depleted medium, 995 kg of USP-derived impurities, 4,839 kg of DSP waste, more than 60,000 kg of liquid effluents from CIP, and approximately 11,500 kg of gaseous emissions.

Waste Stream Mapping and Circularity Integration

Waste stream mapping was conducted using the WSV framework established by Brenner et al. (2025), which was applied to every unit operation in the platform process. Each stream was assigned to one of the five WSV categories (plastic, media/nutrient, organic, CO_2 off-gas, and heat/energy), and the corresponding valorisation routes were integrated into the process flows shown in Figure 3. The mapping creates a process-wide resource and effluent structure that supports circularity assessment, TEA/LCA coupling, and infrastructure planning.

Plastic waste originates from single-use components, including bioreactor bags, filtration modules, pipettes, tubing, protective consumables, and packaging materials. These materials differ in polymer type, contamination load, and reprocessing feasibility. Mechanical recycling is applicable for low-contaminated PP and PE components, while multilayer and heavily contaminated plastics require chemical recycling or energy-integrated incineration (Markarian, 2019; Hollands, 2024; Ringa, 2024). In the WSV framework, PLW streams are routed either to pyrolysis or to solvent-based polymer recovery, as outlined by Brenner et al. (2025), depending on solvent compatibility, bioburden, and sterilisation state. Media and nutrient waste (MNW) represents the most significant mass fraction in USP and DSP. It includes depleted medium from large-scale cultivation, wash buffers, and permeates from filtration or clarification processes. These streams contain salts, amino acids, trace elements, vitamins, lactate, ammonia, and protein degradation products (Hassell et al., 1991; O'Neill et al., 2022). Primary valorization

utilizes tangential flow filtration or ultrafiltration to recover nutrients and remove high-molecular-weight impurities, complemented by ion exchange or neutralization to reduce inhibitory metabolite concentrations. Where complete media upgrading is not viable, anaerobic digestion provides high-conversion, high-stability valorization pathways for nutrient-rich effluents (Bochmann et al., 2020; Millati et al., 2023). These strategies correspond to the MNW flows outlined in Brenner et al. (2025). Organic waste (ORW) arises from biomass residues, extracellular matrix fragments, TFF retentates, centrifugation solids, cell debris, and greywater from cleaning cycles. Solid organic fractions can be diverted to anaerobic digestion, producing biogas and digestate with reduced pollutant load. Liquid organic fractions may undergo aerobic polishing or membrane treatment to increase water recoverability and reduce discharge requirements. Brenner et al. (2025) (submitted; currently under review) highlight the potential for optional protein recovery from certain biomass-rich retentates, provided that regulatory and hygienic conditions are met. CO_2 off-gas (COG) emerges from bioreactor aeration and metabolic activity, as well as from downstream biological oxidation. Valorization pathways include amine-based CO_2 capture, off-gas conditioning for internal reuse, and biological uptake in microalgae systems (Haraguchi et al., 2022; Su et al., 2023). Within the WSV framework, COG can be reintegrated into pH control loops, biomass production, or regional carbon mitigation infrastructure, depending on facility design and regulatory constraints.

Heat and energy waste (HEW) is generated predominantly during the cooling of bioreactors, thermal sterilization, formulation, and extrusion. Heat exchangers, temperature-integration networks, and combined heat-and-power systems can transfer these residual thermal loads into pre-heating of media, CIP water, or facility-level HVAC systems (Götz et al., 2016; IEA, 2024). Brenner et al. (2025) identify HEW valorization as a high-impact lever for reducing external energy demand, particularly in STR-based USP, where metabolic and mechanical heat loads are concentrated.

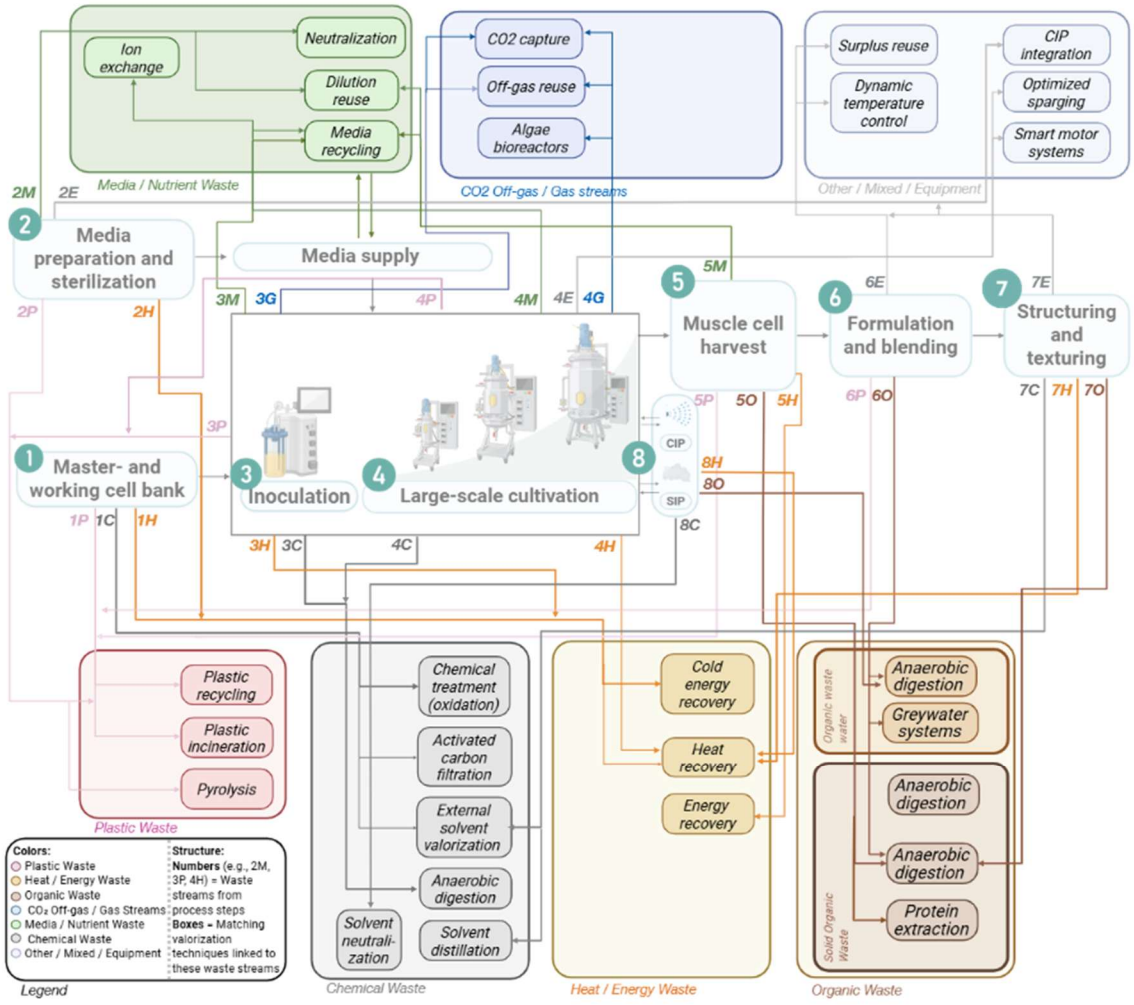

Figure 9: Waste stream valorisation framework of an exemplary CM production process

Among all strategies, TFF-based media recycling, anaerobic digestion, and heat recovery provide the most considerable circularity benefits and the most immediate engineering feasibility. Mapping each waste stream to its recovery pathway enables targeted infrastructure design, informs TEA/LCA parameters, and provides a foundation for evaluating circular manufacturing scenarios in WP4 and WP6 (Figure 9).

