

Trends in cultivated meat scale-up and bioprocessing

A summary of a 2023 industry-wide survey

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Executive Summary

This report presents key findings from a survey conducted in early 2023 involving 30 companies in the cultivated meat industry, including both producers and suppliers. The primary focus was to assess the current state of the industry's production capabilities and to outline the projected scale-up plans over the next few years by deeply surveying industry trends related to equipment and material usage, current and future production facilities, food safety considerations, and proliferation, differentiation, and harvesting strategies for scale up.

Key insights include:

- The industry currently operates at a small scale, with most production capabilities at the kilogram scale. An encouraging number of companies are scaling up their operations with plans to install bioreactors with capacities from tens to hundreds of thousands of liters in larger facilities within the next three years, enabling hundreds to thousands of tons of cultivated meat production annually.
- Companies are actively exploring various bioprocessing techniques and bioreactor designs to optimize their processes. These include using diverse types of bioreactors such as stirred-tank or air-lift, employing different modes of operation such as fed-batch or continuous, and implementing recycling and filtration strategies to reduce costs.
- Some companies acknowledged knowledge gaps in regulatory affairs, and some attributed this to the fact that relevant information is lacking or difficult to obtain. Those in the cultivated meat industry should collaborate

with regulatory agencies, leveraging insights from approved companies to establish the regulatory framework.

 Cultivated meat companies predominantly favor scale-up over scale-out strategies in the survey, potentially indicating a shift from R&D scale, while also exploring different bioreactors and operational modes. While variations in cell types, products, and risk tolerances suggest a universal bioprocess and scaling solution may not apply in the cultivated meat industry, there is a need for more techno-economic models and experimental data to optimize bioprocesses for each product type.

The survey underscores the critical need for ongoing innovation and investment in bioprocessing technologies to enable the cultivated meat industry to scale efficiently. It identified specific areas where suppliers, manufacturers, and researchers can accelerate the industry's expansion and reduce production costs.

Key recommendations and R&D opportunities

We provide the following recommendations to cultivated meat suppliers, manufacturers, investors, and R&D labs to accelerate the scale-up of cultivated meat and reduce the cost of production.

Recommendations to suppliers and manufacturers:

- Improve accessibility and cost-effectiveness of growth factors, basal media, and other added factors (such as peptides or other recombinant proteins) and equipment such as bioreactors and filtration devices.
- 2. Design scalable, cost-effective bioreactors and equipment for upstream and downstream processes.
- 3. Collaborate with industry stakeholders to adopt food-grade materials or other innovative methods to lower equipment expenses and establish standards and specifications for materials and equipment tailored to cultivated meat production, leveraging expertise from both the food and pharmaceutical industries.
- 4. Determine the bottlenecks or tradeoffs that exist for using more affordable 304 stainless steel alloys compared to 316 alloys for bioreactors and other equipment.
- 5. Adopt food-grade materials or other innovative methods to lower equipment expenses, moving away from reliance on pharmaceutical-grade standards.
- 6. Raw material contamination and product defects may not be the primary sources of batch failure, but they contribute to it. Suppliers must ensure maximum safety and produce high-quality products to minimize these risks as much as possible.

Recommendations to researchers and R&D teams:

1. Develop affordable and effective media components such as growth factors to

enhance production efficiency. Optimize media formulations by leveraging artificial intelligence and developing genome-scale metabolic models bolstered by metabolomics, proteomics, and transcriptomics datasets.

- 2. Implement simulation and modeling techniques to optimize bioprocesses.
- 3. Design automated cell separation systems, enhance tangential flow filtration systems, design fit-for-purpose bioreactors, and create high-throughput methods for harvesting.
- 4. In this survey, cultivated meat companies have identified process contamination as the primary source of microbial contamination, with microbial testing and contamination checks being the most common analyses conducted. As the industry grows, the risk of contamination becomes a significant economic concern, presenting an opportunity to mitigate and control these risks.

Recommendations to investors:

- Support research, companies, and startups dedicated to developing cost-effective growth factors, peptides, and recombinant proteins at scale and reducing the cost of basal media.
- 2. Fund companies innovating novel methods and strategies for lowering equipment costs, particularly in developing scalable bioreactors with fit-for-purpose designs and cost-effective materials, or by incorporating strategies such as filtration and recycling to reduce production costs.
- Invest in research areas crucial for the growth of cultivated meat, such as investing in companies or research groups working on optimizing bioprocessing through simulation and modeling.
- 4. Build up co-manufacturing capacity and shared testbed facilities to defray the cost burden required for new pilot-scale infrastructure and equipment required for each manufacturer.

Overall, we advise suppliers, companies, and academic labs to collaborate on creating affordable serum-free media options and fit-for-purpose equipment, to streamline efforts, enhance efficiency, and promote sustainable growth.

Findings at a glance

This survey provides insights into cultivated meat production globally, covering bioprocesses, capacities, and challenges manufacturers face. It includes data on production facilities, food safety, and bioprocessing throughout different stages. Below is a summary outlining the key findings from the survey related to the critical components involved in cultivated meat bioprocessing.

Proliferation

According to the survey, **stirred-tank reactors were the most commonly used**. However, some companies reported using air-lift, rocking-bed, fixed-bed, or hollow-fiber reactors. The most common methods include single-cell suspension, followed by growth in aggregates, adherent microcarriers, and adherence to a scaffolding substrate. Among companies with production capacities anticipated to exceed 100 kg in 2023, **single-cell suspension** and **growth in aggregates** were the dominant choices.

Differentiation

We found that most companies incorporate differentiation into their processes, often involving both adipocytes and myotubes. Differentiation often, but not always, occurs in a separate bioreactor from the proliferation stage. Among the various bioreactor types, **perfusion** emerged as the preferred choice for differentiation.

Scaling strategies

Scaling strategies vary among the respondents, and cultivated meat companies are using both scale-up and scale-out strategies. **Scale-up was the predominant choice** in this survey, but this may reflect the industry's current state. Since many companies are in R&D and small-scale production, many may need to scale up first before considering scaling out. Scaling challenges encompass timely access to equipment and supplies, achieving desired texture, selecting appropriate equipment types, and ensuring an animal-free process.

Harvesting methods

Harvesting techniques present an important area for innovation, as indicated by half of respondents who expressed a need for novel or customized equipment. The pursuit of advanced cell separation methods via different methods, such as automated processes and sophisticated filtration techniques, highlights the ongoing need for research and development in refining and optimizing fit-forpurpose cell harvesting procedures.

Cost and availability of bioreactors

The survey outlines the cost landscape of bioreactor capacities, indicating an approximate **average of \$100,000 for every 100 L of bioreactor capacity** (based on limited data from six respondents). Smaller volumes tend to incur a higher cost per liter, while larger volumes are associated with a cost reduction trend of around 50%. Respondents who sourced their reactors from commercial suppliers reported a 6-12 month acquisition lead time.

Novel fit-for-purpose equipment and talent

Our survey highlights a significant demand for improved bioreactor designs, experienced professionals in bioprocessing, and enhanced equipment for large-scale cell culture, particularly in filtration devices and high-density cell culture.

Modeling and simulation

The industry's exponential growth underscores the urgency to develop advanced modeling and simulation capabilities to meet the burgeoning demand.

The path toward price parity

Regarding the path toward cost competitiveness with conventional meat, there is a general consensus among respondents that products could achieve such **competitiveness when production capacities reach thousands of tons**, and proliferation vessel volumes expand into the tens of thousands of liters.

Media and growth factors

The media's cost, bioreactor type, and operational mode are interdependent factors influencing each other's dynamics. However, the survey emphasizes the **crucial role of media recycling** in tackling cost concerns, with many companies already implementing recycling measures.

Among the challenges in cultivated meat production, the **availability and cost of growth factors** (or alternatives) stand out as the most limiting factor, followed by the availability of talent and affordable bioreactors. There is a significant demand for commercially accessible and cost-effective animal-free media solutions.

Co-manufacturers

Our survey shows that only a minority of respondents currently collaborate with contract manufacturing organizations and contract development and manufacturing organizations (CMOs and CDMOs). Key factors for partner selection include production quality, expertise, and pricing. Preferred locations for CMO/CDMO facilities are the U.S. and Southeast Asia, with Europe as a notable third choice. Common cost-reduction barriers include limited vendor options, capacity constraints, and labor, equipment, and raw material expenses.

Safety

Cultivating meat necessitates tailored safety regulations to achieve the right balance between safety and efficiency. Unlike traditional foods or pharmaceuticals, large-scale cell culture for consumption as food requires a distinct regulatory framework that aligns with its specific requirements. Establishing standards specifically for the cultivated meat industry can guide suppliers to make fit-forpurpose and affordable equipment.

Study design and participants

At the beginning of 2023, we conducted a comprehensive survey to gain insights into the current state of cultivated meat production and the early scale-up challenges faced by the industry. The survey aimed to increase our understanding of production capacities, bioprocessing details, infrastructure, food safety and regulatory-related practices, process development trends, and supplier sentiments. By capturing this information, we sought to assess the global landscape of cultivated meat production and identify key areas that require attention and support. The survey received responses from 30 companies representing various segments of the cultivated meat industry, including suppliers, producers, and CDMOs. Among these, 20 identified as cultivated meat or seafood manufacturers, six as cultivated meat equipment providers, four as cultivated meat CDMO/ CMOs, five as other entities, such as R&D centers, enabling and supporting technology providers, cultivated meat reagent and equipment providers, and platform providers/licensors, and one as a facility engineering and construction firm. Some companies chose more than one option (Fig. 1).



Study design

Fig. 1: Summary of the survey's study design

Survey respondents use a variety of cell types, and some companies work with more than one type of starting cell type (Ravikumar et al. 2023). In this survey, 15 companies responded that they work with mammalian cells, 13 work with avian cells, and 11 work with fish cells, including five companies working on invertebrate products (Fig. 2).

Cell lines



Some companies work with more than one type of cells

Fig. 2: Summary of the cell lines used by the survey respondents.

While most companies work on cultivated meat and seafood (n=22), eight companies responded that they work on other categories, such as fat or milk. Most companies that produce cultivated products (13 out of 22) work on more than one product.

This diverse company representation provides valuable insights into the current state of the cultivated meat industry, capturing a range of perspectives and expertise. While the survey may not encompass the entire industry, it offers a representative snapshot with comprehensive information on production capacities, bioprocessing details, food safety, and industry sentiment. However, caution is advised when interpreting survey results, as respondents may exhibit optimism in their responses to forward-looking questions.

Unifying scale-up terminology

Currently, there are no universally accepted standards for categorizing the various stages of scaling a cultivated meat process, such as proof of concept (PoC), prototype, pre-pilot, demonstration, commercial, or industrial. Many companies and stakeholders within the cultivated meat industry and other sectors use these terms interchangeably, leading to confusion about the actual scale of production. For example, in the microbial fermentation industry, lab scale may refer to less than 1,000 liters, demonstration scale between 1k to 100k liters, and commercial scale above 100k liters (Leman, A., Bess, A., Gale, J., Silvester, D., Rodrigues, S., Gemmell, N., Costa, S. 2023). However, it is unlikely that these same ranges will overlap completely with cultivated meat, where volumes are typically considerably less than microbial cell cultures. To address this issue, we first asked companies to respond in an open-ended format about the terms they use to describe their processes and the criteria they use to categorize them. Nearly every company responded differently, highlighting the need to standardize these terminologies so that the production stage can be accurately described and communicated.

We next asked companies about the importance of different factors when categorizing the stage of their bioprocess (such as pre-pilot, pilot, commercialized, etc.). The scope (purpose) of the process (i.e., technical validation, early development, or full development) was the most important factor, followed by the scale of production and whether the process will be used for acquiring regulatory approval (Fig. 3).



How important is each aspect of the bioprocess in categorizing the stage of your process? *32 respondents, importance (1-10)*

Fig. 3: Importance of various aspects of bioprocessing in categorizing the stage of scaling the cultivated meat process.

How scale is defined also varies widely among companies, with some using mass and others using bioreactor volume. While mass ultimately reflects the amount of cultivated meat produced, bioreactor volume tangibly indicates facility size, regardless of process productivity.

To address discrepancies and unify language for scale-up, we propose the following terminology structure for the scale-up of cultivated meat (Fig. 4):

- 1. R&D: <3L, grams of cultivated meat per production cycle
- 2. Bench-scale: <25L, kilograms of cultivated meat per production cycle
- 3. Pre-pilot: 25<V<100L, 10s of kg of cultivated meat per production cycle
- 4. Pilot: 100<V<1,000L, 100s of kg of cultivated meat per production cycle
- 5. Demonstration: 1,000<V<50,000L, tons¹ of cultivated meat per production cycle
- 6. Commodity: >50,000L or >10 tons of cultivated meat per production cycle

In this categorization, a "production cycle" may include multiple bioreactors with multiple volumes, and the volume of bioreactor refers to the total working volume of production bioreactors in use. The amount of cultivated meat produced in this categorization is an estimated range of cultivated meat based on the total culture volume in one production cycle. The amount of cultivated meat produced largely depends on the bioreactor's mode of operation and its volume. For instance, in perfusion mode, a given bioreactor may yield higher densities and produce larger amounts of meat than if run in fed-batch mode.

For each scale-up stage, models used to inform process development must be validated, processes become further optimized, and new data is acquired to inform moving to the subsequent stage. Because many process-related factors change with scale, this step-wise approach is critical for de-risking scale-up for the manufacturer and investors alike.

¹All tons refer to metric tons throughout this report.





Fig. 4: Unified terminology to describe the scale-up of cultivated meat.

