Title
A sustainability analysis of near-term animal cell-based meat

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Publication Date
2023

Peer reviewed|Thesis/dissertation

A sustainability analysis of near-term animal cell-based meat

By

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Dissertation

Submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in

Food Science and Technology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

Acknowledgements

I would like to thank my family who have been supportive of my PhD research. My wife, Ornramprai who traveled across an ocean to support me during my research. My brother, Kyle who was there during difficult health challenges. My parents, Dan and Kim who have always been supportive in a multitude of ways and opened their homes to me on multiple occasions. I would also like to thank my grandparents, Steve Anna and L.W. for being encouraging and providing genuine perspective during challenging times. Additionally, I would like to thank Ned, the principal investigator I worked under during my PhD who taught me a great deal of hard and soft skills. My committee members, Justin and Karen have also been extremely helpful with understanding the complicated process of commercialization of bioproducts. Finally, I would like to thank my cohort who banded together during the challenging years of the program to learn many valuable lessons from courses taken and rigorous academic challenges overcome.

Abstract

Investment in animal cell-based meat (ACBM) or cultured meat has been increasing at a rapid pace with the total investment being greater than 2 billion USD by 2021. When the investment in ACBM initially occurred ACBM had not been economically vetted. This dissertation contains the first publicly available technoeconomic assessment of ACBM. This assessment was conducted utilizing cellular metabolic requirements and chemical/process engineering conventions. Findings of the first TEA ACBM indicate that nearly all technical hurdles would need to be resolved before economic viability could be achieved. Shortly after publication of the first TEA of ACBM, two other TEAs of ACBM were published and all three were normalized and critically examined for this dissertation. This critical examination is contained within this dissertation to provide readers with a comparison of the methods and assumptions contained within each ACBM TEA. An additional TEA was conducted for an economically viable protein alternative, mycoprotein to validate methods which were utilized in the initial TEA of ACBM. Findings of the mycoprotein TEA indicate that the utilized method provided reasonable estimates for the cost of food produced in bioreactors. The initial ACBM TEA indicated that a substantial quantity of animal cell growth medium may be necessary to produce ACBM at an industrial scale. To understand the potential environmental impact of ACBM production, quantification of the embedded resources contained within the animal growth medium was deemed necessary. Essential 8™ (E8) is a stem cell growth medium that had been suggested as a suitable growth medium for ACBM with some modification. A cradle-to-production gate life cycle assessment (LCA) was conducted for E8. The embedded resources were quantified for each E8 component (when possible) and the environmental impact of a liter of E8 was calculated. Utilizing data obtained from the analyzed TEAs and E8 LCA, a LCA of near-term ACBM was conducted. The

LCA of ACBM indicated that the environmental impact of near-term ACBM was likely greater than commercially produced beef potentially by orders of magnitude.

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Chapter 1. Introduction

The demand for meat is expected to double by 2050 as the population and global affluence continues to increase. (Food and Agriculture Organization of the United Nation (FAO), 2019). This increased demand in meat implies an increase in worldwide livestock production. Livestock are vital to the global food system because they provide the majority of animal protein, they contribute to crop yield via utilization of their manure as fertilizer, and they provide nutrition and income to rural households in low to middle income countries (Gilbert et al., 2018; Robinson et al., 2011).

While livestock provide many individual and community benefits, there are environmental concerns related to these predicted increases in livestock production. Beef which accounted for 22% of global meat production in 2020 is the most impactful from an environmental perspective when the top three meat production systems are considered on a mass basis (poultry, pork and beef/buffalo, ordered respectively) (FOA, 2022; Poore & Nemecek, 2018; Ritchie et al., 2019). The potential environmental impacts of rearing cattle include greenhouse gas emissions (GHG) from enteric fermentation and manure, nutrient loading in the nitrogen and phosphorus cycles, reduction in biodiversity from overgrazing, and land-use change (Gilbert et al., 2018; Steinfeld et al., 2006). The forecasted increase in the environmental impact of global beef production has raised the concerns from global stakeholders which have developed interest in a multitude of solutions including meat alternatives.

Meat alternatives can be broadly categorized into plant, fermentation and animal cell-based meat (ACBM) (Asgar et al., 2010; Post, 2012; E. A. Specht et al., 2018; L. Specht, 2019; Suhlmann et al., 2019; Tubb & Seba, 2019; Tziva et al., 2020). Processed plant-based and fermentation-based meat alternatives are currently available in western markets, and these products have been for several decades (e.g. Tofurky and Quorn®, respectively) (Tziva et al., 2020). However, ACBM has not achieved commercial viability as of the time of this writing. The current concept of ACBM production involves the proliferation of animal cells in bioreactors with the possibility of differentiation of the cells into different cell types (e.g., myotubes, fibroblasts, and adipocytes) and the cells would then be processed and packaged for consumption as an alternative to conventionally produced meat.