Strategic Case Studies

Case Study 1 and 2 – Circular Cell Culture (CCC) Systems and Waste Valorization (ACIB)

A literature study was conducted to identify existing possibilities of CCC systems for CM/CSF production as a waste valorization approach. Please refer to the extensive analysis of the topic in our review, available as a preprint (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5310265). Soon, the existing variants will be based on microalgae species that can propagate in waste cell culture medium, consuming ammonia and, if genetically modified, also lactate produced by the cultivated mammalian cells (Figure 10). Two main disadvantages of the published CCC systems were identified: (1) GFs, necessary for the growth of mammalian cells, were produced by the RL34 rat liver cells, which can hardly be used in the production of food; (2) microalgae species used have a low doubling time (8 hours to several days). We thus propose to utilize yeast as a recycling organism in a CCC system. Its doubling time is only 1,5-2 hours; it can be very easily genetically modified to produce required GFs and to metabolize ammonia and lactate; and it is a GRAS organism, with some species used in the food industry for several hundred years (S. cerevisie), and others are more and more widely used in recent times (e.g., Komagataella phaffii).

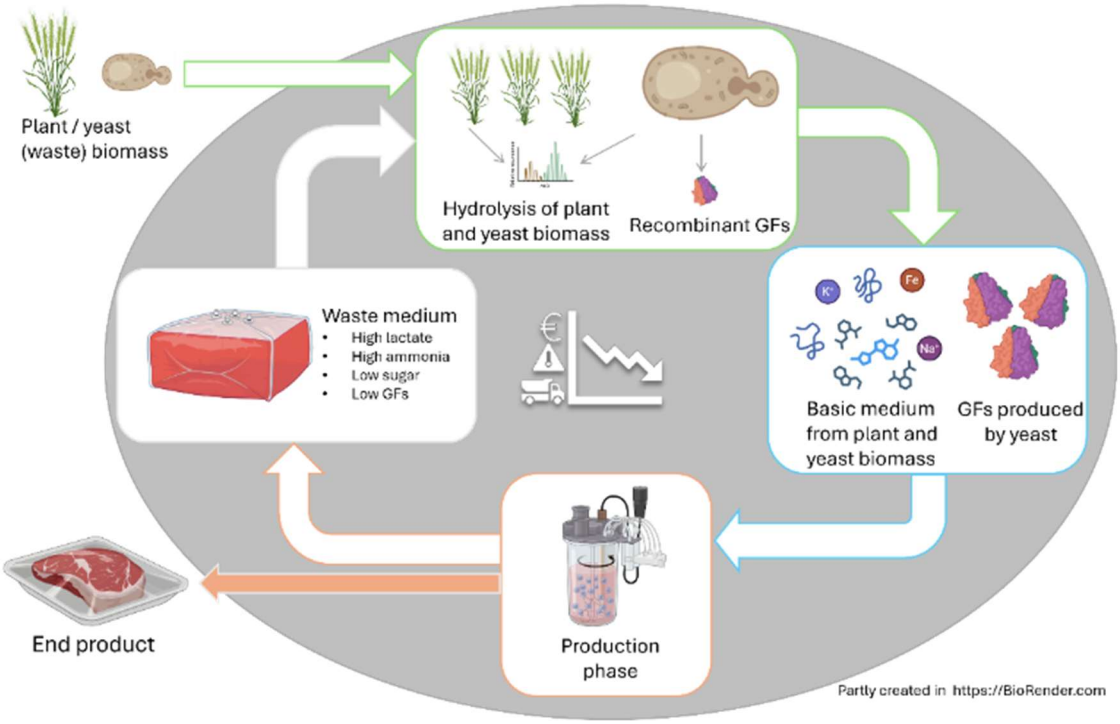

Figure 10: The potential application scheme of using yeast species as medium recycling organisms could be a more efficient and easier-to-adapt system than one based on microalgae.

First work in order to establish such a system was started:

- The efficiency of Komagataella phaffii strain producing a growth factor in the standard cell culture waste vs. in the full cell culture media vs standard yeast media is being evaluated.
- The efficiency of Komagataella phaffii strain producing a growth factor in the cell culture based on the hydrolysates-based basal medium vs standard yeast media is being evaluated.

This case study provides an initial estimate of the suitability of the yeast species for CCC systems and the data for comparison with existing algae-based CCC systems.

Case Study 3 – Media Recycling (VM)

In the context of sustainable biomanufacturing and reducing the environmental footprint of cell culture processes, this study examines the feasibility of recycling spent media. The project focused on two main objectives: (A) determining cellular tolerance rates to key metabolic byproducts, and (B) evaluating methods for removing toxic compounds. The media recycling phase involving VM addressed two critical aspects: defining tolerance thresholds for cells and assessing the removal of toxic compounds. The two primary toxic byproducts accumulated in the media are lactate (produced by cellular metabolism) and ammonium (Pereira et al., 2018), which result from both cellular metabolism and the degradation of glucose, respectively, as well as glutamine (Tritsch and Moore, 1962). This study concentrated on these two compounds. To mimic the conditions of media recycling, where spent or conditioned media is separated from harvested cells and reused for a new production batch, we focused on the effects of exogenously added lactate and ammonium. Tolerance rates for cells.

A) Cellular tolerance rates to lactate and ammonium

To assess tolerance, cells were cultured for three days in media supplemented with increasing concentrations of ammonium (Figure 11) or lactate (Figure 12). The concentrations tested were selected based on observations from high-density, five-day cultures. Based on these trials, it was observed that for the chicken cell line, increasing doses of lactate and ammonium concentrations led to reduced cell growth, with a 50 % reduction in cell growth attained theoretically at approximately 11 mmol/l of ammonium or 14 mmol/l of lactate. The limited cell growth could not be explained solely by cell death, as cell viability decreased only slightly with increasing concentrations of the two toxins. This indicates that extracellular

lactate and ammonium act primarily as cytostatic agents (inhibiting growth) rather than cytotoxic agents (inducing cell death).