Current and near-term cultivated meat production capacity

In 2021, McKinsey published a report that suggested the cultivated meat market could achieve between 1k and 75k tons of production by 2025, which could increase to between 400k to 2.1M tons by 2030, depending on different growth scenarios (Brennan et al. 2021). To put these numbers into perspective, the average production of a conventional slaughterhouse in the US is estimated to be around 31.5k tons per year, based on data from FAO.²

According to data from the United Nations Food and Agriculture Organization (FAO), the total global meat production in 2020 was approximately 337.8 million metric tons (MMT). Similarly, fish and seafood production in the same year amounted to around 176.6 MMT ("FAOSTAT" n.d.). Combining these figures, global total meat and seafood production reached over 514 MMT in 2020 and is expected to rise to 531 MMT by 2030 (Brennan et al. 2021). Thus, based on McKinsey's predictions, the entire cultivated meat industry's production volume in 2025 could be best compared to a single slaughterhouse, and even under high growth scenarios through 2030, the total production volume of the cultivated meat industry would correspond to just 0.4% of the anticipated global conventional meat and seafood production volume.

To understand cultivated meat companies' current and near-term production capacity, we asked companies for their anticipated production volumes by the end of both 2023 and 2026. When considering the expected production volumes for cultivated meat through 2023, responses from the survey indicated varying scales of expected production by the end of 2023. Out of the 24 companies that responded, three companies expect to produce between 1-10 tons, seven companies between 100-1,000 kilograms, three companies between 10-100 kilograms, eight between one to 10 kilograms, and three companies anticipate production of less than one kilogram (Fig. 5).

What amount of cultivated meat is your company "expected" to produce?





Fig. 5: Amount of cultivated meat that companies expect to produce by the end of 2023.

²Search criteria: red meat (i.e., "USA" + "Production Quantity" + "Bovine Meat" + "Mutton & Goat Meat" + "Pigmeat" + "2020"). Based on these search criteria, total US red meat production was approximately 25.3M tons in 2020. <u>According to USDA</u>, 95% of red meat in the US comes from 800 federally regulated slaughterhouses.

Looking ahead to 2026, when larger production facilities are expected to become operational, the survey results indicate that most companies envision significantly larger annual production capacities, with estimates on the order of tons. Out of 19 companies that responded to the question, two companies expect between 5,000-10,000 tons, one company between 1,000-5,000 tons, four companies between 100-1,000 tons, five companies between 10-100 tons, three companies between 1-10 tons, and two companies anticipating less than one ton (Fig. 6).



By end of 2026; 19 respondents



Fig. 6: Amount of cultivated meat that companies expect to produce by the end of 2026

Estimated cultivated meat production



By the end of 2026; the production of 19 cultivated meat companies

Fig. 7: The estimated cumulative cultivated meat production of 19 cultivated meat companies by the end of 2026 compared to an average U.S. slaughterhouse.

Disclaimer: Caution is advised when interpreting the figures presented, as they are based on a subset of 19 responses, not representative of all CM companies, and do not fully reflect the industry. Scaling challenges and optimistic projections can lead to varying production capacity estimations. Technological advancements and process refinements may accelerate industry growth. The projections do not account for potential innovations or new industry players, which could greatly influence future production capacity. The higher and lower bounds in the figure refer to the ranges of options selected by companies. "Max capacity" refers to maximum production capacity, and "estimated production" refers to expected production amount.

The average range of cultivated meat production estimates by 2026, based on the data from 19 companies and considering today's technology, points to a projected output of approximately 25k tons of cultivated meat (low 13.4k tons; high 39.5k tons) (Fig. 7). Assuming that the 19 responding companies provide a representative sample of the industry, we can extrapolate from the reported 25k tons by multiplying it by a factor of five to account for roughly 100 global B2C companies (Bomkamp et al., 2023), resulting in an estimated industrywide total of approximately 125k tons of cultivated meat produced by the end of 2026.

When making comparisons, it is important to note that McKinsey's projections are for 2030, whereas we requested estimated production by the end of 2026. If the cultivated meat industry is projected to reach around 125k tons by 2026, it would need to experience a growth of 3.2 times by 2030 to align with McKinsey's low-growth scenario or an increase of 16.8 times by 2030 to meet the high-growth scenario. Accordingly, based on the currently available data, the cultivated meat industry will likely be tracking toward McKinsey's low growth scenario.

Validation of these estimations in future operational, large-scale facilities is required before reaching conclusions on the expected pace of scale-up. In the interim, these production estimates can help inform industry roadmapping efforts, allowing stakeholders to anticipate infrastructure requirements and plan capital investments accordingly.

Limitations to production capacity estimations

Numerous factors may contribute to uncertainty in these data and projections, which are best viewed as low-confidence estimations at this early stage of the industry's development. As noted in the study design, companies may respond optimistically when answering forward-looking questions, resulting in overestimations due to failure to account for hurdles they may encounter during scale-up. Similarly, some companies may report anticipated production capacities in their facilities rather than actual production. Estimates for capacity may not fully align with actual production, which will depend on acquiring the necessary equipment and having production runs operate on schedule. On the other hand, advancements in bioprocessing, reduction of media costs, cell line optimization, or other breakthroughs in the next three years could result in underestimating these projections.

These projections were based on 19 companies, with those companies representing approximately 100 companies developing cultivated products around the globe. However, the global number of companies could decrease due to failure to raise sufficient funds or increase as additional companies are founded. Changes in the overall funding environment will also influence the speed and scale at which new infrastructure is built, but predicting these future market dynamics is outside this report's scope.

Lastly, this survey asked specifically about the production of cultivated biomass rather than total product weight. Many companies aim to enter the market with hybrid products composed of varying fractions of plant-based ingredients mixed with cultivated cells. Accordingly, hybrid products may significantly increase the total volume of cultivated meat and seafood products on the market compared to strictly measuring the production of cultivated cells.

All of these factors could significantly impact the industry's growth and potential production capacity in the future. The industry needs further production capacity data acquired from future operational facilities to validate these projections. Nevertheless, these ballpark estimations can be a useful starting point for understanding industry growth and needs in the coming years.

Constructing and commissioning a production facility

The first wave of cultivated meat companies was founded in 2015, and by the end of 2022, there were over 100 companies globally (Bomkamp et al, 2023). At the current stage of the cultivated meat industry's development, many companies are planning or moving into their first pre-pilot or pilot-scale production facilities to obtain regulatory approval in different regions. To assess progress, we asked several questions related to facility planning, construction, and commissioning.³

Facility planning

The survey results show that of 24 respondents, 16 use or plan to use an existing warehouse with only the exterior structure (the shell) but no interior infrastructure when constructing their first facilities. In contrast, six companies use or plan to use an existing manufacturing or pilot facility with interior equipment and utilities. One company said they used an existing pharmaceutical facility (they did not specify whether with interior equipment or not), and one said their facility was entirely greenfield (Fig. 8).

What was the state of the facilities prior to the construction of your first facility? *24 respondents*



Fig. 8: State of cultivated meat first facility prior to construction.

³Companies that were still in the planning stages for their first facility were instructed to answer according to their expectations. The data presented in Figures 8-10 represent a combination of real-world responses and best guesses.



Based on these findings, using an existing warehouse or facility is the preferable option that companies considered, with the decision to use existing interior equipment and utilities over an external shell possibly dependent on factors such as location, process design, and budget for CAPEX.

Facility construction time

We asked cultivated meat companies about the construction time of their first facility. As shown in Fig. 9, from 24 respondents, 10 companies

said it took six months to a year, eight companies said between one to two years, and six companies said less than six months. Construction time greatly depends on the state of the facility before construction (i.e., greenfield vs existing facilities), facility size, and production stage (i.e., R&D vs manufacturing). However, one to two years may be more realistic for constructing most research and manufacturing facilities, given typical delays, such as supply issues, permit delays, or other unforeseen circumstances.

What was the construction time for your current pilot/production facility?

24 respondents



Fig 9: Construction time of the first or current pilot/production facility.

Time to reach full capacity

22 respondents

Most companies expect to or have been able to reach full capacity for their first facility within a year, and some expect to or have achieved this milestone in less than six months (Fig. 10). For a significant portion of cultivated meat companies, it took or is expected to take more than a year to reach full capacity. Often, unexpected delays in planning, constructing, and commissioning facilities result in a longer time to reach full capacity than initially calculated and planned. Delays can also occur due to unexpected equipment and supply chain interruptions or delays in issuing permits. Cultivated meat companies and stakeholders should consider these factors when planning new facility construction or forming expectations around cultivated meat industry growth.

54.5% 22.7% 4.5% 4.5% Count 1 12 5 4

What was the time to reach capacity for your current pilot/production facility?

Fig. 10: Total time to reach full capacity of the first or current pilot/production facility.

Second (main) production facility

The term second facility refers to the main production facility that will enable companies to reach a broader set of consumers or additional global markets compared to the limited production capacities of a company's first facility or R&D center. These second production facilities are crucial for scaling cultivated meat production. However, there is limited information about these facilities beyond announcements from <u>UPSIDE Foods</u> and <u>Believer</u> <u>Meats</u>. To better understand how companies approach the scale-up of cultivated meat, we asked numerous questions to understand the costs, size, equipment, sustainability, and other considerations for these larger production facilities.

Out of 24 companies, 14 are planning or have already planned to build a second facility, with eight of the 14 working with a contracted engineering firm to design their second facility.

Costs

The median anticipated total capital investment for these second facilities is \sim \$90M, based on a limited number of responses (n = 6). Although it is difficult to discern exact capital investment values, publicly

Selecting all that apply; 10 respondents

announced facilities from well-funded companies such as <u>UPSIDE Foods</u> and <u>Believer Meats</u> are also within a similar magnitude of this estimate, costing \$141M and \$123M, respectively. Given the recent downturn in the funding environment, other companies may take a more capital-light approach to scaling, investing considerably less than these totals. More data is needed to understand the expected investments for second facilities accurately.

Equipment

Seven out of 10 companies plan to use stirred-tank bioreactors as the largest proliferation vessel for their second facility, while two companies plan to use air-lift bioreactors, and one did not specify. Ten companies provided information about the sizes of bioreactors they plan to use in their future second facility (Fig. 11). The most commonly mentioned size was 10,000-50,000 L bioreactors, mentioned by five companies. Three companies reported bioreactors between 5,000–10,000 L, and three companies reported bioreactors smaller than 1,000 L. One company anticipated using bioreactors between 50,000–100,000 L, and one company mentioned bioreactors between 100,000–300,000 L.

23% 23% 23% 23% 8% 8% 8% 8% 100 L 5,000-10,000 L

What sizes of bioreactors are you using or planning to use for your future second facility?

Fig. 11. Sizes of bioreactors cultivated meat companies are using or planning to use for their second (production) facility.



Out of 11 companies, eight intend to purchase off-theshelf bioreactors, five plan to purchase off-the-shelf bioreactors with modifications, and two will build on-site. Some companies are considering multiple options for their bioreactors. Two of the surveyed companies identified <u>ThermoFisher</u> and <u>Applikon</u> (now Getinge) as bioreactor suppliers.

The survey asked about the anticipated total cost of the largest bioreactor in the second facility, including installation cost. The responses varied with one company estimating ~\$18-20 million, another company mentioning \$10 million, and three companies estimating one to three million dollars. Some companies aim to reduce costs by implementing food-grade contact surfaces and reducing unnecessary instrumentation while ensuring compliance with food-grade regulations. Overall, more data is needed to determine the expected costs of these large bioreactors.

Facility capacity and commissioning time

Eleven companies reported their anticipated total annual production for their second facility. One said 10,000-20,000 tons, one said 5,000 - 10,000 tons, four said 1,000-5,000 tons, two said 10-100 tons, and one said 1-10 tons (Fig. 12). To put these numbers in perspective, 20,000 tons of cultivated meat production is comparable to the average annual production of a conventional slaughterhouse in the US which is estimated to be around 31.5k tons.

What is your anticipated annual production for your second facility?

11 respondents



Fig. 12: Anticipated annual production for the second (production) facility

Based on 11 responses, seven companies estimate the commissioning time for their second facility to be between six months to one year. Two companies expect the commissioning time to be less than six months. One company foresees a commissioning time of one to two years. Another company projects a commissioning time of two to five years.

To put this in perspective, BioProcess International collected estimates from engineering firms regarding construction times for biopharmaceutical plants (Dodelet 2008). One firm estimated that completing a proposed 10,000 ft² biopharmaceutical plant would take about one year. Another engineering group specializing in various plant designs indicated that the time from construction to start-up for a similar 10,000 ft² plant would be 18–36 months, and six-12 months for a 10,000 ft² non-GMP plant.

Importantly, cultivated meat facilities may be significantly larger than existing pharmaceutical

facilities. For example, <u>Believer Meats</u> and <u>UPSIDE</u> <u>Food</u> are preparing to build facilities at 200,000 ft² and 187,000 ft², respectively. Companies should know that such large facilities could lead to delays during construction.

Sustainability considerations

Cultivated meat is anticipated to use significantly less land and result in lower air and water pollution than conventional meat production. While cultivated meat can have fewer emissions and use less water compared to beef, how its emissions and water use compares to chicken and pork production is sensitive to how certain practices are implemented at manufacturing facilities (e.g., use of renewable energy, recycling of water; (Sinke et al. 2023)). Accordingly, we asked several questions to better understand how companies are considering the sustainability of their future manufacturing facilities.

Renewable energy

Cultivated meat manufacturers have enormous control over the carbon footprint of production, as most of their emissions are anticipated to be scopes 1 and 2. For example, running a facility on renewables could reduce the carbon footprint of cultivated meat production by 70% compared to a facility that runs on a conventional energy mix (Sinke et al. 2023).

We asked companies about their energy-sourcing strategies in their second facilities (Fig. 13). Two companies stood out for their commitment to sustainability, as they plan to install their own renewable energy source and use it as the facility's primary energy source. Another company stated that they are aiming for carbon and water neutrality. They mentioned using solar energy, water recycling and reuse, and advanced cooling technologies. Six companies responded that they anticipate relying on the standard energy mix available at the facility's location, with five of those companies anticipating a location with less than 50% renewable energy availability and one company anticipating a location with more than 50% renewable energy availability. Six other companies demonstrated a proactive approach by planning to set up their own renewable energy production capacity at the facility to supplement their energy needs. One of them elaborated further that their goal is to reach 100% renewable energy, and they plan to achieve this by using solar, wind, geothermal, and biogas. These findings highlight the growing importance of considering renewable energy options in the cultivated meat industry's expansion, which will likely have co-benefits in cost reduction and environmental impact.