Contemporary interest in this technology can be linked to the development and demonstration of a proof-of-concept ACBM hamburger in 2013 (Kupferschmidt, 2013). This 140-gram ACBM patty was reported to cost over 270,000 USD to develop, and the demonstration was reported to be more of a commercial product unveiling rather than a peer-reviewed scientific inquiry with debate and discussion (Kupferschmidt, 2013). Two years later the first ACBM company, Memphis Meats (now Upside Foods) was founded with the aim to commercialize ACBM and the company produced the first ACBM meatball in 2016 as a proof-of-concept (Schwartz, 2016). By 2021, there were over one hundred ACBM companies even though ACBM products were not widely available to consumers (Cohen et al., 2022). Small-scale production of ACBM in Singapore is occurring at an economic loss, however animal serums such as fetal bovine serum are being utilized in their production and these products are not widely available (Hasiotis, 2022). Other companies appear to be moving toward a first to market strategy, likely the result of investor pressure, despite hesitancy in scientific community about the commercial viability of these products (Holmes et al., 2022).

During the time between the contemporary inception of ACBM in 2013 and 2022 (time of this writing) a total over 2 billion dollars has been invested in ACBM companies (Turi, 2021). This stakeholder excitement can be linked to bullish analyst reports on meat alternatives with some reports stating that 60-70% of ground beef would be displaced by meat alternatives by 2030-2040 (Suhlmann et al., 2019; Tubb & Seba, 2019). These reports were released near the start of this research project in 2019 and these predictions seem to have been tempered with more modest recent reports predicting the replacement of a half of a percent of conventional meat with ACBM by 2030 (Brennan et al., 2021). The United States produced 12.6 billion kg of beef in 2021 and even these more conservative predicted displacements would have a substantial economic and environmental impact on the food system (Maples, 2021). Given the level of investment and the potential environmental impacts of ACBM, the authors of the following studies within this dissertation determined that an in-depth, critical assessment of the

potential sustainability of near-term ACBM production was necessary. The following studies were conducted in an effort to provide this sustainability assessment.

Chapter 2, "A preliminary techno-economic assessment of animal cell-based meat" provides the basis for which all other chapters are derived. At the time of this study (2019-2020), no publicly available technoeconomic assessment (TEA) of ACBM existed. This study examines the capital and operating expenses of a potential industrially scaled ACBM production system (121,000,000 kg/year) utilizing scientific and engineering conventions. It is a limited study due to the degree of uncertainty that existed and still exists with potential ACBM production. Despite being limited in scope, several key takeaways were gleaned from the study which linked the individual cell metabolic needs with the required growth medium volume. This TEA also used the composition of Essential 8™ growth medium (E8) to inform the pricing estimates of the estimated growth medium requirements. These early findings informed later life cycle assessments of Essential 8™ growth medium (E8) and ACBM.

Chapter 3, "Technoeconomic assessments of cellular agriculture" examines and compares the three TEAs of ACBM available at the time of its commission. During the time that the Risner et al. TEA (referred to as UC Davis TEA in Chapter 3) was being conducted, another TEA had been commissioned by the philanthropic group, Open Philanthropy and was conducted by Dr. David Humbird, a bioprocessing consultant (Humbird, 2020, 2021). The Humbird TEA was a more complete TEA with full inclusion of capital expenditures and utilization of chemical engineering conventions for the scaling of growth medium component production (Humbird, 2020, 2021). Despite some differences in assumptions, the overall conclusions of the Risner, et al. and Humbird TEA were similar. Seemingly in response to the Risner, et al. and Humbird TEAs, a third TEA was commissioned by the Good Food Institute and conducted by CE Delft, a consultancy agency for sustainable development (Odegard et al., 2021). Chapter 3 normalizes the results of each TEA and provides critical examination of the assumptions utilized by

each TEA allowing the reader to draw their own informed conclusions on the economic viability of ACBM.