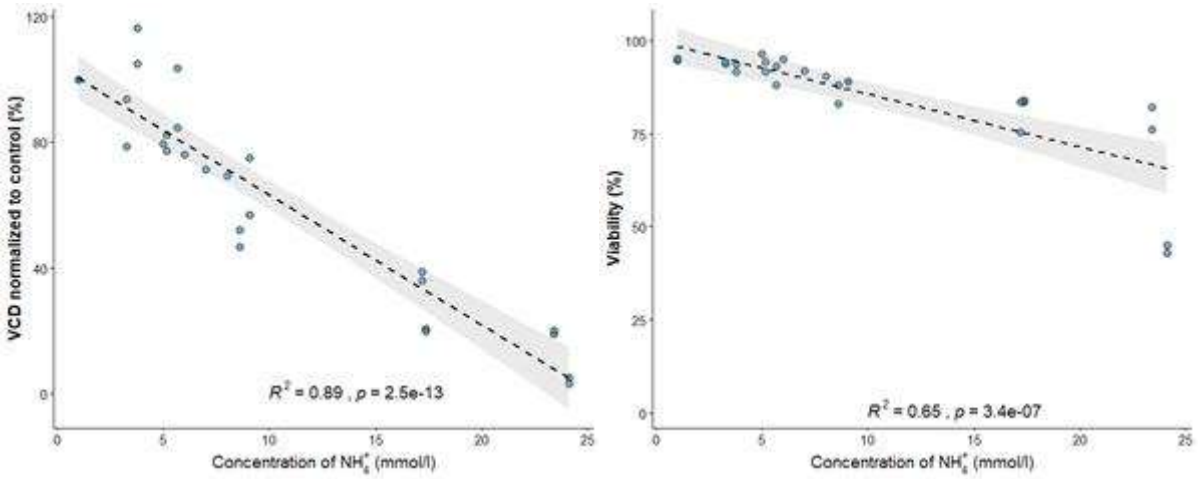

Figure 11: Correlation between ammonium (NH_4^+) concentration and normalized viable cell density (VCD) and viability. Normalized VCD and viability after three days of culture in media supplemented with different doses of NH_4^+. Control is at 1 mmol/l of NH_4^+. The black dashed line indicates the linear regression fit, with the shaded gray area representing the 95% confidence interval. The coefficient of determination (R2) and the p-value are inset, demonstrating the statistical significance of the negative correlation. Cell count and viability were assessed by the NORMA XS from Iprasense.

These results provide insight into the expected effects when lactate and ammonium concentrations reach high levels after several days of dense culture. It is important to consider that pH and osmolality are tightly related to the toxicity of these compounds. This is particularly relevant in bioreactors, where lactate production is often compensated by NaOH addition to maintain stable pH, which further increases osmolality. This study represents the impact of exogenous lactate and ammonium addition, which does not perfectly reflect the gradual accumulation seen in culture (where intracellular concentrations rise before extracellular ones). Furthermore, potential cell adaptation or reprogramming mechanisms to limit toxic impact were not explored here, neither was the study of other cell lines, or different media.

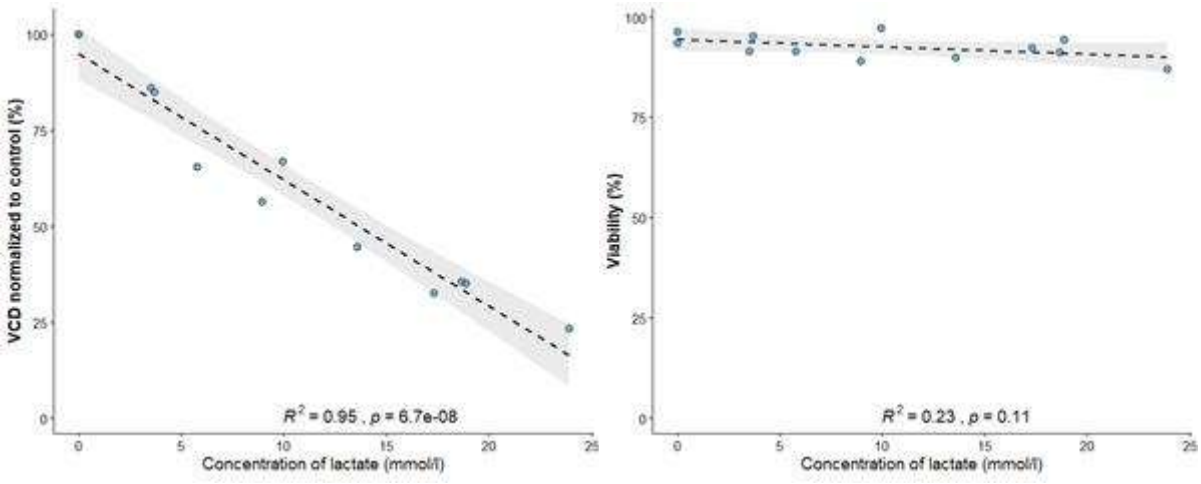

Figure 12: Correlation between lactate concentration and normalized viable cell density (VCD) or viability. Normalized VCD and viability after three days of culture in media supplemented with different doses of lactate. Control VCD is at 0 mmol/l of lactate. The black dashed line indicates the linear regression fit, with the shaded gray area representing the 95% confidence interval. The coefficient of determination (R2) and the p-value are inset, demonstrating the statistical significance of the negative correlation. Cell count and viability were assessed by the NORMA XS from Iprasense.

B) Toxic compound removal

A major hurdle to reusing spent media is the high concentration of ammonium and lactate. This section focuses on the specific removal of ammonium using zeolite. The removal of lactate, being significantly more complex, was not explored in this study. Zeolite is a mineral with a porous structure that is capable of trapping cations, making it a suitable candidate for removing ammonium. However, it is important to note that other cations, proteins, and media components may be non-specifically removed along with ammonium. This will not be presented in the following results. Two zeolite formulations were assessed for their capacity to remove ammonium from the media (Figure 13). It was observed that powdered zeolite (20 g/L) removed 5 mmol/L of ammonium in less than 30 seconds. However, the ammonium concentration plateaued at 5 mmol/L, likely due to zeolite saturation. In contrast, while pelletized zeolite offered easier handling and separation, it exhibited slower kinetics, requiring over 1,200 seconds to achieve a comparable 5 mmol/L reduction.

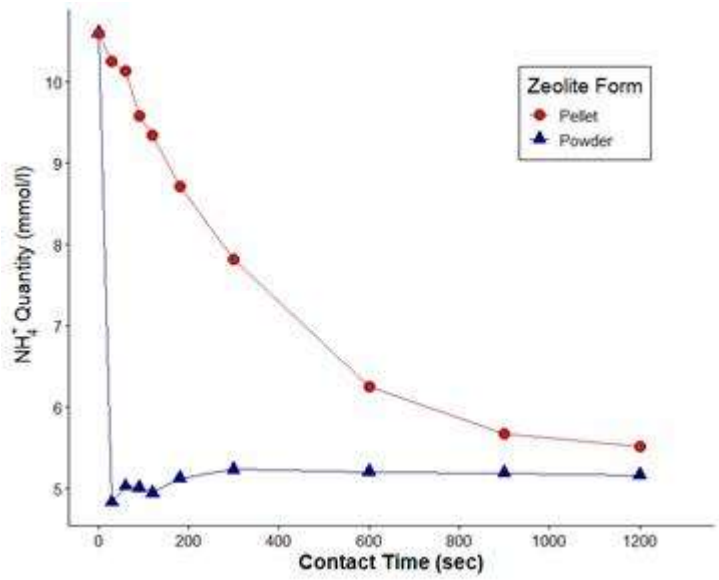

Figure 13: Effect of zeolite formulation on ammonium (NH_4^+) removal kinetics. Two different zeolite formulations: Powder (blue circles) and Pellet (red squares) were used on a media set at around 10 mmol/l of NH_4^+. The ammonium concentration was measured at different times on the NOVA flex system.

Following the validation of ammonium removal, cells were cultured for three days in mixtures of fresh and conditioned media (0–70%), using either non-treated conditioned media or zeolite-treated conditioned media.