43%

36% 14% 7% 14% 7% 14% Energy mix at facility site (<50% renewable)</td> 1 6 2 Installing own renewable energy to supplement primary energy source Installing own renewable energy source

How do you plan on sourcing energy for your second facility?

Selecting all that apply, 9 respondents

Fig. 13: Anticipated energy source for the second (production) facility for cultivated meat companies

Water recycling, waste management, and other considerations

Cultivated meat processing requires large amounts of water and media, and companies have various options for sourcing and managing their water, media, and spent (i.e., used) media. Therefore, in addition to renewable energy, companies are implementing water recycling and reuse practices to enhance efficiency and achieve sustainability goals.

Companies may choose to recycle or dispose of spent media directly. Spent media disposal practices vary among cultivated meat companies. In some cases, liquid waste may require additional processing and pH adjustments before disposal to adhere to regulatory guidelines. Therefore, certain companies opt to treat the spent media further before disposal.

Among 22 respondents, four companies mentioned discarding media after pH adjustment or additional processing, while one reported repurposing spent media for non-cultivated meat-related processes (e.g., <u>spent media can</u> <u>be used to cultivate microalgae</u>). Conversely, the rest indicated recycling the media, which one may attribute to the media's relatively higher cost.

Companies are considering other factors to improve the efficiency and sustainability of their processes as well. Some companies are focused on co-locating production facilities with media processing facilities, neutralizing waste streams, and accessing raw materials within minimal shipping distances to reduce environmental impact. Heat recovery and proximity to food manufacturing and headquarters locations of other food and beverage companies can also contribute to overall sustainability goals. The respondents did not mention to what extent they incorporate these considerations into their processes. Nevertheless, they highlighted these considerations in designing and building cultivated meat facilities.

Overall, media remains a substantial cost factor, making recycling a crucial aspect of cultivated meat processing. The survey reveals that many cultivated meat companies are already engaged in recycling media, highlighting its importance within the industry.

The most important considerations for the second facility

Based on the survey results, many companies' key priorities for their second (main) facility center around minimizing expenses, navigating regulatory requirements, and ensuring access to renewable energy sources (Fig. 14).

How important are these factors in site selection of your second production facility?

12 respondents, importance (1-10)



Fig. 14: Most important factors in selecting cultivated meat facility sites.

The overarching goal for cultivated meat companies is to reduce the cost of cultivated meat, and they employ various strategies to achieve this objective. One of the primary strategies involves cost-cutting measures by <u>addressing technological gaps</u>, such as the need for fit-for-purpose bioreactors and access to large, low-cost quantities of media. These technological advancements are crucial for streamlining production processes and reducing expenses.

Another critical aspect of cost reduction and sustainability is adopting renewable energy sources. Although the specific pathways are yet to be seen, many companies are exploring the integration of renewable energy to minimize costs and improve their environmental footprint, which signifies a commitment to sustainable practices within the cultivated meat industry.

Lastly, cultivated meat companies expressed a great interest in receiving government support, which could be financial, such as with tax incentives, or by receiving support in navigating the complex regulatory landscape. To overcome these challenges, governments and cultivated meat industry stakeholders should collaborate closely to develop regulatory and supportive frameworks that <u>facilitate innovation while</u> ensuring safety and quality.

Bioprocessing

In this section, we have compiled a summary of the survey results that specifically address questions related to the finer details of bioprocessing. This portion covers findings related to both upstream processes, such as media development and proliferation, and downstream processes, including harvesting and recycling. It also encompasses insights into various aspects of bioprocessing crucial for reaching price parity and increasing the production yield, such as automation, monitoring process parameters, sterilization techniques, controlling contamination, and employing modeling and simulation techniques.

Media

Antibiotic use and media sterilization

The cultivated meat sector aims to achieve largescale cell cultivation while minimizing contaminationrelated batch losses and ensuring product safety. The biopharmaceutical industry's experience suggests this is feasible, with contamination accounting for $\sim 3\%$ of total batch failure, where the average interval between failures was estimated to be 58 weeks in 2022. Numerous cultivated meat startups also confirm this potential. Companies can adapt the strategies used in the biopharmaceutical realm to <u>prevent and identify contamination</u> for cultivated meat, except possibly in early-stage R&D and cell line development where antibiotics are used sparingly compared to other contexts.

In our survey, 23 companies responded. Eleven companies do not use antibiotics or antimycotics.

Nine companies use antibiotics or antimycotics only during pre-production (i.e., during cell line development or cell banking). Three companies said they use antibiotics or antimycotics during pre-production and production. However, these companies are not yet producing cultivated meat commercially and thus may include antibiotics to mitigate losses in their upstream R&D efforts or during pilot runs. The three companies who elaborated said they use penicillin-streptomycin. Importantly, the two cultivated meat products approved for sale in the U.S. are produced without antibiotics, and we expect this to remain true for <u>future products</u>.

As the most predominant input into the cultivated meat production process, media sterilization prior to entering the bioreactor is an important aspect of limiting contamination. Several sterilization methods exist, but certain ingredients have limitations for what sterilization method can be applied. For example, heat-labile vitamins and proteins may not be able to undergo <u>high-temperature short-time</u> (HTST) treatment.

To understand how cultivated meat manufacturers deal with these limitations, we asked about their sterilization methods. Almost all 21 respondents reported using 0.2 µm filters for media sterilization. Two said they use 0.1 µm filtration⁴, one reported using irradiation, and three said they use HTST treatment (Fig. 15). Due to high costs, nanofiltration for viral retention will likely not be used in the cultivated meat industry, with viral contamination instead mitigated by <u>testing and other quality control</u> <u>measures</u> implemented prior to cell banking.

⁴0.2 µm filters can retain most bacteria, whereas 0.1 µm filters are necessary to retain smaller, common contaminants such as Mycoplasma.

How do you sterilize the media?

Selecting all that apply, 21 respondents



Fig. 15: Media sterilization methods used by 21 cultivated meat companies.

*Media is presterilized commercially

Contamination sources for bioreactors can be identified and addressed at their entry points, drawing from established practices in the food, beverage, and pharmaceutical sectors. Notably, the pharmaceutical industry often grows cells in bioreactors without antibiotics at significant scales. The challenge for cultivated meat firms is to achieve this economically, ensuring product safety without relying on antibiotics, while also manufacturing in a food-grade environment with lower-grade clean rooms than those found in the pharmaceutical industry. While the true threat of contamination on cultivated meat operational costs has yet to be determined, there's potential for further research and development to discover new ways to reduce contamination risks or to address contamination using innovative approaches like antimicrobial peptides.

Metabolites and values monitored during the bioprocess

It is important to keep track of metabolites' concentrations during bioprocessing to monitor the level of nutrients and accumulation of toxic waste products, assess the overall quality of media and cells' health, and take action accordingly. For instance, as a fed-batch cell culture progresses, incomplete glucose fermentation leads to lactate accumulation, which causes the pH to drop. Sodium bicarbonate can be added to the cell culture as a basic buffer to neutralize the acid and maintain the pH. Similarly, the concentration of certain amino acids can be measured to ensure their levels do not fall below those needed to support maximal cell growth. If these parameters are not measured and monitored precisely, the changes in media composition and metabolites' concentrations can drastically impact the viability of cells. However, measuring all values and parameters is impractical and can increase the cost and complexity of the bioprocess. Therefore, cultivated meat companies may monitor different parameters and metabolites based on their needs.

We asked cultivated meat companies about the parameters and metabolites they monitor to identify the most critical ones. All 22 respondents indicated that they track pH, and most also monitor glucose, lactate, pO2, ammonium, pCO2, osmolality, and glutamate (Fig. 16). Half of the respondents said they monitor amino acids. Other compounds are only monitored by a few companies. The most common measurement instruments are <u>Cedex Bio</u> and <u>NovaFlex</u>. Eleven out of 19 companies also use antifoam in their cell culture process.

Based on these results, measuring pH, glucose, lactate, ammonium, and the partial pressure of oxygen and carbon dioxide is the most critical. Sensors and reagents used for these measurements are often expensive, and many cultivated meat companies may not yet be able to "fine-tune" their cell culture media. Therefore, there is a need to develop sensors and other measurement equipment and materials that are cost-effective and fit for purpose. Better measurement and monitoring of such values can integrate into automation control systems and further facilitate cell culture. Lastly, collecting large metabolite datasets from multiple runs provides an opportunity to apply machine learning and artificial intelligence to optimize bioprocess controls.

What metabolites and parameters do you monitor during your bioprocess?



Selecting all that apply, 22 respondents

Fig. 16: Metabolites and parameters monitored by cultivated meat companies.

Serum replacements

<u>Cell culture media</u> significantly impacts cultivated meat production's cost and environmental sustainability. While basic formulations can sustain cell life briefly, efficient long-term proliferation has traditionally required animal sera, notably fetal bovine serum (FBS), which poses contamination and ethical concerns due to its undefined composition and sourcing. Replacing animal products in media improves ethical standing, decreases contamination risks, and enhances batch consistency.

Serum replacement strategies vary, including assessing hydrolysate-based media for optimal raw materials, analyzing feed-grade ingredients' performance, comparing species-specific growth factors, advancing recycling technologies, adapting cell lines for lower growth factor needs, and discovering plant-based analogs ("Cultivated Meat Media and Growth Factor Trends" n.d.). GFI's recent analysis of media and growth factors highlighted the projected high demand for certain recombinant proteins, potentially surpassing that of current industrial enzymes. The report indicated that to meet this demand efficiently and affordably, the industry must explore new production platforms and innovative solutions (Swartz n.d.).

Out of the 23 companies surveyed, 17 are actively working on developing serum replacements, reflecting a strong <u>trend toward serum-free bioprocesses</u> (Fig. 17). Only four companies reported that their processes still involve serum, while two others have adopted commercially available serum replacements. Some companies employ a combination of animalderived serums and their own serum replacements. These findings align with a previous survey conducted by GFI, which found that 64% of surveyed companies had adapted at least one cell line to serum-free conditions (Ravikumar et al. 2023).

Among the respondents, 14 companies disclosed the specific serum replacements they use. Eight companies use recombinant proteins, while nine are integrating plant-based alternatives. Additionally, two companies are employing fungal-based serums, and one is deriving serum from algae.



What serum replacement do you use?

23 respondents



The prevalence of in-house serum replacement development could indicate higher costs of commercial products or specific media needs for distinct cell lines. This trend of independent formulation could slow down progress due to duplicated efforts, given the infrequency of companies sharing proprietary recipes. However, this situation also promotes more innovation. It shows a significant demand for commercially accessible and costeffective animal-free media solutions. Companies specializing in cultivated meat cell banking and media development could play an important role in producing economically viable, cell-specific, and animal-free media for the industry to address this critical need. Cultivated meat manufacturers may also license media formulations or ingredients to generate additional revenue streams.

Proliferation

A sequential process using seed train vessels is often employed prior to large-scale proliferation. Numerous parameters impact proliferation, including cell type, media composition, how media is added or waste is removed, and the mixing and aeration method. Depending on the scale and purpose of proliferation (e.g., for recombinant protein production or for cultivated meat), <u>bioreactors with different designs</u> and modes of operation have been produced.

For many pharmaceutical and biotech companies, the goal is to optimize the final titer (i.e., concentration) of the products produced by cells, such as antibodies and other therapeutic proteins. In cultivated meat production, however, the objective is to optimize and/ or maximize proliferation, as the cells are the product. Proliferation is, therefore, a vital step in cultivated meat processing, involving the rapid multiplication of cells to increase biomass. The yield, or the amount of biomass produced, is a key metric during this stage.

Production yield

Yield is a critical parameter influenced by various factors, including bioprocess design, cell type, media formulation, and bioreactor type. Achieving high yield involves optimizing these factors to enhance cell proliferation and biomass production, which directly influences the efficiency and economic viability of cultivated meat production.⁵

We received responses from nine companies regarding the yield of their cultivated meat or seafood production (Figure 18). Among the respondents, five companies indicated that they observe or expect a yield between 30 to 100 g/L, while three companies indicated a 200-300 g/L yield. The remaining company expects a minimum yield of 5-10 g/L, while the highest reported yield was 300 g/L. Overall, the survey indicates that most companies observe production yields between 20 and 100 grams of wet cell biomass per liter (g/L) in the proliferation phase.

In addition to the values recorded by the survey, Sinke et al. estimated that the average yield across the industry may be able to reach approximately 150 g/L in larger-scale facilities by 2030 (Sinke et al. 2023). Believer Meats recently published the only example of yield, achieving 360 g/L for their cultivated chicken grown in 2L bioreactors (Pasitka et al. 2022). However, this required high rates of perfusion that may be too costly at scale due to the large amounts of media used. In an interview in March 2023, Ever After Foods said that they could produce more than 10 kilograms of cultivated meat with a 35 L bioreactor, corresponding to a yield of 285 g/L. Overall, there needs to be more peerreviewed data on cultivated meat yields achieved at larger production scales.

These findings suggest that there is significant variation in the expected or achieved yield in the cultivated meat industry. Such variations may reflect differences in the bioprocessing techniques used, such as the bioreactors' mode of operation, the production scale, or the progress attained for a given production process. There remains difficulty in predicting the yields the industry will ultimately achieve. The numbers cited above can serve as a useful baseline for tracking yield progress as more information becomes available.

⁵While yield for animal cell cultures is often reported as millions of cells per milliliter, this convention can be misleading (Humbird 2020), as cells used in cultivated meat production can vary greatly in their size (e.g., shrimp cells are smaller than cow cells). These differences can be corrected by reporting yield on a mass basis, in grams per liter (g/L).

What is your production yield?

9 respondents



Fig 18: Reported and estimated yield of production of nine cultivated meat companies. Each bar represents one response.

The survey question was presented as open-ended.