Chapter 4, "A techno-economic model of continuous mycoprotein production: A journey to price parity with beef protein" was a study with two goals. The first goal was to test the validity of the TEA method utilized in Chapter 2 by applying the method to an existing product whose base ingredient is grown in an industrial scale bioreactor. The base ingredient, mycoprotein is a fungal meat alternative produced utilizing the fungal strain *Fusarium venenatum* A3/5/3 and is cultivated utilizing industrial scale airlift bioreactors (~155m^3) (Moore et al., 2021). The TEA method applied in Chapter 2 was utilized to model continuous mycoprotein production to allow for a better understanding of the economic viability of the product for interested stakeholders (Maroulis & Saravacos, 2007). The results of the modeled mycoprotein production system was then compared with the real world economics of commercially available mycoprotein products to test model validity (Moore et al., 2021). The second goal was understanding how an existing meat alternative could be utilized to replace a commodity protein source like beef. These lessons could then be extrapolated to understand the potential of ACBM production if key technical hurdles such as, a limited specific growth rate are overcome.

Chapter 5, "Cradle to production gate life cycle assessment of cultured meat growth media: A comparison of Essential 8 and Beefy-9" was a study deemed critical based upon the results of Chapter 2. Results from Chapter 2 indicated that when individual cell metabolism is considered, the volume of growth or the mass of growth medium components (if the bioreactor is operating utilizing a fed-batch method) is substantial. To understand the potential environmental impact of ACBM, it would be critical to understand the environmental impact of the growth medium utilized to produce ACBM products. E8 is a defined growth medium which was designed for stem cell culture and has been promoted as an economically viable growth medium for mass ACBM production with minor formulation adjustments (Chen et al., 2011; Kolkmann et al., 2020; L. Specht, 2019; Verbruggen et al., 2018). E8 production was examined from a cradle-to-production gate view to understand the embedded resources within E8's life

cycle, which in turn can be utilized to help determine the environmental impact of ACBM or other animal cell products produced utilizing the E8 growth medium or other similar growth media.

Chapter 6, “Environmental examination of near-term animal cell-based meat: Is kill-free meat sustainable?” is a study that combines the results and methods of chapter 2 and 5 as well as the Humbird TEA which was critically examined in chapter 3 to assess the environmental impact of near-term ACBM production. The existing life cycle assessments were reviewed and evaluated (Mattick et al., 2015; Tuomisto et al., 2014; Tuomisto & Teixeira de Mattos, 2011). These previous environmental assessments were performed before the formation of the first ACBM companies (Mattick et al., 2015; Tuomisto et al., 2014; Tuomisto & Teixeira de Mattos, 2011). As companies begin to develop proof-of-concepts and protypes of ACBM products it has become clear that an updated environmental assessment is necessary to reflect the ground truth of how these early ACBM products are produced. The necessity of an updated, critical environmental assessment becomes clearly evident as governments and private entities provide capital to ACBM companies with stated environmental objectives (Zimberoff, 2022). In short, chapter 6 is a cumulation of the research conducted for this dissertation and provides an environmental assessment based upon that research utilizing the framework of life cycle assessment as defined by the International Organization of Standardization (ISO) standards 14040 and 14044 (International Organization for Standardization, 2006b, 2006a).

These chapters should provide the readers with an informative examination of near-term ACBM production and identify economic and environmental challenges related to near-term ACBM production. The chapters can be read independently given that they are intended for publication or are published in peer-reviewed literature sources.

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Chapter 2. Preliminary techno-economic assessment of animal cell-based meat

Journal article formatted for and published in: Foods (2020)

Article

Preliminary techno-economic assessment of animal cell-based meat

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Received: date; Accepted: date; Published: date

Abstract: Interest in animal cell-based meat (ACBM) or laboratory grown meat has been increasing, however the economic viability of these potential products has not been thoroughly vetted. Recent studies suggest monoclonal antibody production technology can be adapted for the industrialization of ACBM production. This study provides a scenario-based assessment of the projected cost per kilogram of ACBM produced in the United States based on cellular metabolic requirements and process/chemical engineering conventions. A sensitivity analysis of the model identified the nine most influential cost factors for ACBM production out of 67 initial parameters. The results indicate that technological performance will need to approach technical limits for ACBM to achieve profitably as a commodity. However, the model also suggests that low-volume high-value specialty products could be viable based on current technology.