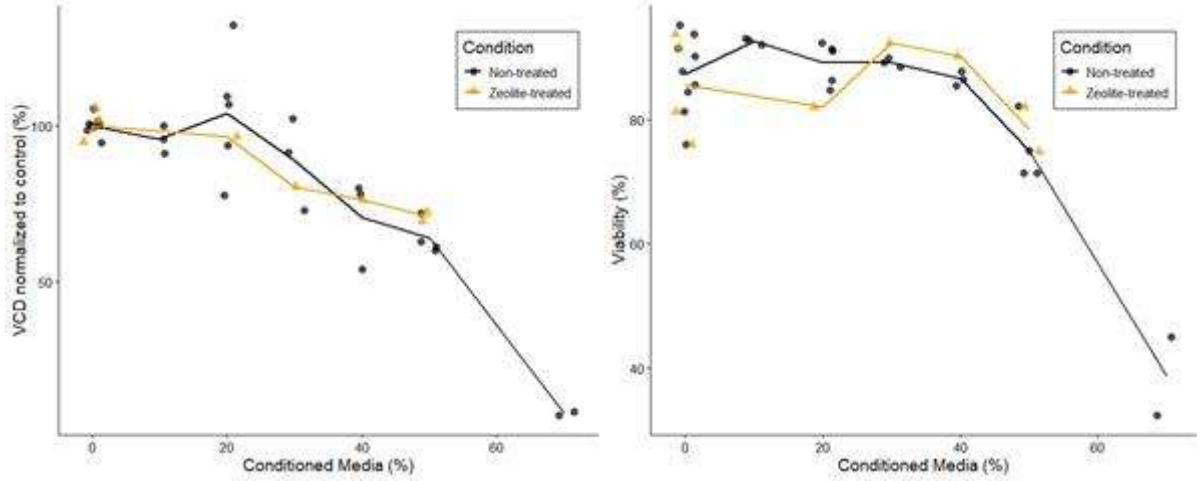

Figure 14: Impact of zeolite treatment on Conditioned Media toxicity. The graphs display the normalized Viable Cell Density (VCD) and viability of cells cultured in increasing concentrations of conditioned media (0-70%). Conditioned Media was either non-treated (black circles) or zeolite-treated (orange triangles). Cell count and viability were assessed by the NORMA XS from Iprasense.

Despite the effective removal of ammonium by the zeolite treatment, the conditioned media exhibited the same growth inhibition profile as the non-treated control (Figure 14), starting at a concentration of 30% conditioned media. Notably, this growth inhibition was not associated with a significant decrease in cell viability which only started at 50% conditioned media concentration. Consequently, the potential to reuse 20% of non-treated conditioned media represents a significant initial step toward minimizing waste generation and water consumption.

This case study demonstrates that the chicken cell line is sensitive to the accumulation of ammonium and lactate, with both metabolites acting as growth inhibitors. While zeolite treatment (particularly the powder formulation) proved highly effective at rapidly reducing ammonium levels, this removal alone was insufficient to restore cell growth in recycled media. Since zeolite-treated media showed similar growth inhibition to non-treated media, we conclude that ammonium is not the sole, or perhaps even the primary, limiting factor in this recycling context. The persistence of growth inhibition suggests that lactate accumulation (which was not removed), other toxic metabolites, or the depletion of essential nutrients plays a dominant role in limiting cell proliferation. Future media recycling strategies must therefore address lactate removal or nutrient supplementation alongside ammonium reduction to be effective.

Case Study 4 – Perfusion System Optimization (TUM/UM)

This case study includes three main activities, namely A) hardware assessment and development of a perfusion prototype for lab-scale cell culture experiments, B) assessment of tubing materials for peristaltic perfusion in cell culture applications, and C) assessing the impact of shear stress on in vitro cell cultures. Together, these activities provide insight into the definition of technical, biological, and material-related performance criteria for dynamic cell cultures.

A) Hardware assessment and development of a perfusion prototype for lab-scale cell culture experiments

Peristaltic pumps are widely used in biomedical research and bioprocessing because they provide sterile, closed-loop medium handling, eliminating the exposure of fluids to mechanical components. However, most commercial peristaltic systems are not designed for in-incubator use, meaning they are not suitable for prolonged (several days to weeks) continuous operation under standard cell culture conditions, where high humidity, elevated CO2, and restricted airflow can negatively affect mechanical and electronic components. To address this gap, we conducted in-incubator experiments and defined technical specifications for an incubator-compatible perfusion module, which is currently being co-developed with external subcontractors. We assembled a simple test platform consisting of an Ismatec® multi-channel peristaltic pump head mounted on a custom holder and driven by different stepper motors (Nema-17, Nema-23, and Nema-34). The entire assembly was operated inside a standard cell culture

incubator for up to 4 weeks at a time. It was also maintained idle for up to 6 months to evaluate performance, robustness, ease of aseptic handling, and the impact of the mechanical/electronic components on the incubator environment. Key findings include:

- Torque and thermal stability for motor selection are essential. While smaller motors may provide sufficient torque to maintain prolonged peristalsis, this requires higher current settings, which increases heat generation and can cause temperature disturbances within the incubator. With an 8-channel pump head, only the largest motor (i.e., Nema 34) allowed stable perfusion without noticeable heat generation (i.e., touching the motor did not result in a higher temperature than its environment).
- Stepper motors generate heat when stationary under current. An automatic power cut logic during idle states is therefore beneficial for preserving system stability.
- Many materials corrode faster under cell culture conditions, including bearings, gaskets, mechanical couplers, electronic connectors, cables, etc. To avoid degradation, cross-contamination, or particle release, the planned setup utilises corrosion-resistant materials where possible and will ideally be removed from the incubator when not in operation.
- Hardware that is intended for incubator use represents a hazard for aseptic work. Therefore, the design must avoid cavities, slits, and other complex features that could trap moisture or biological contaminants. The surfaces of the planned setups are smooth and accessible for cleaning, disinfection, and maintenance.

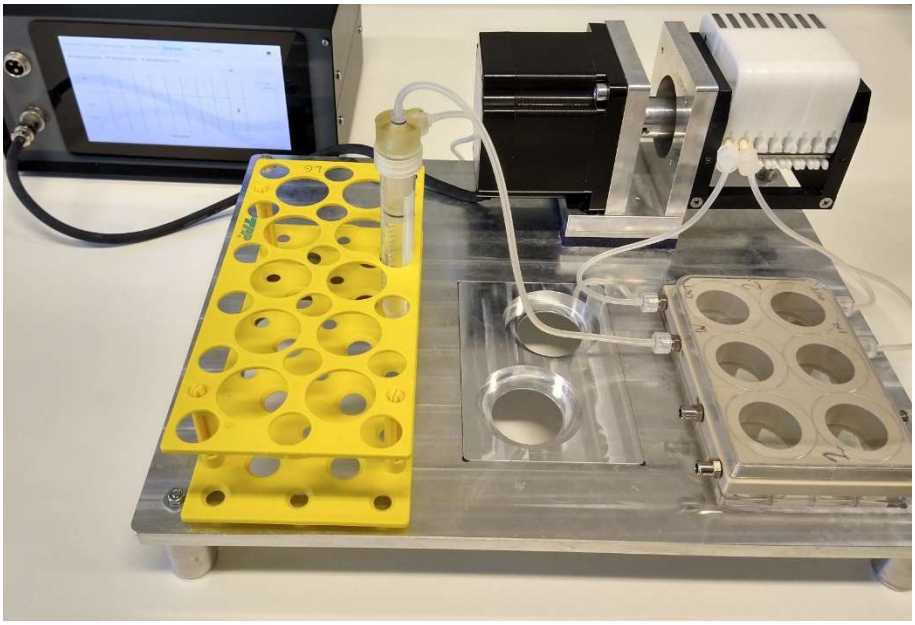

Figure 15: photo of the peristaltic set-up used for testing and specification development, assembled using a Nema 34 stepper motor and Ismatec ® 8-channel peristaltic pump head.