Disclaimer: The information presented here is based on limited data. Variations in reported production yields may result from differences in bioprocesses, including bioreactor mode of operation and scale, as well as the types of cells or organisms used. Some companies may have reported expected and desired values rather than experimental data.

*Sinke et al modeled that the average yield across the industry may be able to reach approximately 150g/L in larger-scale facilities by 2030. **Believer Meats' published the yield of 360 g/L for cultivated chicken in 2L bioreactors.

The length of the proliferation phase

The proliferation phase most often takes between one and four weeks; however, it can last less than a week or as long as two months. The length of proliferation can depend on factors such as the species (e.g., <u>cells</u> <u>from aquatic animals tend to divide slowly</u>) and the bioreactors' mode of operation. If toxic metabolites such as ammonia are removed and fresh nutrients are added, cells may be grown for longer periods. Fig. 19 shows the length of proliferation before differentiation or harvesting. In this graph, the dark green designates responses from companies that currently operate larger bioreactors (> 100L) to better understand the differences in timelines based on bioreactor size.

How long do you currently grow cells?

In proliferation bioreactors before harvesting or moving to differentiation phase, 19 respondents



Fig. 19: The length of cell proliferation from the beginning to the end of the proliferation stage, including the seed train.

Relatively smaller bioreactors, such as shake flasks, and relatively larger bioreactors, such as those used for pilot studies, stratify data because cell culture equipment and techniques may be vastly different at different scales, with certain modes of operation more likely to be employed at larger scales.

In addition to the proliferation phase, we asked companies to specify the length of cell growth during seed train alone, before inoculating vessel N, the final production/proliferation vessel. Furthermore, we asked about the length of cell growth in vessel N, as opposed to the entire length of cell growth during proliferation.

Based on these results, many companies proliferate cells in seed trains for a period of two to six weeks.

Various bioreactors that companies may use for seed trains explain the wide range. In addition, cell growth can be significantly slower immediately after thawing cells. In contrast, companies spend less time during the last stage of proliferation in vessel N, often ranging from two to four weeks. The different lengths of proliferation could be due to operational details that were not specified, including but not limited to the mode of operation.

Cell density from seed train to harvest

The cell density for inoculation varies based on the process stage. Determining the optimal cell densities requires experimental calibration and is further influenced by the specific cell line. We inquired about cell densities (cells/mL) during various bioprocess phases: post-inoculation of the initial seed train culture, after inoculating their final production vessel (N), and just prior to harvest.

Typically, cells are seeded at a low density following cell thawing. Based on our survey, 12 companies ranged from 10,000 to two million cells/mL. However, the majority of companies fall within the range of 100,000 to 500,000 cells/mL.

Inoculation of vessel N generally occurs in much higher densities, as reflected by responses from 12 companies. Responses vary, but one, 10, and 20 million cells/mL were among the responses. The wide range in the responses could be due to different cell types, bioreactors, or vessel sizes.

When asked about the target cell densities at harvest, the responses exhibited significant variation. However, major cultivated meat producers (i.e., companies at larger production stages) aim for high cell densities, often surpassing 50 million cells/mL and occasionally reaching as high as 400 million cells/mL. One company also reported 150 cells/microcarrier.

Attributable factors for these varying numbers include production scale, bioreactor type, and operational methods. Different bioreactor operation modes, like perfusion, can achieve notably higher densities due to continuous fresh media introduction compared to batch or fed-batch processes. The cell line's intrinsic or selected characteristics are also a significant factor influencing the target densities.

Cell growth during proliferation

There are different methods for growing cells in bioreactors based on their type and intended purpose, such as for proliferation or differentiation. These methods include single-cell suspension, growth in aggregates, or adherence to microcarriers or other scaffolding substrates. The choice of approach can depend on many factors, including the cells' intrinsic properties and the final product's specific characteristics, such as whether it requires a textured structure or involves differentiation into tissues.

From 23 respondents, the most common method, reported by 13 companies, is single-cell suspension. Adherent microcarriers, growth in aggregates, and adherence to a scaffolding substrate were also quite common, being used by 10, nine, and four respondents, respectively (Fig. 20). Looking at companies with relatively larger production capacities (greater than 100 kg in 2023), single-cell suspension and growth in aggregates were the most common.



Selecting all that apply, 23 respondents



Fig. 20: The method of cell growth during the proliferation phase. Please note this graph is representative of data collected from all respondents, including companies in various stages of maturity.

Differentiation and scaffolding

After proliferation, cells can be differentiated into various cell types, often using <u>scaffolds</u> or microcarriers, and then harvested to be prepared for the final product. Companies may use differentiated muscle, fat, fibroblasts, or combinations of these cells and may use diverse techniques and materials to assemble scaffolds to aid the differentiation process (Bomkamp et al. 2021). Harvesting can also be challenging at large scales and may require new, fit-for-purpose designs for cultivated meat. We designed this section of the survey to gain a deeper understanding of these processes and to identify existing gaps for further exploration.

Based on the survey's results, of 21 respondents, 15 said that their company includes a differentiation or maturation phase in its manufacturing process, with the most common post-differentiation cell types being myotubes (10 companies) and mature adipocytes (nine companies). Interestingly, seven companies said they work with both myotubes and mature adipocytes.

The length of differentiation and maturation

Differentiation may be crucial for generating structured cultivated meat products with matched textures. However, not all companies pursue differentiated products. This step can occur either concurrently with proliferation or after it. Differentiation often involves scaffolding and necessitates specific considerations in bioreactor and bioprocess design, including factors such as the mode of action, bioreactor type, and the incorporation of growth factors and media additives.

According to our survey, nine of 13 respondents said that differentiation for cultivated meat and seafood typically occurs over a 5-10 day period, three companies reported slightly shorter timelines (two-five days), and one company reported longer timelines (10-15 days) (Fig. 21).

What is the length of time for differentiation?

13 respondents





The state and stage at which differentiation occurs

Differentiation can occur either in suspension (for instance using microcarriers) or as adherent cells using functionalized (i.e., coated) <u>scaffolds</u> that facilitate cell adherence. Additionally, proliferation and differentiation may occur in the same vessel or using different bioreactors.

When asked about the state in which differentiation occurs, six out of 12 respondents reported that differentiation occurs in the adherent state. Companies producing cultivated meat in the range of 100-1000 kg typically use this method. Four companies reported using the suspension method, and two used the aggregate method.

Of the companies that reported having a differentiation stage, eight out of 12 respondents said that differentiation takes place in a separate bioreactor from proliferation. Four companies also indicated that they use bioreactors with volumes of less than 100 L, and three are working with or planning to work with much larger equipment (1,000-5,000 L bioreactors).

These findings highlight the varied approaches used throughout the cultivated meat industry and suggest that different differentiation methods may be necessary for optimizing specific products.

Using scaffolds for differentiation

Scaffolds are used in cultivated meat to support cell attachment and growth, replicating natural tissue development and aiding in the formation of desired textures and structures in the final product.

Of 13 companies, eight said they produce multicellular tissues using scaffolds. Four of these eight respondents said their differentiation process involves creating a multicellular tissue by combining multiple cell types from upstream process stages, namely myoblasts, fibroblasts, and adipocytes.

Materials and methods to produce scaffolds

Scaffolds are usually made in-house from polysaccharides (e.g., cellulose), proteins (e.g., collagen, zein, silk, keratin, laminin), or complex natural products (e.g., mycelia, lignin, or soy hydrolysate), and they are usually edible so they can remain in the end product (Bomkamp et al. 2021).

We asked companies which of these three material categories they use for their scaffolds. Interestingly, the majority of the eight respondents chose all three categories, which could be due to differentiating cells with different requirements, such as myocytes, fibroblasts, or adipocytes, or it could be indicative of companies still performing R&D to determine the most efficient way to differentiate cells.

Methods of scaffold production

The most common scaffold biofabrication method is polymer spinning, specified by five out of seven respondents. Four said hydrogels, one said extrusion, and one said 3D printing (Fig. 22). Several companies use other methods that they did not specify.

Of the eight respondents, the majority, six companies, fabricate their scaffolds outside the bioreactor before sterilizing and positioning them inside. In contrast, two companies use bioreactors with permanently fixed scaffolds.

How are your scaffolds developed and used?

45.5% 36.4% 9.1% 9.1% Count 5 4 1 1 Polymer spinning Hydrogels **3D** printing Extrusion

Selecting all that apply, 7 respondents

Fig. 22: Methods of scaffold production

Sterilization is a crucial step in using scaffolds, typically with the use of irradiation, heat, or chemical treatment. Of seven respondents, four specified they sterilize their scaffolds using heat and irradiation, one using only heat, one only irradiation, and one using irradiation and another method they did not disclose.

These findings suggest that companies use various methods for scaffold biofabrication and sterilization, depending on the product and the cell type used. Furthermore, companies may fabricate their scaffolds outside the bioreactor to ensure precise control over scaffold properties before sterilizing and integrating them into the bioreactor for subsequent cell culture.

Harvest

After proliferation, differentiation, and scaffolding, harvesting cells becomes crucial for the final stages of production. Although cell harvesting is a common step in bioprocessing for biotech and pharmaceutical companies, the challenge escalates when dealing with potentially millions of liters of culture in future cultivated meat production. As our survey results show, there is a need for further innovation and development of fit-for-purpose harvesting processes.

The best time to harvest

Harvesting cells in bioprocessing depends on various factors tailored to a specific company's goals. Depending on the process, cells may be harvested after they reach a certain viability, density, or length of culturing time. To understand these nuances further, we asked cultivated meat companies whether their harvesting criteria were time-based or density-driven.

Our survey results show that of 13 respondents, five companies harvest when cells reach a certain density, while three harvest after a certain amount of time. Three companies use either method. One company noted that they harvest when cell viability drops below a certain point. Another company said they use a handful of cell quality and media consumption markers to decide the harvesting time.

Our survey did not specify harvesting techniques for various scenarios, such as differentiated products, adherent cells, or cells grown in suspension. Different cell types and processes may require distinct approaches. Nevertheless, deciding when to harvest is commonly based on parameters like cell density or culture duration.

Cell viability prior to harvest

When cells are harvested with lower viability, it leads to more debris from dead cells in the media, which can cause downstream complications such as clogged filters during harvesting. The importance of final viability varies based on downstream processes, such as further differentiation, which this question did not specifically address. From 20 responses, all but one company reported pre-harvest cell viability over 80%, with seven companies harvesting between 80-90% viability (Fig 23). A majority of companies, 12 out of 20, opt to harvest cells when they are predominantly alive, with viability rates exceeding 90%. However, one company said they harvest at low cell viability (10-30%). This is an outlier, and the reason for it was not specified. But it could be due to prolonged cell culture length to maximize the biomass production. The end product does not necessarily have to be live cells.



What is the viability of your cells prior to harvest?

20 respondents

Fig. 23: Cell viability at which companies harvest the cells

Harvesting method

From 15 respondents, the continuous centrifuge was the most popular harvesting method used by 10 companies. The other five companies use batch centrifuges. Interestingly, seven companies use other methods, such as settling and filtration, or removing the final product from a tissue bioreactor (such as a hollow-fiber bioreactor) (Fig. 24). We asked about the need for novel processes or equipment for harvesting. Of 15 respondents, seven said they either use a novel method or think there needs to be novel or customized harvesting equipment. Respondents identified several areas for improvement and innovation, such as developing more automated cell separation processes, tangential flow filtration (TFF) systems, and high-throughput filtration-based approaches.



How do you harvest the cells or tissues at the end of the process?

Selecting all that apply, 15 respondents

Fig. 24: Different methods of harvesting cells and tissues used by cultivated meat companies.

*Seven companies use other methods, such as settling and filtration, or removing the product from a tissue bioreactor.

These responses demonstrate the need for further research and development to improve filtration and other processing methods for harvesting cells, or develop fit-for-purpose centrifuges, considering the large volumes required for cultivated meat cell culture.

Implementing a washing step

A washing step is an optional downstream process following harvesting, typically carried out using water or simple buffers. This step helps remove residual media components from the final product, such as growth factors. The washing process can be implemented pre- or post-harvest.

Based on our survey, of 22 respondents, 15 said they implement a washing step either before or after harvest, while seven said that they do not include a washing step at all. Most respondents did not report developing any novel equipment or methodology for washing and instead use simple buffers (e.g., a simple isotonic buffer or phosphate-buffered saline).

These details are provided by companies that may not currently produce cultivated meat for consumption using an approved process by regulators. Their focus may primarily be on scaling up and optimization, so these specifics could evolve when they transition to focusing on regulatory approval.

Recycling and Filtration

Recycling processes involving media, water, and other components can reduce environmental impact and potentially lead to cost savings (Yang et al. 2023). Some companies use recycling to optimize resource utilization and minimize waste. However, such strategies might also result in higher initial investment costs, particularly when considering filtration methods (e.g., <u>alternating</u> tangential flow filtration (ATF) and TFF) for recycling. These filtration approaches can also contribute to increased consumable expenses.

In our survey, 12 out of 23 respondents say that their company's process involves some form of recycling. As mentioned by 11 of the 12 companies that recycle, media components are the most commonly recycled. Four companies also recycle water and two recycle metabolites. Recycling tends to be a greater concern for larger production facilities, possibly accounting for its lower frequency among respondents, especially regarding water recycling. Nevertheless, companies are clearly interested in adopting media recycling practices.

Several companies offered additional insights into their practices. These include treating and supplementing spent media and reclaiming water from waste for purification and reuse. Some companies reflow media through reactor vessels and use various markers to blend spent media optimally for continued growth. One company mentioned recycling an entire media batch, while another shared their strategy of concentrating the waste stream to remove water and recycle organics through a microbial process. This process involves digestion and potential metabolite recycling, which requires in-house technology development.

Of 13 companies that reported on their filtration systems, seven said they use TFF, two said they use ATF, and three reported using both. One company also said that they are working on novel filtration architecture that is being co-developed with a filtration company.