Keywords: Cultured meat; Cell-based meat; Techno-economic assessment; Bioreactor; Process engineering; Bioengineering; Biomanufacturing

1. Introduction

Global population growth and economic development are expected to double the demand for meat products by 2050 [1]. Meanwhile, the United Nations Food and Agriculture Organization (FAO) estimates that beef and dairy cattle may be responsible for up to 5.0 gigatonnes of CO_2-equivalent emissions, or 9% of total greenhouse gas (GHG) emissions [2,3]. These reported emissions are considered generalizations and a nuanced examination of an individual production system must occur to quantify CO_2-equivalent emissions for each system [4]. Concerns over global warming, animal welfare, and human health have prompted interest in the development of "meat alternatives" which have the organoleptic qualities of meat, but whose origin is not from a slaughtered animal [5–8]. The environmental costs of ACBM production are still being determined and debated [9–14] , however significant economic interests in ACBM products has developed. Analyst reports are bullish on growth in the meat alternatives sector and have predicted a significant displacement of conventional ground beef, with some reports predicting a 60-70% decrease over the next 10-20 years [7,8]. The predicted shift to meat alternatives would represent a disruption of a highly valuable market. In 2018, the United States processed 12.1 million tonnes of beef, including 8.5 million tonnes of retail cuts valued at US$106 billion [15].

Plant- and fungal-based meat alternatives are already widely available, but producers and consumers are looking to animal cell-based meat (ACBM) as the next frontier for meat alternatives. While ACBM has yet to be scaled commercially, it is currently perceived as a core component of this "2nd domestication of plants and animals"[7]. In

fact, ACBM companies have received significant early stage investments in excess of US$230 million [16,17]. This level of economic investment suggests the need for a rigorous assessment of the pathway to profitability for the sector.

Proposed ACBM production systems suggest existing pharmaceutical technologies could be employed for mass ACBM production [6,18]. Industrial-scale bioreactors would be used to proliferate myoblasts or myosatellite cells which were harvested from animals [6,18]. These cells then undergo a differentiation and maturation process (i.e. myogenesis) supported by a scaffolding process to form the final ACBM meat product (Figure 1) [19–21]. ACBM first became a reality in 2013 with an initial public demonstration of a 140 g "hamburger" that cost over US$270,000 to produce [22]. The high cost of production remains a significant challenge for the ACBM industry. A number of technological hurdles to lower production cost have been identified but not extensively quantified (e.g. cell senescence, high cost of growth factors, time and nutrients required for cell growth/differentiation/ maturation, and scalable scaffolding processes) [20,23,24]

Figure 1. Myogenesis sequence for ACBM production.

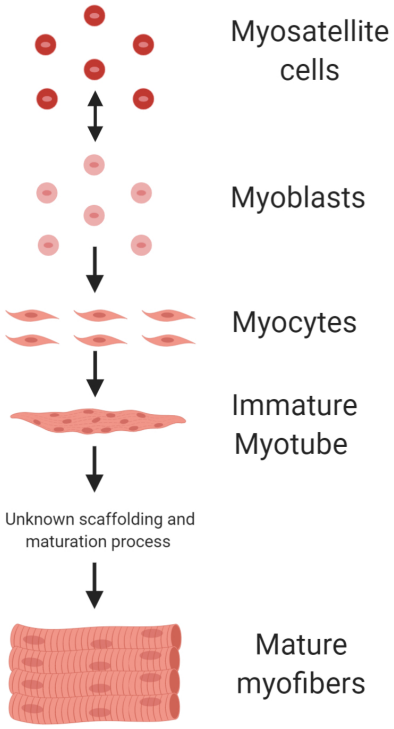
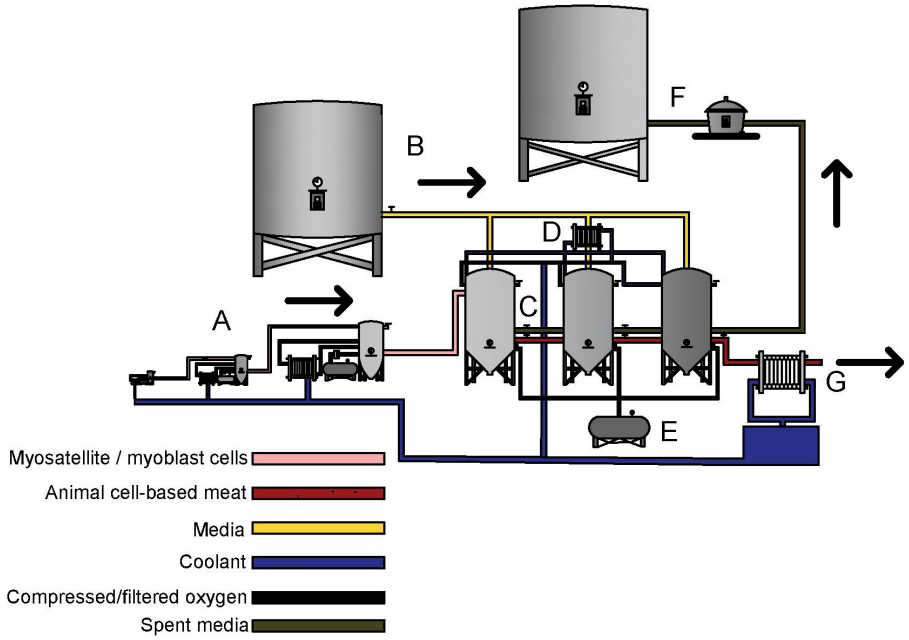