A simple experimental layout, based on direct motor-driven actuation, shows promise for various modes of dynamic culture. In addition to peristaltic pumping, where medium flow-rate and shear stress on the exposed surfaces can be controlled (further described in section C), a simple stepper motor can be used inside cell culture incubators to drive: wave-based (rocking motion) bioreactors to provide a more gentle perfusion through oscillating liquid flow in a closed system (suitable for suspension cultures and tissue scaffold cultures), and rotating chamber bioreactors – closed system with continuous rotation and even nutrient consumption suitable for cell suspensions and microcarrier-based cultures.

The diversity of cultivation approaches required in cellular agriculture (suspensions, microcarriers, scaffolds) necessitates different perfusion strategies, which can, however, be implemented using a similar base system that allows for comparative experimental studies. Based on the obtained findings, we propose a single multi-mode perfusion prototype for in-incubator use that supports peristaltic flow, rocking-based gravity perfusion, and continuous rotation. The prototype is currently in co-development with subcontractors. Complete documentation, required for building and operating the prototype (including mechanics, electronics, assembly, and software), will be released under an open-source licence after development is completed by the end of WP3.

B) Assessment of tubing materials for peristaltic perfusion in cell culture applications

While peristaltic pumps offer advantages for aseptic handling in a wide range of applications, this type of operation subjects tubing to repeated compression–release cycles, this mechanical stress can cause spallation, i.e., the shedding of nano- and microplastic particles from the inner tubing surface. Given the need for stable and continuous perfusion, it is important to understand how tubing materials behave under realistic cell culture conditions and how their performance/wear may impact the production of food in cellular agriculture. To evaluate the suitability of tubing for incubator-based perfusion, we conducted a systematic abrasion and contamination study using the peristaltic pump set-up for in-incubator use (as described above). Six commonly used autoclavable tubing types were tested, including Tygon® LMT-55, PharMed® BPT, Tygon® SI 3350, standard silicone, peroxide-cured silicone, and Viton®.

Each tubing type was perfused under two flow rates:

- 1 mL/min for 96 h
- 10 mL/min for 24 h

Three liquids were used to evaluate how fluid composition affects tubing abrasion, namely:

- ultrapure water (control medium),
- Advanced Dulbecco's Modified Eagle Medium – ADMEM (Gibco; widely used cell culture medium, compatible with many different cell cultures),
- Human Endothelial Cell Medium (Cell Applications, Inc.; suitable for use with endothelial cells, which are naturally exposed to flow and related shear stress).

To distinguish mechanical abrasion from passive leaching, static controls were prepared by incubating the same media inside tubing segments without peristalsis. All collected liquids were analyzed for pH and conductivity changes, as well as particle size distribution using dynamic light scattering (Anton Paar Litesizer 500) in collaboration with the Laboratory for Characterization and Processing of Polymers (LCPP, UM). Selected results are shown in Figure 16 and Table 5. Key findings:

- All tubing materials released particles during peristaltic pumping.
- Spallation was higher in complex media compared to water, indicating medium-driven acceleration of wear.
- Particle-size distributions span from the nanometer to micrometer range.

- Flow rate, medium type, and tubing material all significantly influenced particle release.

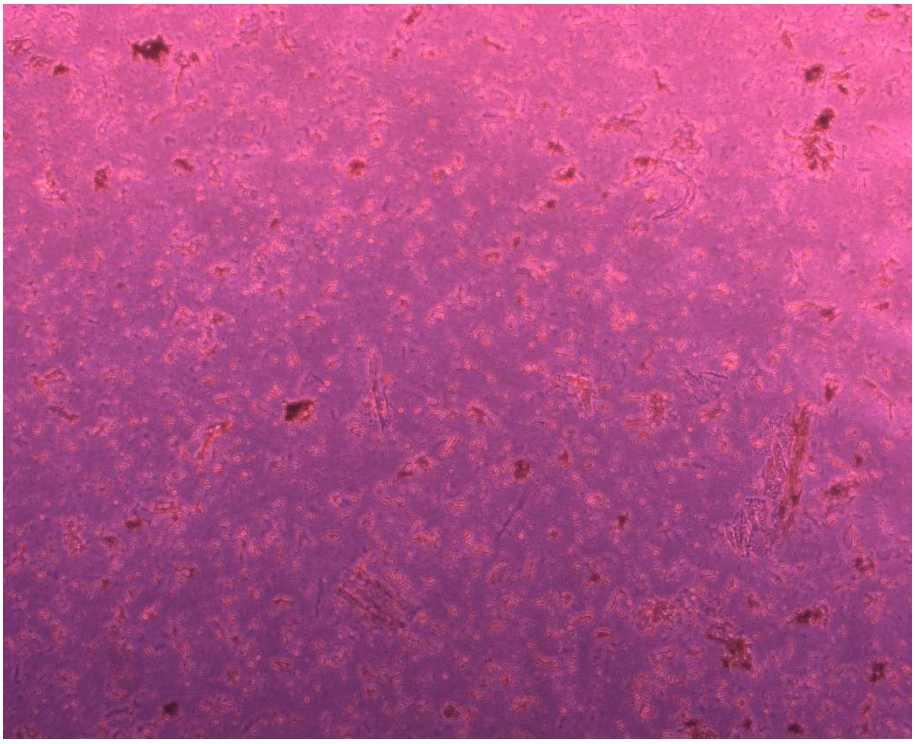

Figure 16: A micrograph of spallation particles after a peristaltic pumping experiment.

The presence of nanoparticles in the tested media is especially critical, as reliable filtration by standard filters may be difficult. A portion of the particles is also small enough to allow internalization by cells, which could affect both the production process in cellular agriculture and pose a hazard to the final consumer.

Table 5: Comparison of selected particle size distributions after perfusion with different media and flow rates.

	Peak 1			Peak 2			Peak 3				
SAMPLE	Fraction [%]	Size [nm]	Size SD [nm]	Fraction [%]	Size [nm]	Size SD [nm]	Fraction [%]	Size [um]	Size SD [um]	pH	Conductivity [**mS/cm**]
Ultrapure water (reference)	/	/	/	/	/	/	/	/	/	5,21	0,19
Ultrapure water (10 ml/min) – Tygon LMT 55	32.27	295,10	49.11	62,73	2024,00	342,60	/	/	/	3,89	0,06
ADMEM (10 ml/min) – Tygon LMT 55	7.38	16.65	4.22	86,77	478,40	139,72	5,84	6,83	0,995	8,65	68,60
Endo medium (10 ml/min) – Pharmed BPT	7.27	18.44	5.33	89,45	243,90	89,45	/	/	/	8,48	69,10
Endo medium (1 ml/min) – Tygon LMT 55	23,55	26,59	7,73	52,86	422,90	68,49	23,59	11,89	1,715	8,52	67,90

The obtained results highlight the importance of selecting appropriate equipment and materials, as well as implementing preventive contamination control, compared to downstream filtration, which may be insufficient in some cases for cellular agriculture and other food production processes that rely on continuous perfusion. The findings of the described activities will be presented at the 3rd International Conference of the Portuguese Association for Cellular Agriculture (November 2025 in Portugal).