Overall, several companies have emphasized the necessity for continuous research and innovation in filtration and recycling. This underscores the importance of creating cost-effective filtration devices that can enable more efficient recycling on a larger scale.

Bioprocessing monitoring and quality control

Online sample preparation allows immediate analysis on the same bioprocess workstation where the sample is prepared, while offline preparation requires transferring samples for later analysis, which may take hours or days after collection. Online analysis may offer real-time results, whereas offline occurs in an external lab setting. The choice between online or offline is specific to each company's needs, prompting us to inquire with cultivated meat manufacturers about their bioprocess monitoring preferences.⁶

Seventeen out of 23 respondents said their companies use a combination of both methods, while four said they exclusively use online monitoring, and two exclusively use offline monitoring.

As the cultivated meat industry continues to evolve, a combination of different analysis methods may be required. With the ongoing trend toward using automation in bioprocessing, we may anticipate a shift toward increased reliance on online and real-time monitoring. This can potentially streamline operations and enhance the efficiency, precision, and safety of cultivated meat production.

Quality control is also an important part of cultivated meat bioprocessing that can be performed in-house or outsourced. Of 19 respondents, 11 said they have an in-house QC department, two reported outsourcing QC, one reported using a CMO/CDMO, and the rest said they use some combination of these three.

Modeling and simulation

Modeling could play a crucial role in developing cultivated meat by significantly reducing the time and costs associated with large-scale bioreactor experiments. It offers a cost-effective solution for prototyping and process development, which is traditionally labor-intensive, time-consuming, and expensive. For example, modeling can enable cultivated meat scientists to alter variables like nutrient concentrations, scaffold structures, and temperature in a virtual environment to study the effects on cell growth and differentiation. This approach may also help decision-making and de-risking investment into scale-up.

According to the survey results, companies expressed enthusiasm for applying modeling and simulation for their bioprocesses. The 18 responses highlighted various areas where companies saw the potential benefits of simulation and modeling.

Companies frequently mentioned using modeling to optimize cultivated meat production by fine-tuning various parameters, such as yield, shear stress, pH, dissolved oxygen, or oxygen transfer rate. Numerous parameters and variables must be adjusted to optimize the bioprocess for maximum biomass production while minimizing time and costs. However, not all parameters can be feasibly experimentally optimized. For instance, finding the appropriate agitation speed is crucial. While increased stirring can improve dissolved oxygen and uniform mixing, it can also cause shear stress, potentially damaging the cells. Modeling can help to find the balance, ensuring optimal growth conditions to maximize the yield. Using modeling and simulation to study shear stress, as some companies have suggested, can help determine the optimal mixing rate or design bioreactors with improved geometries.

<u>Companies also indicated</u> using modeling to analyze cellular metabolism and metabolite concentrations would be beneficial. By simulating these concentrations and validating the models through experiments, as well as developing metabolic flux analyses, it is possible to create more efficient and cost-effective media (Gomez Romero and Boyle 2023). Lastly, several companies mentioned that they are already building computational fluid dynamics and modeling capabilities as a part of their R&D or through a consulting firm.

These findings highlight an urgent need for better models and enhanced simulation capabilities. Developing such models has begun to take place through the <u>Cultivated Meat Modeling Consortium</u>, where industry and academic scientists can collaborate to advance cultivated meat through computational modeling.

⁶To read more on the differences between online, inline, at-line, and offline, please visit this website.

Other methods may require the bioreactor to withstand a higher pressure or temperature. Engineers of bioreactors consider durability, resistance to harsh

Bioreactor sterilization

hydrogen peroxide or ethylene oxide.

Bioreactor sterilization is important to ensure

the safety and integrity of the product and can

be achieved using different techniques. Thermal

Alternatively, in chemical sterilization, bioreactors

and parts are treated with sterilizing agents such as

chemicals, prevention of cross-contamination, and

How are your bioreactors sterilized?

avoiding early corrosion while ensuring product safety.

sterilization involving steam is common in biopharma.

Clean-in-place (CIP) and steam-in-place (SIP) are common techniques for thoroughly cleaning and sterilizing processing systems without dismantling bioreactors. By employing a combination of chemicals, heat, and water, these methods effectively sanitize equipment, including pipes, filters, and fittings.

In this survey, 16 out of 22 respondents said they use SIP, CIP, or both for sterilization. Among those using other methods, autoclave sterilization and radiation were common, and a few companies use single-use bags instead of sterilization (Fig. 25). When asked to specify the cleaning solution used for CIP, two companies said sodium hydroxide and one said detergent (e.g., <u>Cipton</u>).



Fig. 25: Bioreactor sterilization methods used by cultivated meat companies

gti

Respondents reported a similar assortment of methods for sterilizing other equipment, such as media preparation vessels and mixing tanks. Regarding the sterile operation of equipment besides bioreactors, out of 14 respondents, five use both CIP and SIP, two rely on CIP, while the rest employ alternative methods like radiation or opting for single-use liners.

Sterilization is a pivotal aspect of bioreactor operations, directly influencing product safety, operational expenses, and the overall cost. While several techniques, including thermal and chemical methods, are prevalent in industries like biopharma, each approach comes with its specific requirements and implications, from the use of harsh chemicals like sodium hydroxide to the need for equipment to endure high pressures or temperatures. The choice of sterilization method can also influence the bioreactor material selection and cost. Given the existing high costs associated with bioreactors, the cultivated meat industry faces a pressing need to <u>develop sterilization</u> <u>methods</u> that are both safe and economically efficient.

Process automation

Automation plays an important role in modern bioprocess design. By integrating automation, companies can achieve considerable benefits such as reduced manual labor costs, reduced contamination risk, more efficient use of reagents and lab space, and minimized batch-to-batch variation. However, the initial cost of setting up automated systems can be high. Recognizing the impact of automation on the industry, we asked how cultivated meat companies employ automation in their process design.

Based on the responses received, it is evident that automation plays a significant role in various aspects of bioprocessing for cultivated meat companies. The most commonly automated processes include bioreactor feeds, clone picking, cell culture, pH control (i.e., addition of acid or base), dissolved oxygen control, online measurements, and basic operations such as CIP and SIP. Companies are progressively transitioning from manual operations to high-throughput cell and tissue culture, aiming to automate more processes from seed train to harvest. This move toward automation is not only driven by increased efficiency and control but also by the desire to ensure sterility at large scales, as specified by one of the respondents.

An annual survey of biopharmaceutical manufacturing found that in the past, batch failures were linked to equipment problems or contamination, in which case the solution was fixing or upgrading the equipment. However, the report notes that operator errors will be more challenging to mitigate, and the time needed to find, train, and keep the right operators may result in operator errors continuing to be the primary cause of batch failures in the coming years. According to this annual biopharmaceutical survey, operator error was the leading cause of batch failure in 2022, accounting for a 3.8% batch failure rate in both commercial and clinical scales. These findings suggest that automating bioprocesses in the cultivated meat industry to reduce operator errors and manual handling, as well as interaction with equipment, could be a solution to mitigate costly batch failure rates in the future.

As the cultivated meat sector evolves, integrating automation will be important in minimizing labor and sterility expenses, especially as production scales. The industry needs to model bottlenecks in bioprocessing that should be prioritized for automation and engineering. Undoubtedly, automation will play a huge role in reducing the cost of operating bioreactors, and more research and development are needed to fully harness the potential of automation.

Shear stress control

Shear stress in bioreactors, caused by agitation during mixing or aeration, can damage sensitive cells like mammalian cells by exerting force on them. Managing this stress through bioreactor design and process optimization is crucial to protect the cells and ensure efficient bioprocesses.

Among the 18 companies that responded, 17 have taken steps to prevent cell damage from shear stress caused by aeration or stirring. These measures include:

• Equipment and bioreactor design:

- Designing agitators and aeration strategies suitable for shear-sensitive cells
- Implementing different impeller types and large pore sizes in spargers
- Computational and mathematical approaches:
 - Utilizing computational fluid dynamics (CFD) modeling and empirical shear tests
 - Performing calculations to evaluate cell resilience to shear stress and stirring

• Chemical methods or scaffolds:

- Using pluronic acid as a shear protectant
- Exploring encapsulants, macromolecular crowding agents such as methylcellulose (Schenzle et al. 2022), and other technologies to reduce shear stress on cells
- Relying on scaffolds, including microcarriers, to protect cells from shear stress

• Diagnostic and testing approaches:

- Immunostaining diagnostics and cell viability monitoring
- Monitoring senescence and cell viability closely

The diverse approaches employed by companies to mitigate shear stress-related cell damage suggest that effective strategies are needed. Additionally, using modeling and simulation techniques like CFD highlights the importance of developing better computer models for the cultivated meat industry.

Food-grade vs pharmaceuticalgrade chemicals and equipment

While cultivated meat companies originally adapted technologies designed for pharmaceutical use, they are now developing their own fit-for-purpose equipment and materials. Initially, the culture media used in cultivated meat production were borrowed from biomedical research, often with high costs associated with pharmaceutical-grade standards. Now, researchers and companies are developing their own food-grade media that are much more affordable and scalable (Kanayama et al. 2022). This shift is also seen in equipment; for example, the bioreactors in the pharmaceutical industry are built with materials to withstand extreme conditions required for sterilization and CIP/SIP, which may not be necessarily required for food production. Using food-grade rather than pharmaceutical-grade materials and reagents can significantly reduce cultivated meat production costs.

In our survey, seven out of 25 respondents report using both pharmaceutical-grade and food-grade equipment for their bioprocesses, with five using only food-grade equipment and four using only pharmaceutical-grade. Seven companies said they use a combination of feed, food, and pharma-grade materials.

The most common reason reported for using pharmaceutical-grade equipment was a lack of high-quality food-grade options. Sometimes the difference between food grade and pharmaceutical grade is not clearly defined, nor in the material quality or production method but rather the additional certifications that pharmaceutical-grade materials and equipment need to obtain. Further exploration into substituting pharmaceuticalgrade materials with food-grade alternatives while maintaining safety is essential.⁷

Establishing standards specifically for the cultivated meat industry can guide suppliers to make fit-forpurpose and affordable equipment. This endeavor would necessitate a collaborative effort among food and beverage scientists, cultivated meat process development experts, and industry standards and regulations agents.

⁷Use of food-grade materials are discussed further in sections of the report focused on bioreactors

Bioprocess scaling strategy

Economically scaling cultivated meat production is essential for its competitiveness against conventional products. The industry employs two main strategies for this: scaling up and scaling out. Scale-up involves increasing working volumes from small to production scale, which can make product quality more consistent by reducing the number of batches and, therefore, batch-to-batch variations. Larger bioreactors can also reduce capital spend and lead to reduced depreciation as the cost is spread over a larger production volume (Yossi et al. 2023, n.d.). However, scale-up presents technical challenges such as careful consideration of agitation and aeration, and operational risk, as batch failures in larger reactors due to contamination or other errors will be expensive.

Conversely, scale-out maintains constant bioreactor size throughout pilot and commercial manufacturing, expanding production by increasing the number of bioreactors and production lines of the same size. This approach can reduce the operational risks from batch losses as material from unaffected bioreactors and production lines can still be harvested. Overall, the scale-out strategy may hold several advantages, including enabling single-use bioreactor technology, mitigating scale-up risks, and accommodating multiple product types and market demands under one roof.

In addition to risk tolerance level, the choice between scale-up and scale-out can also depend on cell type, which can determine whether the cells are grown in adherent or suspension platforms. While suspension platforms have long been favored for scale-up, adherent platforms may offer advantages in creating <u>microenvironments</u> that mimic solid tissue growth. Stirred tank bioreactors are likely the most used system for the large-scale culture of mammalian cells. While they are often used with suspension cell lines, microcarriers can provide a surface for the attachment of adherent cells. Other types of bioreactors, such as hollow-fiber or rocking-bed bioreactors, can also be used to scale adherent cell production. However, the capacity of these bioreactors for scaling up is limited or unexplored.

In our survey, out of 25 respondents, three said that scaling out is their primary strategy, 12 are relying on scaling up, and the rest use a combination of strategies. Fifteen out of 24 respondents said that they have done a techno-economic assessment of their scaled production process, but most do not plan on sharing these publicly.

Some companies are exploring large bioreactors with <u>volumes such as 200,000 liters</u> while others are contemplating the development of bioreactors with the potential to scale up to <u>millions of liters</u>. Conversely, other companies may initially opt for a more conservative approach, initially scaling up to volumes ranging from hundreds to a few thousand liters, and scaling out the production from there. The "sweet spot" where the techno-economic models indicate a profitable operation will be dependent on factors such as the final product, cell type, and other process considerations.

Overall, cultivated meat companies are using both scale-up and scale-out strategies. While scale-up was the predominant choice in this survey, this may reflect the current state of the industry as companies move beyond R&D rather than the end goal for scaling. It is unlikely that there will be a universal solution applicable to all companies, as each will have its own business strategy and risk tolerance.

Achieving price parity in cultivated meat production at scale

We asked companies about the required production capacity (tons) and the volume (size of the largest proliferation vessel) at which their cultivated meat could become a viable conventional product cost competitor. Companies provided the following observations and expectations:

- High-value cultivated products like foie gras and unagi may reach cost parity at ton-scale production.
- Mass-market seafood such as tuna and salmon are projected to reach cost competitiveness at kiloton-scale production.
- Hybrid products with lower cell content are expected to be competitive at ton-scale cell mass production.
- Cultivated meat products that are commonplace, like beef burgers, are estimated to achieve price parity when produced at tens of kilotons per year.

Generally, respondents (n=12) estimated that their products will become cost-competitive with conventional meat once production capacities reach thousands of tons and proliferation vessel volumes reach tens of thousands of liters.⁸ Companies also mentioned other ways to reach price parity faster, such as producing hybrid products or developing more cost-effective and efficient equipment and materials.

One company highlighted that the key lies not just in scale, but in productivity over time and supply chain improvements. They emphasized that an optimized process can make a smaller reactor as effective as one ten times larger if executed correctly. Lowering media costs through reducing the cost of basal media and using viable growth factor substitutes is another challenge that impacts the scale at which cultivated meat reaches price parity. The growth of the supply chain ecosystem alongside industry scale-up is crucial to tackle these cost issues effectively.