C) Assessing the impact of shear stress on in vitro cell cultures

Continuous medium exchange allows maintenance of in vitro cultures with higher cell densities and larger volumes, which is essential for tissue engineering, both in the context of cellular agriculture and biomedical research. However, perfusion also creates shear forces that can act as a stimulus or cause damage and detachment of (adherent) cells. To investigate the correlation between shear stress and cell culture, a study was conducted comparing computational fluid dynamics (CFD) simulations with an in vitro experimental study in collaboration with the Laboratory for Advanced Computational Engineering and Experimenting (UM). CFD simulations (using Ansys CFX) were made for a steady-state laminar flow to quantify wall shear stress at flow rates between 0 and 100 mL/min in a geometry that mimics flat-bottom ibidi™ microchannels (µ-Slide I Luer), which showed a linear relationship between flow rate and average wall shear stress, enabling accurate prediction of shear values from flow rate alone. To validate computational predictions and characterize biological responses, human umbilical vein endothelial cells (HUVEC) were cultured in ibidi™ flow channels (µ-Slide I Luer) under five flow regimes: 0.0 (control), 0.1, 1.0, 5.0, and 10.0 mL/min, corresponding to shear stresses spanning 0–0.64 Pa. Cells were perfused for 72 hours using the in-incubator peristaltic pump setup (described in section A) with ADMEM (Gibco), supplemented with 5% FBS. The medium was recirculated in a closed loop with a degasser permitting adequate gas exchange. After perfusion, cell morphology was evaluated using brightfield and fluorescence microscopy (the latter following actin staining using phalloidin). Selected results are shown in Figure 17.

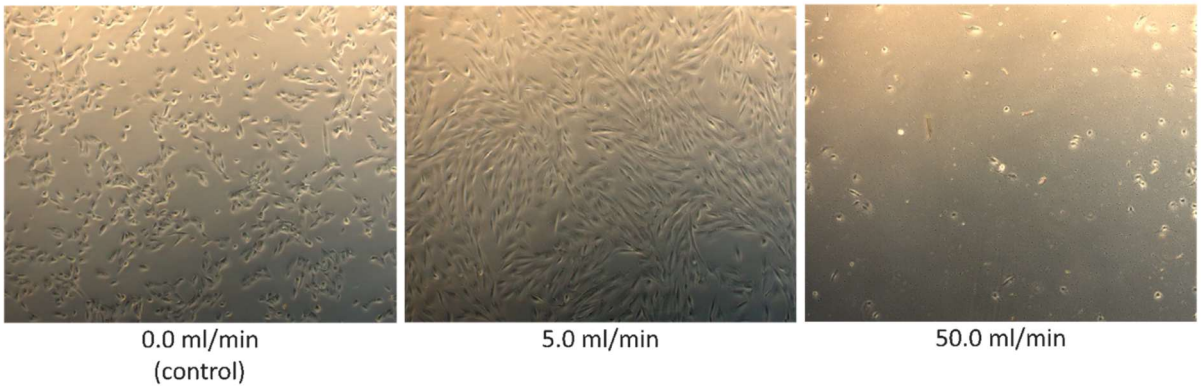

Figure 17: Comparison of HUVEC morphology under different flow regimes.

Under low shear regimes (≤1mL/min), the cells maintained comparable morphology to control samples, with some signs of cytoskeletal alignment. Under intermediate shear (flow rates of 5–10 mL/min), the cells exhibited pronounced elongation and alignment in the direction of flow. Flow rates of 50 mL/min or higher caused cell detachment, suggesting that the created loads exceeded the adhesion capacity on standard culturing surfaces. The obtained results are currently under internal review, and the manuscript is planned for submission in 2026.

The optimisation of continuous perfusion cultivation was performed by quantifying how the perfusion rate, specific growth rate, and metabolite tolerance jointly determine the steady-state operating point of an ATF-retained stirred tank. Alternating tangential flow (ATF) retention typically supports perfusion intensities of approximately one reactor volume per day, which for a two cubic meter culture corresponds to an exchange of 1,600 liters of medium per day, equivalent to 66–70 liters per hour. Under a net specific growth rate of 0.035 per hour, corresponding to a twenty-hour doubling time, the biomass bleed required to maintain a constant working volume is approximately 55 litres per hour, resulting in a required combined feed rate of around 120 litres per hour to keep the system at steady state. This defines the baseline perfusion window within which nutrient supply and metabolite removal remain balanced. It also sets a maximum allowable residence time of roughly twenty-four hours for extracellular medium, while the adequate biomass residence time is governed by the specific growth kinetics rather than volumetric turnover. Growth kinetics have a first-order effect on achievable volumetric productivity. Literature values for mammalian perfusion systems indicate doubling times ranging from twelve to forty-eight hours. These correspond to net specific

growth rates between 0.058 and 0.014 per hour. Within this range, the modelled production rate changes by approximately a factor of three. At a doubling time of twenty hours, biomass accumulation rates on the order of ten to thirteen kilograms per hour can be supported before either oxygen transfer or metabolite accumulation becomes limiting. At a twenty-four-hour doubling time, this rate decreases proportionally to eight to nine kilograms per hour, and at forty-eight hours it falls to approximately four to five kilograms per hour. These values illustrate that perfusion systems exhibit a strong dependency on μ-controlled residence time extension, which determines both volumetric productivity and biomass washout stability.

Metabolite inhibition defines the upper boundary for sustained high-density perfusion. Published data for mammalian production systems indicate that lactate concentrations above approximately 50 millimoles per liter and ammonia concentrations above 2 to 6 millimoles per liter significantly reduce specific growth rate and oxygen uptake capacity. Increasing the permissible ammonia concentration from 2 to 6 millimoles per liter expands the feasible biomass productivity range substantially. For twenty-hour doubling times, this shift increases the theoretical productivity limit from approximately five kilograms per hour to more than ten kilograms per hour, until oxygen transfer capacity becomes the limiting factor. These values reflect the coupling between inhibitor accumulation, the oxygen mass transfer coefficient, and the maximal dry cell weight (DCW) attainable under constant perfusion. Tolerance improvements, therefore, effectively expand the steady state operating window and allow operation closer to the oxygen constraint. Perfusion rate, specific growth rate, and metabolite tolerance jointly determine the adequate residence time of cells and extracellular metabolites. At a fixed perfusion intensity of one reactor volume per day, the hydraulic residence time is approximately twenty-four hours; however, the biological residence time is significantly longer when μ is high, allowing cell densities that can surpass the limits of fed-batch systems. Increasing metabolite tolerance increases the slope of the washout curve, shifting the inhibition boundary such that the process becomes oxygen-limited before it becomes metabolite-limited. This behaviour is consistent with mechanistic models of mammalian perfusion systems, where the dominant constraint transitions from metabolic inhibition to oxygen transfer as μ increases.