⁸As noted elsewhere in this report, production capacities are not anticipated to reach the ton scale until larger facilities are constructed and become operational, likely in the latter half of this decade.

Process development challenges

We asked respondents to rate the importance of various factors that can make scaling up cultivated meat production difficult (20 respondents, Fig. 26). Additionally, we asked companies to share their main challenges in designing their bioprocesses in an open question.

What challenges did you face during process development and scaling up?

20 respondents, challenge (1-10), 10 being the most challenging



Fig. 26: Top challenges faced by cultivated meat companies.

The leading challenge to scaling is timely access to supplies and equipment. Achieving the desired texture, access to the right type of equipment, and avoiding animal-derived materials are also leading concerns.

Eighteen companies shared their insight and challenges in designing their bioprocesses, which are summarized below:

• Design-related challenges

- Filtration and separation processes especially for adherent cultures or large scales
- Designing fit-for-purpose bioreactors and other equipment

• Cost-related challenges

• High cost of media and bioreactors

• Cell culture challenges

- Developing a high cell density process rapidly
- Media formulation
- Poor cell metabolism

Logistical challenges

- Bioreactor and equipment lead time
- Lack of talent, especially bioprocessing

• Scientific challenges

• Lack of species-specific literature, especially for novel cell lines

Interestingly, cultivated meat companies did not express significant concerns about GMO labeling and batch failure due to contamination. Avoiding GMO labels may have been influenced by the geographic location of some respondents, as restrictions for GMOs vary considerably around the world.

The lack of concern regarding batch failure could result from many companies not being in the large-scale production phase. However, it can also indicate that the rate of failure in cultivated meat companies is likely not higher than that of pharmaceutical companies, and may even be lower. This is a positive signal for the industry but will need to be verified as additional facilities become operational. To add perspective, according to survey results from biopharmaceutical companies in 2022, the leading causes of batch failure in clinical scales were equipment failure, operator error, and contamination, accounting for 4.3, 3.8, and 2.9% failure rates, respectively. For later-staged commercial biopharmaceutical manufacturers, the leading causes of batch failure were operator error, equipment failure, and contamination, accounting for 3.8, 3.3, and 3.2% failure rates.

Overall, the responses highlight significant demand for improved bioreactor designs, experienced professionals in bioprocessing, enhanced equipment for large-scale cell culture—particularly in terms of filtration devices—and addressing concerns related to rapidly growing cells reaching high densities. A deeper <u>understanding of these cells' metabolism</u> and media requirements is also crucial for advancement.

Proliferation bioreactor

Mode of operation during proliferation

Bioreactor operation modes are crucial considerations in cultivated meat production, impacting efficiency and cost. Each mode has advantages and disadvantages, influencing the size and complexity of the bioreactor setup. The mode of operation directly affects media usage, which is a significant cost and environmental impact factor in cultivated meat production. More complex modes may require additional equipment, such as recycling and filtration units, which can either reduce overall costs or increase consumables expenses. A recent techno-economic analysis by Ark Biotech explores the interconnectedness of media cost, bioreactor scale and design, and mode of operation, shedding light on the path to price parity in cultivated meat production (Yossi et al. 2023, n.d.).

Cells can be proliferated using various modes of bioreactor operation. Batch processing involves

combining a specific quantity of cells and growth media as a single unit, processing them together until completion, and then harvesting or further processing the entire batch. Fed-batch involves real-time delivery of growth media to cell batches as they grow. Continuous processing allows for cell harvesting while others continue to grow. Perfusion, a type of continuous processing, maintains cells in a bioreactor while continuously exchanging the culture medium, ensuring a steady supply of nutrients and waste removal during cultivated meat production.

According to the survey data, the most common mode of operation is fed-batch, followed by continuous processing and simple batch processing. Perfusion is currently less common among cultivated meat companies (Fig. 27). From 22 responses, many companies indicated they use multiple modes of operation during their proliferation process.

What is your mode of operation in your proliferation process?

Selecting all that apply, 22 respondents



Fig. 27: Mode of operation during proliferation used by cultivated meat companies.

Two respondents said they were poised to produce between one and ten tons of cultivated products in 2023. One of these companies specified multiple modes of action, while the other employed continuous processing. Furthermore, among the companies that expect to produce between 100 to 1,000 kg of cultivated products in 2023 and only specified a single mode of operation, one company uses batch processing, two companies use fed-batch processing, and one company uses a perfusion process.

These findings highlight the diverse bioprocessing techniques companies adopt to achieve their production targets, encompassing continuous, batch, fed-batch, or perfusion processes. However, there are still uncertainties and unexplored areas in this domain. The interconnection between the mode of operation and media cost calls for advancements in low-cost media production to enable the exploration of less conventional scaling strategies. Additionally, designing fit-for-purpose bioreactors or auxiliary equipment, such as cell retention devices and filtration systems, can significantly impact the efficacy of common bioprocessing modes and potentially revolutionize the cultivated meat production landscape. Bioreactor selection depends on the specific process requirements, including the cell type, desired production scale, and expected bioprocessing outcome — for instance, whether the final product requires a differentiation step to produce textured meat and tissues.

The survey showed that stirred-tank reactors were the most commonly used (20 out of 22 responses). This prevalence may be attributed, in part, to the pharmaceutical industry's adaptation of this technology. However, 10 companies reported using air-lift, rocking-bed, fixed-bed, or hollow-fiber reactors (Fig. 28).

These results suggest the cultivated meat industry exhibits a diverse landscape of bioreactor types being utilized and explored. While stirred-tank bioreactors were the most common, other bioreactor types, such as air-lift and hollow-fiber, remain relatively unexplored for cultivated meat production. A rigorous, comparative assessment of the technoeconomics of these systems for scaling cultivated meat production would be valuable for the field.

What kind of bioreactor do you use for proliferation?

Selecting all that apply, 22 respondents



Fig 28: The types of bioreactors used by cultivated meat companies for proliferation

Bioreactor construction

Bioreactors can be built on-site, purchased off-the-shelf, or purchased off-the-shelf with modifications. Interestingly, the vast majority (20 out of 22) of survey respondents indicated that they work with third-party designers and builders for their bioreactors, although about half of those respondents also make their own design modifications. Respondents who sourced their reactors from commercial suppliers reported a 6—12 month lead time for acquiring them.

While only seven companies reported that they design and build their own reactors in-house, they often still rely on third parties for key parts of the design, or they purchase bioreactors from a third party with modifications. These findings suggest that third-party collaborations and suppliers play a crucial role in bioreactor design and construction, while some companies prefer in-house development with support from external partners. This highlights an important opportunity for suppliers to build fitfor-purpose equipment by considering cultivated meat companies' design needs.

Seed vessel sizes

Seed train processes are used to procure enough cells for bioreactor cultivation by growing a small sample of cells taken from a cell bank in consecutively larger vessels. Seed trains involve multiple steps of proliferation, and the scale typically increases by a factor of 10 (or sometimes five) at each seed train proliferation stage.

Our survey results indicate that a typical starting volume is 100 mL, and this must scale to 1000-2000 L to reach bioreactor capacities that many companies aim to achieve in their first facilities (though not all companies are scaling this far yet).

Production bioreactor sizes

To better understand the industry's production scale, we asked cultivated meat companies about the size(s) of bioreactors they use for production. Specifically, we excluded smaller vessels primarily used for research and development purposes, such as screening and media optimization. However, smaller bioreactors may be used for production purposes during seed train expansion, and companies may have included such cases in their responses.

What size(s) of bioreactor is (are) your company using for production?

Selecting all that apply, 21 respondents



Fig. 29: Bioreactor sizes used for cultivated meat production (excluding bioreactors used for R&D purposes).

*This size is being built out in a pilot facility. Not currently operational.

The survey results reveal a diverse range of bioreactor sizes employed by companies, reflecting varying stages of development and production needs (Fig. 29). Small bioreactors (1-10 L) dominate for prototyping and development purposes, while larger bioreactors (>100 L) are crucial for initial scaleup, product development, and data collection for regulatory submission. Expectedly, most companies are in the early stages, focused mainly on R&D or small-scale pilot studies, and larger companies still use small-scale bioreactors for the same purposes in addition to their larger bioreactors.

Cultivated meat production currently relies on bioreactors in the low-thousand-liter range. Whether companies opt for scaling up or scaling out, it is likely that much larger bioreactors ranging from tens to hundreds of thousands of liters will be essential. Notably, some companies are planning to build such large bioreactors soon, as demonstrated by previously presented survey data on anticipated production in companies' second facilities. As the industry is still in the early stages of scaling, a substantial expansion in bioreactor capacity is essential to meet global demand. The roadmap for growth is becoming clearer as some companies actively plan to integrate larger bioreactors in the coming years.

Materials used in the proliferation bioreactors

Large bioreactors are typically made from stainless steel, which can be manufactured in multiple alloys with different properties that can be leveraged for different use cases. The most commonly used steel alloys in food and pharmaceuticals are 316 and 304. Unlike 304 alloy, the 316 alloy has molybdenum, which enhances its resistance to harmful substances such as acids, alkalis, and chloride pitting, resulting in increased durability and protection for crucial components. Additionally, its high chromium and nickel content provides superior strength and corrosion resistance in demanding environments, such as high salt. The 304 alloy has similar properties but is more prone to corrosion when frequently exposed to prolonged exposures to high salt or chloride-containing solutions.

Selecting the appropriate bioreactor material involves considering various factors, including cost, sterilization techniques (e.g., heat versus harsh chemical clean-in-place processes), and safety and regulatory compliance. While 316 alloys are more durable, they are also more expensive, with <u>price</u> <u>points 40% higher than 304</u>. For example, one study estimated a 20.000 L stirred-tank bioreactor made of 316L⁹ stainless steel could cost ~\$1.5M (Humbird 2021), while another study estimated that a 42,000 L and 211,000 L stirred-tank bioreactor made of 304 stainless steel could cost only ~\$900,000 and \$2.38M, respectively (Negulescu et al. 2022). Therefore, the cost savings from switching from 316 to 304 stainless steel could be considerable even within a single facility. Reducing the exposure of 304 alloys to corrosive cleaning chemicals may also be possible through the use of gas sterilants such as chlorine dioxide. The cost-sensitive nature of the cultivated meat industry will likely drive bioreactor materials and sterilization methods into different forms than what is currently practiced in the pharmaceutical sector.

Out of the 24 companies that provided information about their proliferation bioreactors, 18 mentioned stainless steel as the material of choice, nine indicated the use of single-use bioreactor bags, and two indicated glass (Fig. 30). Five companies indicated both stainless steel and single-use bioreactor bags, which could indicate various bioreactors used for different stages, such as R&D, process development, and production.¹⁰ Additionally, seven of the companies that use stainless steel specified the use of 316 alloy, while four indicated the 304 alloy.¹¹

When purchasing or designing bioreactors, companies prioritize factors such as durability, adherence to safety standards, and budget considerations. Stainless steel, especially made with specific alloys, is often chosen for its strength and resistance to rust. Yet, the expense of premium alloys like 316 has led some businesses to look at more cost-effective alternatives, such as the 304 alloy. The reason behind the limited adoption of lower-cost, food-grade options remains ambiguous. We speculate this could be due to borrowing technology from the pharmaceutical sector, which typically uses 316 alloy for cell culture or higher resistance of the 316 alloy to harsh chemicals used during CIP. An important research need is determining whether 304 stainless steel is suitable for long-term cultivated meat production or if its drawbacks are limiting.

What type of materials are your proliferation bioreactor(s) made of?





Fig. 30: Material used for proliferation bioreactors

Selecting all that apply, 24 respondents

⁹<u>316L</u> alloy is similar to 316 alloys, except that it has lower carbon content, is more resistant to corrosion, and does not require post-weld annealing.

¹⁰For smaller reactors, single-use bags are more prevalent.

¹¹The 304 alloy is sometimes referred to as "food-grade." In actuality, the definition of "food-grade" for materials can be difficult to pinpoint. In this case, companies that use food-grade stainless steel state that their materials have food contact certifications.

Differentiation bioreactor

Mode of operation during differentiation

We asked companies about the operation mode for their differentiation vessel. Eight companies responded, and three reported multiple modes. This could be due to having multiple bioreactors in R&D and process development. Of eight respondents, seven companies use perfusion as the operation mode for differentiation, while four use batch, two use continuous processing, and only one use fed-batch processing. Based on the survey data, perfusion is the most common differentiation operation mode.

Types of bioreactors used for differentiation

We asked companies about the type of bioreactors they use for differentiation. Few companies responded to this question, and several specifically said "other", did not specify, or said they could not disclose. Of six companies that specified, three said stirred-tank, two said fixed-bed, and one said hollow fiber reactors.

The hesitation to disclose and lack of certainty in responses, such as choosing multiple options for mode of operation, could signal that companies are less certain about this process and/or are more protective of their innovations and data regarding the type and mode of operation of differentiation bioreactors. This can highlight an open-source knowledge gap that, if filled, has the potential to accelerate cultivated meat development.

Materials used for differentiation bioreactors

Of seven respondents, six said they use stainless steel reactors for differentiation, typically with pharmaceutical-grade steel and 304 or 316 stainless steel alloys. Only one company uses single-use bioreactor bags, and only two companies use stainless steel food-grade material (304 or 304L¹² stainless steel), but the material has food contact certification.

Obtaining differentiation bioreactors

Of eight respondents, six companies purchase their differentiation bioreactors from a third party. Two companies built the bioreactor in-house, one of which said they use third parties to provide the key parts.

This finding emphasizes the importance of collaboration and partnerships in the bioprocessing industry for cultivated meat and seafood. Cultivated meat companies are urged to pay attention to bioreactor lead times. While we asked about the lead time of bioreactors for differentiation, only one company responded, specifying four months until commissioning. Companies are also encouraged to frequently contact the suppliers to ensure on-time delivery of bioreactors.

¹²304L stainless steel has a lower carbon content and is structurally weaker than standard 304 alloy.

The financial investment

Several techno-economic studies have indicated that capital expenditure for equipment is expected to be a major cost driver of cultivated meat production (Vergeer, R., Sinke, P., Odegard, I 2021; Humbird 2021; Negulescu et al. 2022). We aimed to gather insights into the significant cost and investment factors related to the major equipment used in cultivated meat production, such as bioreactors, media mixing tanks, and centrifuges. The responses provided were descriptive and covered a range of information regarding various equipment. However, the reported data should be considered individual data points rather than indicative of average industry standards.

Cost of major equipment

Bioreactor cost

6 respondents

From the data provided by 10 companies, the cost of bioreactors exhibited a significant range, spanning from \$50,000 to \$4 million. Notable specifics include a \$100,000 investment in a 1,000 L bioreactor, \$300,000 for a 200 L single-sue bioreactor, and a \$1M expenditure for a combination of 200 L and 2,000 L bioreactors. Another company allocated around \$200,000 for a stainless steel 20-40 L bioreactor and \$400,000 for a 500 L single-use bioreactor. Generally, companies paid several hundred thousand dollars for bioreactors ranging in size from a few hundred to around a thousand liters, while those closer to the 1,000-liter capacity mark incurred costs near the million-dollar range. We also inquired whether companies rented their bioreactors; of 24 respondents, only three have done so.

With limited data points and several exceptions, the trend suggests an average cost of approximately \$100,000 per 100 L bioreactor capacity (Fig. 31). Economies of scale become a factor with larger reactors, as smaller volume reactors (<500 L) tend to cost more than average per liter, while larger reactors (>500 L) cost less than average. For instance, a 2000 L bioreactor was purchased for \$1 million. The numbers cited here diverge significantly from previously cited techno-economic analyses (Negulescu et al. 2022; Humbird 2021), suggesting more data is needed to accurately assess the current costs of bioreactors.



Approximate cost of bioreactors

Fig 31: Approximate cost of bioreactors based on data from six companies.

Disclaimer: Caution should be exercised as this figure is derived from a subset of the data and may not accurately represent industry-wide averages.

Various factors play a role in determining the final price of a bioreactor, including the desired working volume, the required level of process monitoring and control, the number of moving parts such as impellers, strict quality and sterility standards (e.g., resistance to heat and harsh chemicals), adherence to regulatory guidelines, customization for specific processes, and ongoing expenses like maintenance and consumables. Furthermore, bioreactor delivery and installation can be a long and costly process. The delivery and installation of large bioreactors involve careful transportation planning, site preparation, precise installation, calibration, and testing to ensure proper function. Training personnel is also essential to operate and maintain the bioreactor effectively, and validation procedures are conducted to meet regulatory standards. To read more about a case study for successful bioreactor installation, please see this article from BioPharm.

There are several key areas to explore to reduce the cost of bioreactors. The mode of operation can significantly impact costs, and optimizing the mode of operation while simplifying complexity can effectively lower expenses. Using more affordable materials and sterilization techniques, as well as integrating automation, are other approaches to cost reduction. Scaling up bioreactors can also help reduce depreciation expenses as the cost is spread over a larger production volume. Further, increasing the reactor scale enhances production efficiency, reducing facility costs by requiring fewer vessels and parts, fewer personnel and utilities for the same output, and less maintenance. For example, Ark Biotech plans to minimize capital spending primarily by using larger bioreactors (Quint, Y., Rauch, A., Sands, D., Hoffner, K., Rubio, N., Huang, Z. 2023).

Mixing tank cost

In biopharmaceutical and food manufacturing, a mixing tank is a specialized vessel designed for precise blending and homogenization while maintaining sterile conditions. For cultivated meat, mixing tanks are most frequently used to prepare complete media on site. These tanks are constructed from materials compatible with bioprocess fluids, equipped with agitators for controlled agitation, and may feature temperature control capabilities. They may include ports for sampling and monitoring parameters like pH, as well as connections for adding components and transferring materials. Mixing tanks often incorporate clean-in-place (CIP) and steam-inplace (SIP) systems for easy cleaning and sterilization.

Mixing tank expenses vary widely among companies (and not all included volumes in their responses): one reported an investment of \$1 million, another \$100,000, and one \$50,000. Additionally, one invested \$20,000 in a 500 L tank, and one company spent \$40,000 for a 600 L tank and \$60,000 for a 100 L tank. Companies could expect to spend tens to hundreds of thousands of dollars on mixing tanks at their facilities. However, due to limited data and several exceptions, it's challenging to conclude an overarching trend from these figures.

Centrifuge cost

Centrifuges are specialized machines used in the pharmaceutical industry and cultivated meat production. They separate components in liquids or suspensions based on their density. The pharmaceutical industry uses them for various purposes, including separating cell cultures, isolating proteins, and purifying drugs. In cultivated meat production, centrifuges can separate muscle or fat cells from growth media and other components to harvest and concentrate the cultured cells.

The cost of centrifuges can vary based on factors such as the type of centrifuge (e.g., filtration or sedimentation), processing capacity or size (i.e., product throughput), and the manufacturer. Five companies reported spending between \$100,000 and \$200,000, with one investing \$400,000 and another \$1.5 million. High-capacity centrifuges, such as decanter-type or disc stack centrifuges that can process upwards of 1000 liters per minute, may cost over one million dollars.

Bioprocess major cost drivers and availability challenges

Reduced bioprocess expenses across bioreactors, growth factors, and media are needed for cultivated meat to achieve commercial viability. However, some items pose a more urgent challenge for businesses because of their high costs and limited availability, potentially leading to bottlenecks in operations.

To gain insights into the most pressing challenges in terms of cost and availability, we asked cultivated meat companies to prioritize limiting factors among the currently available options. We also inquired about the items they would like to see become more affordable the fastest.

Of the 20 respondents, a substantial number flagged growth factor cost and availability as a primary challenge (see Figure 32). This was followed by talent availability, underscoring the pressing need to invest in training the next generation of experts via specialized programs and integrating relevant curriculums in academic institutions.

10

8.6

What are the most limiting factors in terms of availability or cost?



20 respondents, importance (1-10)

Fig. 32: Most limiting factors in terms of cost and availability from the options already available

When questioned about the urgency of cost-cutting for certain products, the results from the 20 participants indicated recombinant proteins, peptides, and growth factors need to be prioritized (refer to Fig. 33). Bioreactors, basal media, and other additives were also identified as critical areas requiring urgent cost reduction. Interestingly, cost reduction in scaffolds was considered less crucial, potentially because the current focus is on less structured cultivated meat products or the cost of bioreactors and media is significantly higher than other materials and equipment.



20 respondents, importance (1-10)



Fig. 33: The products companies want to see a cost reduction for most urgently.

From another subset of 16 respondents, 13 indicated that the primary expense drivers on the <u>bill of</u> <u>materials</u>, excluding capital expenditure, are the media and/or growth factors. A few also brought attention to costs associated with filters, bags, and various raw materials.

These results aim to offer suppliers a clearer perspective on the primary obstacles encountered by cultivated meat businesses, enabling them to better anticipate and address needs. Concurrently, it equips cultivated meat companies with valuable intel on current challenges, aiding strategic planning. In summary, the primary challenges largely center on the costs associated with media and bioreactor designs, which are interconnected as the mode of operation of a bioreactor can directly affect media consumption needs. Moreover, other pressing issues like the availability of skilled talent or high labor costs can be partly addressed through automation and refining bioreactor handling and operation. Nonetheless, it is important to exercise caution when interpreting the survey results, as the current emphasis on rapid and cost-effective scaling may overshadow other future bottlenecks. Therefore, it is crucial to consider the evolving landscape of cultivated meat production and remain attentive to emerging challenges and priorities.

The role of CMOs/CDMOs

Cultivated meat companies, like pharmaceutical and biotech firms, may leverage contract manufacturing organizations (CMOs) or contract development and manufacturing organizations (CDMOs) to outsource their production needs. Currently, there are few CMOs/CDMOs that offer services for cultivated meat around the globe, although notably, GOOD Meat's cultivated chicken product approved in the U.S. is manufactured by JOINN Biologics, a CDMO based in California, and several other companies have signed agreements with Esco Aster, a CDMO based in Singapore. In today's down funding environment, CMOs/CDMOs could offer pilot production capacity that enables companies to take a more capitalefficient approach to scale up without investing millions of dollars into their unique facilities.

While partnering with a CMO/CDMO could offer advantages such as cost reduction, quicker time to market, scalability, and supply chain support, there are also drawbacks. Among the cons are the potential loss of profit, diminished control, compliance risks, the need to ensure the CMO's qualifications and ethics, and possible communication challenges, especially concerning the transfer of IP or know-how. This decision depends on each company's specific circumstances and priorities.

We asked cultivated meat companies if they work with a contract manufacturer, and from 17 respondents, only four said they work with a qualified CMO/CDMO for manufacturing. All those using a CMO/CDMO are using it for full production, and half are also using it to form the final product. One respondent reports using a CMO/CDMO for cell banking.

Since there are many factors involved in deciding the right contract manufacturer, we asked companies to rank the importance of different factors (Fig. 34). Based on our survey results from 17 companies, the most important factors that cultivated meat companies are looking for in the contract manufacturers are quality of production, having the right expertise, and price. However, the results indicate relatively little variation in how much companies prioritize various factors in selecting a CMO/CDMO.

How do you rate what you value in a cultivated meat CMO/CDMO?

17 respondents, importance (1-10)



Fig. 34: Most important factors in selecting a cultivated meat CMO/CDMO.

We then asked companies to identify their first, second, and third choices in selecting a cultivated meat CMO/CDMO based on its location (Fig. 35). The U.S. and Southeast Asia were equally popular first choices, followed by China, Europe, and India.



17 respondents, importance (1-10)



Fig. 35: Cultivated meat companies' preference for the location of partner CMO/CDMO.

Additionally, respondents were asked about the greatest barriers to lowering the cost of CMO/CDMO contract work. Common responses indicated the following:

- Availability of alternate vendors: The small number of <u>currently available CMO/CDMOs</u> <u>for cultivated meat</u> drives up the price of their services.
- Capacity of CMO/CDMO services: Larger bioreactor volumes would help reduce prices.
- Costs of labor, equipment, and raw materials

Our survey indicates that a minority of companies currently work with CMOs and CDMOs. Key factors for choosing CMO/CDMO partners include production quality, expertise, and pricing, but we observed a small difference between answers. The preferred locations for CMO/CDMO facilities are the U.S., Southeast Asia, and China, with Europe as a third choice. These findings suggest many opportunities for additional CMO/CDMOs. For existing CMO/ CDMOs, stronger partnerships with cultivated meat companies will enable service providers to meet their needs better.

Food Safety

Food safety is a top priority in cultivated meat production and is central to developing new technologies. There are various factors in assessing food safety and quality, such as the types of tests used and regulatory rules that can differ between countries. Companies also consider Good Manufacturing Practices (GMP) certifications. Even though this survey does not solely focus on food safety, it is an integrated part of all bioprocesses. As such, we dedicated a section to highlight the safety measures cultivated meat companies are taking.

For more information on food safety considerations in cultivated meat, see this report by the <u>UN FAO</u> and this report by <u>GFI-Brazil</u>.

Post-production QC and safety evaluation of cultivated meat products

We aimed to know what safety and quality tests cultivated meat companies carry out after production. Different companies conduct varying tests depending on their production stage and focus (e.g., B2B, final product, supplier). Out of the 15 companies that responded, common analyses include checking for contamination, assessing composition, analyzing spent media, and determining shelf life (Fig. 36). The frequency of tests does not always indicate their importance, as some companies might not yet be in the production stage.

What kind of post-production (for QC/safety) analysis do you perform on your product? *15 respondents*



Fig. 36: Post-production analyses performed by cultivated meat companies.

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Microbial testing and contamination checks are most frequently conducted, underscoring the focus on product safety and minimizing contamination risks. Testing for lipid composition and other nutrients is crucial for validating and comparing the nutritional value to traditional meat. Analysis of spent media is also vital, as it indicates media efficiency and cell culture health. Meanwhile, antibiotic testing is less common, likely due to the minimal use of antibiotics, which, if used, occurs mainly in early cell line development rather than during full-scale production.

Contamination risk and management in cultivated meat

Various forms of contamination, such as bacteria, mold, yeast, viruses, and mycoplasma, can infiltrate the production process at different stages. Contamination can arise from raw materials like media, during processing due to equipment issues, sampling, adding culture components, or harvesting, as well as during final product processing (FAO & WHO 2023).

From the 22 respondents, a majority (13) identified process contamination as the main source of microbial contamination. In addition, seven identified final product contamination and two identified raw material contamination as the primary sources (Fig. 37).

What do you think is the biggest source of microbial contamination risk with cultivated meat? *22 respondents*



Fig. 37: Major sources of contamination reported by 22 cultivated meat companies.

Out of the 19 companies that discussed preventing contamination sources, mold was often considered the most challenging to manage, while bacteria were considered the easiest to handle.

Some companies provided additional feedback regarding their experience managing contaminants which we summarize below:

- Sterility and contamination risks: Handling and media changes can increase the risk of contaminating sterile cell lines; human error during sampling or setup can also breach sealed processes.
- **Raw material and media safety:** Sterile culture media and high-quality raw materials are key to preventing contamination; extensive testing ensures contaminated materials are discarded.
- Scale-up challenges and cell bank integrity: Managing contamination at scale is challenging; regular testing of cell banks is vital, with contaminated ones being discarded.
- **Bioprocess monitoring and final product safety:** Many cultivated meat companies said they have rigorous contamination checks and prevention strategies, ensuring final products are food-safe.

Preventing cell culture contamination is not a unique concern to cultivated meat. Life sciences companies are familiar with ways to identify and prevent contamination. Our results also highlight the importance of sterility and quality-controlled raw materials such as media, additives, and bioreactor bags in ensuring a contamination-free process.