The implications for facility-scale waste generation are substantial. Techno-economic analyses indicate that achieving 100,000 tonnes of biomass per year requires approximately 20,000 cubic metres of bioreactor volume in batch mode. In contrast, perfusion reduces the cumulative reactor capacity to around 2,600 cubic metres. This corresponds to an almost eight-fold decrease in stainless steel demand, as well as cooling infrastructure, floor space, and associated embodied environmental impact. Although perfusion processes typically consume 12 to 14 litres of medium per kilogram of biomass, compared to 10 to 11 litres per kilogram for batch, concentrated-feed perfusion, and intensified retention systems can reduce medium consumption by 20 to 50 percent. Extending perfusion duration increases annual output; however, the effect shows diminishing returns. Beyond ten to twenty days of continuous processing, doubling the perfusion length increases annual biomass production by only about five to nine percent. Together, these quantitative relationships show that perfusion system optimisation is governed by three tightly linked variables: perfusion rate, which sets hydraulic residence time; specific growth rate, which defines biological residence time and washout stability; and metabolite tolerance, which determines the upper limit of sustained DCW and the proximity to oxygen-transfer constraints.

Discussion

The modeling results demonstrate that large-scale CM production is primarily constrained by bioreactor availability, oxygen transfer capacity, cooling demand, and metabolite inhibition, which jointly define the feasible operating window for both batch and perfusion systems. Perfusion reduces the installed reactor volume and the demand for stainless steel eightfold. However, it shifts the burden toward higher medium consumption and increased utility requirements, illustrating the central trade-off between volumetric productivity, medium intensity, and energy use. While batch production benchmarks cumulative capacity needs above 20,000 m^3, continuous perfusion illustrates a fundamentally different planning logic that reduces installed fleet volume demand by nearly 90%, shifting the decisive focus from reactor counts to shared facility limits such as cooling, gas corridors, and commissioning realism (Humbird 2021; Yang et al. 2023; Joseph et al. 2024). Industrial throughput remains anchored in 20 m^3 stainless systems for production ceilings, whereas smaller 2 m^3 pilot nodes drive faster commissioning cycles, process iteration, and industrial learning, not total output equivalence (Rischer et al. 2020; Humbird 2021). Waste recovery and circular media loops become viable when effluent fractions are routed directly into on-site resource conversion units,

such as anaerobic digestion and utility heat reintegration, but this is contingent on process stability, predictable hydrodynamics, and constant effluent quality rather than single-compound separation assumptions (Bhat et al. 2022; Joseph et al. 2024). Long-term European deployment planning must therefore lock convergence between modeled utility demand corridors, stainless fabrication capacity, and commissioning time envelopes until 2050. The key contribution of this work is the integration of platform process modelling, perfusion optimisation, waste stream mapping, and infrastructure scaling into quantitative frameworks that link WP3 process engineering with WP6 sustainability analysis. Remaining research challenges include refining metabolite tolerance data for relevant production cell lines, improving medium recycling efficiency, modeling dynamic utility loads, and validating oxygen transfer limits at an industrial scale. The results will be incorporated into the final FEASTS deliverables as inputs for TEA sensitivity analysis, LCA boundary conditions, infrastructure scenario modelling, and the circularity assessment spanning WP3.4, WP4, and WP6.

Conclusions and future perspectives

This report outlines the key process and infrastructure determinants that shape the feasibility of industrial cultured-meat production. The bioprocess modelling results indicate that scale-up remains primarily limited by oxygen transfer capacity, achievable cell densities, medium turnover, and the substantial utility demands characteristic of large stainless-steel bioreactor systems, consistent with observations from large-scale mammalian cell culture (Humbird, 2021). At the facility level, cooling capacity, compressed-gas supply, and wastewater handling remain decisive constraints. The analysis of waste streams shows that meaningful circularity requires dedicated valorization units integrated directly into the process architecture, rather than downstream add-ons. Perfusion offers clear reductions in installed reactor volume; however, its advantages are contingent upon metabolite tolerance, perfusion stability, and medium-recycling performance. Trade-offs between productivity, resource efficiency, and operational robustness therefore persist at every stage. The integration of process models, infrastructure scaling, and waste-stream analysis provides a coherent basis for subsequent techno-economic and life-cycle assessments within FEASTS. Future work should prioritize quantitative modeling of media-recycling efficiencies and validation of high-density perfusion at the pilot scale.

The results generated here contribute directly to the final FEASTS deliverables by defining realistic engineering boundaries for the circular and scalable production of CM and CSF. The following lists the papers emerging from Task 3.5 and the key exploitable results based on modeled European scale-up and scale-out production insights.

Papers:

- Scale-up paper (in writing): Comparative assessment of industrial scale-up and distributed scale-out reactor systems for European production networks.
- CM Platform Design and Process Modelling (in writing)

- WSV paper (submitted): Waste stream recovery routes for cultured biomass at scale.

- Bioreactor parameters paper (published): Industrial stirred tank thresholds, utility ceilings, and process assumptions for cultured biomass: https://www.researchgate.net/publication/396667763_Bioreactor_parameters_and_systems_for_cultured_meat_production

- KPI framework paper (to be submitted)

- Perfusion evaluation paper (in writing): Perfusion versus batch productivity scenarios comparing medium use and required bioreactor volumes.

- Value chain infrastructure paper (in writing): European fabrication capacity, utility hotspots, and facility scale requirements for cultured biomass supply planning.

- Production planning paper (in writing): Centralized 20 m^3 infrastructure scale up versus localized 2 m^3 system network logics for Europe.

Key Exploitable Results (KERs):

- End to end bioprocess model (SuperPro): Full mass and utility flows including 90:10 and 50:50 sterilization split sensitivity, media staging, expansion cascades and downstream intensity.
- Local 2 m^3 plant model (SuperPro): Material and utility deltas for localized facility classes with adapted media volumes and intensified downstream blocks.
- Steady state perfusion model (SuperPro): Continuous biomass concentration scenarios run over 33 days with annual productivity calibration.
- 100 kTA annual capacity model (SuperPro): Total bioreactor volume comparison for batch versus continuous formats at equal annual output assumptions.
- Split sensitivity utility model (SuperPro): Quantified reduction corridors for water, thermal loads and cleaning utilities depending on sterilization split and tank resizing constraints.
- Excel scaling model: Facility clustering feasibility mapped in a tabular scale-out network for Europe using the conventional meat demand capture assumptions, plant productivity scenarios, and reactor size classes. (This replaces the previous line and reflects the actual Excel model output you built.)

References

Alves, C. et al. (2022) 'Bioreactor design strategies for large-scale mammalian cell culture', *Journal of Chemical Technology and Biotechnology*, 97, pp. 1885–1898.

Allan, S.J., De Bank, P.A. and Ellis, M.J. (2019) 'Bioreactor technologies for the expansion of mammalian cells', *Biotechnology Letters*, 41(7), pp. 1255–1267.

Bélanger, A., Miller, T. and Doran, P. (2023) 'Oxygen transfer limitations in high-cell-density mammalian bioreactors', *Biotechnology Progress*, 39(1), e3315.

Bellani, C.F. et al. (2020) 'Scale-up technologies for the manufacture of adherent cells', *Frontiers in Nutrition*, 7, 575146.

Bielser, J.M. et al. (2018) 'Continuous mammalian cell culture for bioprocessing', *Biotechnology Progress*, 34(6), pp. 1–15.

Bielser, J.M. et al. (2020) 'Perfusion culture technologies and applications', *Journal of Biotechnology*, 310, pp. 67–76.

Bochmann, G. and Montgomery, L.F.R. (2020) 'Biogas from solid-state anaerobic digestion', *Waste Management*, 103, pp. 208–214.

Brenner, K.J. et al. (2025) 'Waste stream valorization in cultivated meat manufacturing: Pathways toward circularity and sustainability', *Manuscript*, July 2025.

Brenner, K.J., Henkel, M. and FRA team (2025) 'Engineering determinants of bioreactor performance for industrial cultured meat', *Manuscript*, November 2025.

CE Delft (2021) *Sustainability of cultivated meat*. Delft: CE Delft.

Cousin, T. et al. (2021) 'Gas–liquid mass transfer in large bioreactors', *Chemical Engineering Science*, 246, 116993.

Ellis, M.J. et al. (2022) 'Sustainability considerations for cellular agriculture', *Biotechnology Advances*, 56, 107947.

Eurostat (2023) 'European meat consumption statistics'. Brussels: European Commission.

FAO (2019) *Future protein demand and supply projections*. Rome: United Nations FAO.

Ghosh, S. et al. (2023) 'Hybrid modelling frameworks for large-scale bioprocesses', *Computers & Chemical Engineering*, 178, 108402.

Götz, M., Lefebvre, J. and Mörs, F. (2016) 'Renewable power-to-gas: A technological review', *Energy*, 85, pp. 137–149.

Haraguchi, T. et al. (2022) 'CO_2 capture in bioreactor exhaust streams using microalgae', *Journal of CO_2 Utilization*, 58, 101906.

Hassell, T., Gleave, S. and Butler, M. (1991) 'Growth inhibition in cell culture by ammonia', *Cytotechnology*, 5, pp. 63–75.

Hollands, T. (2024) 'Advances in chemical recycling of plastics', *Materials Today Chemistry*, 33, 101072.

Humbird, D. (2020) 'An industrial analysis of cultured meat production', *Good Food Institute Technical Report*, March 2020.

Humbird, D. (2021) 'Scale-up economics for cultured meat', *Biotechnology and Bioengineering*, 118(8), pp. 3239–3250.

IEA (2024) *Industrial heat recovery systems: Global technology review*. Paris: International Energy Agency.

Jain, E. and Kumar, A. (2021) 'Oxygen limitations in mammalian cell bioreactors', *Bioprocess and Biosystems Engineering*, 44(3), pp. 453–468.

Kemp, R. et al. (2023) 'Utility demands of mammalian bioprocesses', *Journal of Industrial Microbiology & Biotechnology*, 50(4), kuad009.

Li, D. et al. (2023) 'Advanced nanofiltration and ion-exchange for media recycling', *Separation and Purification Technology*, 307, 122930.

MacQueen, L.A. et al. (2019) 'Engineering challenges in large-scale cell culture', *Current Opinion in Chemical Engineering*, 26, pp. 120–130.

Markarian, J. (2019) 'Recycling strategies for bioprocessing plastics', *Plastics Engineering*, 75(3), pp. 32–38.

McKinsey (2021) *Cultivated meat: Out of the lab, into the future*. New York: McKinsey & Company.

Miller, T., Mariani, R. and Burner, R. (2020) 'Techno-economic modelling of cultured meat media costs', *Foods*, 9(9), 1156.

Millati, R. et al. (2023) 'Anaerobic digestion of nutrient-rich wastewater streams', *Renewable Energy*, 205, pp. 247–257.

Montgomery, E. et al. (2021) 'Thermal and mixing constraints in stirred tank bioreactors', *Chemical Engineering Journal*, 420, 127650.

O'Flaherty, R. et al. (2022) 'Scalability limits of mammalian cell bioreactors', *Biochemical Engineering Journal*, 181, 108377.

O'Neill, K. et al. (2022) 'Nutrient composition and depletion kinetics in serum-free media', *Biotechnology Progress*, 38(2), e3214.

Pereira, Sara, Helene Faustrup Kildegaard, et Mikael Rørdam Andersen. 2018. « Impact of CHO Metabolism on Cell Growth and Protein Production: An Overview of Toxic and Inhibiting Metabolites and Nutrients ». *Biotechnology Journal*13 (3): 1700499. https://doi.org/10.1002/biot.201700499.

Perez-Pinera, P. et al. (2023) 'Energy intensities of precision fermentation', *Biotechnology and Bioengineering*, 120(5), pp. 1241–1252.

Ringa, N. (2024) 'Pyrolysis-based recycling of single-use plastics', *Journal of Cleaner Production*, 412, 137002.

Risner, D. et al. (2020) 'Metabolic limitations of myoblast culture', *Metabolic Engineering*, 61, pp. 1–13.

Risner, D. et al. (2023) 'Energy and water requirements for cultured meat production', *Journal of Industrial Ecology*, 27, pp. 540–556.

Specht, L. (2020) *An analysis of culture media costs and scale-up feasibility*. Good Food Institute.

Stephens, N. et al. (2018) 'Bringing cultured meat to market: Technical, socio-political and economic considerations', *Trends in Food Science & Technology*, 78, pp. 155–166.

Stout, A.J. et al. (2023) 'Downstream recovery strategies for cultured meat biomass', *Biotechnology and Bioengineering*, 120(1), pp. 143–158.

Su, Y. et al. (2023) 'Biological CO_2 fixation pathways via algae-based systems', *Bioresource Technology*, 389, 129836.

Sverdlov, A. et al. (2024) 'Modelling hybrid mechanistic–ML bioprocesses', *Computers & Chemical Engineering*, 182, 108630.

Tritsch, G. L., et G. E. Moore. 1962. « Spontaneous decomposition of glutamine in cell culture media ». *Experimental Cell Research* 28 (2): 360-64. https://doi.org/10.1016/0014-4827(62)90290-2.

Zhang, F. et al. (2022) 'Separation and purification technologies for cell-based meat processing', *Separation and Purification Technology*, 291, 120928.

FEASTS
Fostering European Cellular Agriculture for Sustainable Transition Solutions

Final Notes and Recommendations

This report confirms that scaling cultured meat (CM) and cell-based seafood (CSF) for Europe must be treated as an infrastructure-first planning problem. Process-mode scenarios model a 100 kTA annual biomass target, demonstrating a need for a bioreactor capacity of over 20,000 m^3 in batch operation versus ~2,600 m^3 in continuous perfusion for equal annual output, resulting in an 87% capacity relief that enables regional clustering strategies. The results also show that 20 m^3 stirred tank clusters remain the throughput backbone for high-capacity manufacturing, while 2 m^3 distributed tanks accelerate commissioning and learning, not production ceilings. Media blending at ~20% fresh-conditioned ratios is feasible without significant viability decline; however, sustained cell growth limitations remain inhibitor-coupled rather than solvable by ammonium removal alone. Scalability is defined by tank size thresholds and shared cooling and cleaning utility limits, not linear reactor multiplication assumptions. Based on these results, the European Union and its funding and regulatory bodies must implement three priorities without compromise: (1) expand hygienic stainless fabrication capacity to support reactor clustering across regions, (2) fund replication logic that models commissioning realism and utility hotspots rather than vessel numbers, and (3) incentivize stable circular waste-to-resource flows instead of isolated single-compound removal pathways. These decisions anchor continental scalability, reduce long-term cost drift, and define the deployment boundary for structured industrial learning and scale-out clustering across Europe to 2050.

Funded by
the European Union