Evaluating microbial risks in cultivated meat versus other foods

Contamination risks are associated with any food processing. Both conventional and cultivated meat are at risk of contamination, although the contamination sources can vary.

To assess the contamination risk of cultivated meat relative to conventional meat and identify any unique challenges, we asked cultivated meat companies to compare the microbial risks of cultivated meat to other foods. Most industry players (23 out of 24 respondents) do not expect unique microbiological risks from cultivated meat production. The one company that expects unique difficulties regarding cultivated meat said they are concerned about the financial risk of large-scale contamination management. They also said viral contamination risk still needs to be assessed for cultivated meat. Nineteen respondents further clarified that they expect microbial safety risk to be lower for cultivated meat than for conventional meat products. At the same time, three said they think the risk is higher, and one said it is the same.

Cultivated meat companies highlighted several advantages that contribute to a lower perceived risk in cultivated meat production compared to conventional meat production. Respondents have indicated that the initial product in cultivated meat, consisting of pathogen-free cells from aseptic processes, is considered cleaner than meat derived from slaughterhouses. As a result, cultivated meat carries a lower risk of introducing contaminants than traditional meats, where animal fecal microorganisms and other contaminants are present. Respondents also emphasized the absence of other risks associated with animal-based meat processing, such as unsanitary slaughterhouses and zoonotic diseases. Furthermore, wellestablished and controlled procedures drawn from biopharmaceutical applications, along with the use of sterilized bioreactors, can further reduce the contamination risk in cultivated meat production. Automated procedures and methods also play a significant role in reducing contamination risks. In summary, these companies view cultivated meat production as cleaner, more automated, and better controlled, leading to a reduced risk of contamination.

On the other hand, companies perceiving higher microbial risk point to risk factors like multiple unit operations or the use of nutrient-dense media prone to contamination, which can potentially lead to the destruction of large lots in the event of contamination. Overall, most companies expect microbial safety for cultivated meat to be lower than that of conventional meat, and they do not see unique challenges in controlling the microbial risks. These sentiments are similar to those expressed in a recent report by the U.N. Food and Agriculture Organization (FAO & WHO 2023). However, companies also acknowledge that cultivated meat bioprocessing is prone to contamination, which can jeopardize the process and lead to significant costs on a large scale.

Maintaining microbial standards for cultivated meat products

The regulatory landscape for cultivated meat is still in development, and new or modified standards and regulations to ensure the safety of cultivated meat around the globe are in the works. Cultivated meat manufacturers and other food safety experts and organizations are essential in shaping these outcomes. To this end, we asked cultivated meat companies about the standards they maintain during their processes.

Of the 20 companies that responded, 17 said that their company maintains a microbial safety standard/specification for their product, with most following criteria set by regulatory authorities or recommendations of expert food safety organizations. Six companies said they set their specific limits, often in combination with following recommendations or criteria set by authorities or experts. Several companies also indicated that they are not yet at the stage to have safety standards and set specifications.

Several companies provided further insights, stating that they adhere to internal reference limits that often surpass regulatory and expert recommendations. Generally, they are committed to maintaining or surpassing the standards of conventional meat production, using methods such as a rigorous 14-day pharmaceutical broth test or achieving <u>bioburden</u> levels lower than conventional meat.

Overall, there is a need for cultivated meat and regulatory groups and authorities to set limits that not only ensure the safety of cultivated meat but also do not slow down its development by enforcing criteria not applicable to the industry or necessary to protect human health. For instance, tight regulations around pharmaceutical-level cell cultures may be pertinent for injectables but not for producing materials meant for consumption as food. Similarly, some limitations or criteria in the food industries may not apply to cultivated meat, given the complexity of large-scale cell culture and its unique safety requirements.

Top markets and regulatory approval process for cultivated meat

The process of obtaining regulatory approval for cultivated meat can vary by country or region, with many frameworks still developing worldwide (reviewed in GFI's <u>State of Global Policy report</u>). These differences impact how companies prioritize market entry for their cultivated meat products. Therefore, we asked companies questions about their top priority markets and their level of understanding of regulatory pathways.

When we asked companies about top priorities for entering the market and seeking regulatory approval, 25 respondents ranked countries listed in Fig. 38. Companies are most interested in gaining regulatory approval to sell cultivated meat in the U.S. and Singapore, likely because these two countries are the only ones to have approved a cultivated meat product. However, companies noted other important markets, including the U.K., Australia and New Zealand, the E.U., China, and Japan.

We then asked about companies' understanding of regulatory food safety requirements in their top

priority markets. Of the 27 companies that responded, 22 respondents perceive their companies to have a "sufficient" or "excellent" understanding of the regulatory requirements in their highest-priority markets. Twelve of those companies acknowledge that there are still gaps in their knowledge of regulatory affairs. Five companies said their understanding of regulatory requirements is lacking because relevant information is difficult to obtain or unavailable. One company said their understanding of these requirements is lacking because they have not reached the stage to consider such regulatory requirements seriously.

Overall, cultivated meat companies' interest in seeking markets in different countries could be attributed to the different levels of governmental support, clarity of regulatory frameworks, and product-market fit. In addition, while most companies say they have a sufficient understanding of regulatory pathways in their top market priority, the regulatory approval process in many countries remains under development. At the same time, the <u>GOOD Meat and</u> <u>UPSIDE Foods regulatory approvals</u> will likely pave the way for other companies and countries internationally.

What are your company's highest priority market(s)?

Actively planning to understand, and apply for, regulatory approval to sell cultivated meat; 25 respondents, importance (1–10)



Fig 38: Companies' highest priority market(s) in which they are actively planning to investigate or apply for regulatory approval to sell cultivated meat.

Safety and preventive measures

Systematic methods to ensure food safety, including identifying potential hazards, developing controls, and implementing a food safety plan, can be applied in the cultivated meat industry ("Assuring the Safety of Cultivated Meat: HACCP Plan Development and Application to a Cultivated Meat Target Product" 2023). We surveyed the industry to assess safety protocols and measures, such as microbial testing, contamination prevention, or adherence to food safety plans.

Of 24 respondents, nine said their company has an operational pilot or production facility. Six of those with an operational pilot or production facility have a hazard analysis and critical control point (HACCP) plan. Of those six, two said their plan is certified by a third party such as <u>ISO</u>, while two said theirs is not, and two did not specify. Three out of eight respondents said their companies have obtained GMP certification, and another three said they are planning on obtaining third-party verification in the future (Fig. 37).

Eight out of nine companies with an operational pilot or production facility said they identified critical

control points within their production process that they feel pose a risk for microbial contamination.

Four out of six respondents have completed five-10 production runs, and two have completed 10-50 production runs. Six companies specifically disclosed their all-cause batch failure rate due to contamination, equipment failure, or other reasons: three said between 2-5%, two between 5-10%, and one greater than 10% (Fig. 39). Eight respondents unanimously say that they perform microbial sampling and testing, with most performing these tests in-house.

Overall, the companies emphasized their commitment to safety by implementing extensive precautions, conducting risk analyses, and performing rigorous post-operation assessments to identify contamination sources. Most companies with a pilot or production facility have plans in place for hazard prevention and safety compliance, such as HACCP and GMP certification. Companies have outlined various strategies to mitigate contamination risks, including rigorous risk analysis, preventive checks, and process control through automation. Learning from initial batch failures, companies have refined their processes, leading to greater stability and significantly reducing contamination events.



Summary of responses regarding safety and preventative measures in cultivated meat companies' facilities

Fig. 39: Summary of responses regarding safety and preventive measures in cultivated meat companies' facilities.

References

- 1. "Assuring the Safety of Cultivated Meat: HACCP Plan Development and Application to a Cultivated Meat Target Product." 2023. GFI Brazil. https://gfi.org.br/wp-content/uploads/2023/10/Assuring-the-Safety-of-Cultivated-Meat_HACCP-plan-development-and-application-to-a-cultivated-meat-target-product_GFI-Brasil.pdf.
- Bomkamp, C., Carter, M., Cohen, M., Gertner, D., Ignaszewsi, E., Murray, S., O'Donnell, M., Pierce, B., Swartz, E., Voss, S. 2023. "2022 State of the Industry Report: Cultivated Meat and Seafood." The Good Food Institute. https://gfi.org/wp-content/ uploads/2023/01/2022-Cultivated-Meat-State-of-the-Industry-Report.pdf.
- 3. Bomkamp, Claire, Stacey C. Skaalure, Gonçalo F. Fernando, Tom Ben-Arye, Elliot W. Swartz, and Elizabeth A. Specht. 2021. "Scaffolding Biomaterials for 3D Cultivated Meat: Prospects and Challenges." *Advancement of Science*, November, e2102908.
- 4. Brennan, Tom, Joshua Katz, Yossi Quint, and Boyd Spencer. 2021. "Cultivated Meat: Out of the Lab, into the Frying Pan." McKinsey & Company. June 15, 2021. https://www.mckinsey.com/industries/agriculture/our-insights/cultivated-meat-outof-the-lab-into-the-frying-pan.
- 5. "Cultivated Meat Media and Growth Factor Trends." n.d. The Good Food Institute. Accessed August 16, 2023. https://gfi.org/ resource/cultivated-meat-media-growth-factor-survey/.
- Dodelet, Jean-Francois Denault Agnes Coquet. 2008. "Construction and Start-up Costs for Biomanufacturing Plants." BioProcess International. February 1, 2008. https://bioprocessintl.com/manufacturing/facility-design-engineering/ construction-and-start-up-costs-for-biomanufacturing-plants-182238/.
- 7. "FAOSTAT." n.d. Accessed August 2, 2023. https://www.fao.org/faostat/en/#data/FBS.
- 8. FAO & WHO. 2023. "Food Safety Aspects of Cell-Based Food." FAO & WHO. https://www.fao.org/3/cc4855en.cc4855en.pdf.
- 9. Gomez Romero, Sandra, and Nanette Boyle. 2023. "Systems Biology and Metabolic Modeling for Cultivated Meat: A Promising Approach for Cell Culture Media Optimization and Cost Reduction." *Comprehensive Reviews in Food Science and Food Safety* 22 (4): 3422–43.
- 10. Humbird, David. 2020. "Scale-Up Economics for Cultured Meat: Techno-Economic Analysis and Due Diligence." https://doi.org/10.31224/osf.io/795su.
- 11. Humbird, David. 2021. "Scale-up Economics for Cultured Meat." Biotechnology and Bioengineering 118 (8): 3239–50.
- 12. Kanayama, Takanori, Keita Tanaka, Haruna Shimada, Koji Ichiyama, Sadaharu Higuchi, and Ikko Kawashima. 2022. "Improving the Safety of Cultured Meat Using Basal Medium Prepared Using Food Ingredients." *bioRxiv*. https://doi. org/10.1101/2022.03.28.486069.
- 13. Langer, Eric S. 2016. "Average Batch Failure Rate Worsens." Genetic Engineering & Biotechnology News 36 (17): 34–34.
- Leman, A., Bess, A., Gale, J., Silvester, D., Rodrigues, S., Gemmell, N., Costa, S. 2023. "Manufacturing Capacity Landscape and Scaling Strategies for Fermentation-Derived Protein." The Good Food Institute. https://gfi.org/wp-content/ uploads/2023/01/SCI23024_FERM-manufacturing-capacity-analysis_Final.pdf.
- Negulescu, Patrick G., Derrick Risner, Edward S. Spang, Daniel Sumner, David Block, Somen Nandi, and Karen A. McDonald. 2022. "Techno-Economic Modelling and Assessment of Cultivated Meat: Impact of Production Bioreactor Scale." *Biotechnology and Bioengineering*, December. https://doi.org/10.1002/bit.28324.
- Pasitka, L., M. Cohen, A. Ehrlich, B. Gildor, E. Reuveni, M. Ayyash, G. Wissotsky, et al. 2022. "Spontaneous Immortalization of Chicken Fibroblasts Generates Stable, High-Yield Cell Lines for Serum-Free Production of Cultured Meat." *Nature Food*, December, 1–16.
- 17. Quint, Y., Rauch, A., Sands, D., Hoffner, K., Rubio, N., Huang, Z. 2023. "CULTIVATED MEAT'S PATH TO PRICE PARITY." Ark Biotech.
- 18. Ravikumar, Maanasa, Dean Powell, Elliot Swartz, and Claire Bomkamp. 2023. "Cell Line Development and Utilisation Trends in the Cultivated Meat Industry." The Good Food Institute.

- 19. Schenzle, Lisa, Kristina Egger, Aleksandra Fuchs, and Harald Pichler. 2022. "Never Let Me down: Optimizing Performance of Serum Free Culture Medium for Bovine Satellite Cells." *bioRxiv*. https://doi.org/10.1101/2022.11.13.516330.
- Sinke, Pelle, Elliot Swartz, Hermes Sanctorum, Coen van der Giesen, and Ingrid Odegard. 2023. "Ex-Ante Life Cycle Assessment of Commercial-Scale Cultivated Meat Production in 2030." *International Journal of Life Cycle Assessment* 28 (3): 234–54.
- 21. Swartz, Elliot. n.d. "Cultivated Meat Growth Factor Volume and Cost Analysis." The Good Food Institute. Accessed August 16, 2023. https://gfi.org/resource/cultivated-meat-growth-factor-volume-and-cost-analysis/.
- 22. Vergeer, R., Sinke, P., Odegard, I. 2021. "TEA of Cultivated Meat: Future Projections for Different Scenarios." CE Delft.
- 23. Yang, Ming, Qiong Wang, Yuyan Zhu, Kuichuan Sheng, Ning Xiang, and Ximing Zhang. 2023. "Cell Culture Medium Cycling in Cultured Meat: Key Factors and Potential Strategies." *Trends in Food Science & Technology* 138 (August): 564–76.
- 24. Yossi et al. 2023. n.d. "Cultivated Meat's Path to Price Parity Ark Biotech." https://www.ark-biotech.com/post/cultivatedmeats-path-to-price-parity.