Figure produced using BioRender.

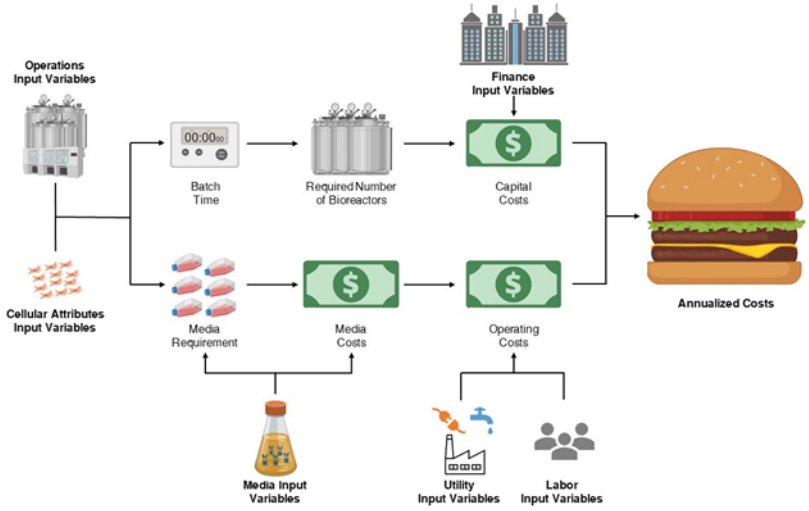
Figure 2 illustrates a potential ACBM production system similar to monoclonal antibody production for bovine myoblasts/MSC expansion [6,25]. We limit our analysis here to the core bioreactor system (section "C" in Figure 2) since industrial-scale scaffolding and maturation systems have not been defined in detail by ACBM producers. Thus, the model presented is a simplified and reduced model whose reported cost should be considered as minimum costs (Figure 3, Eq. 1-47, and Fig. A1-A2). Figure produced using BioRender.

Figure 2. Potential industrialized ACBM production system



This system represents a potential ACBM production process without pumping system shown. A. The bioreactor seed train system 20 L, 200 L and 2000 L B. Media storage system. C. Series of 20,000 L continuous stir bioreactor system with unknown scaffolding processing occurring in bioreactor system. D. Bioreactor temperature control system. E. Oxygen supply system. F. Spent media processing system. G. ACBM cooling system. Capital expenditures only account for C, and therefore a minimum estimate of capital costs. Figure produced using AutoCad.

Figure 3. ACBM simplified economic model flow diagram



ACBM simplified economic model flow diagram. The individual input variables have been grouped into categories (Operations, Cellular attributes, Finance, Media, Utility and Labor) and can be viewed individually in Tables A1a-A1f. Figure produced using BioRender.

Cultured bovine myoblasts using microcarriers (Cytodex® 1 or Synthemax® MC) behave similarly to human mesenchymal stem cells (HMSC) [26]. HMSC bioprocessing is highly complex given the heterogeneity of the HMSC cultures, sensitivity of HMSC to environmental changes, spontaneous differentiation, and the necessary disassociation

of cell aggregates for harvest [27,28]. Meanwhile, the high risk of batch contamination has led many therapeutic stem cell manufacturers to shift to single-use bioreactor systems [28]. However, this study makes the optimistic assumption that advances in MSC/myoblast science will enable the production of MSC/myoblast using large, non-disposable, and semi-continuous bioreactor systems, and that operational issues related to bioreactor sanitation and fill rates are negligible.

2. Materials and Methods

To determine the economic viability of animal cell-based meat (ACBM), we developed a model using standard process and chemical engineering methods. The model system is a semi-continuous-batch production system operating at capacity year around and does not account for fill times, sanitation between batches or any operational downtime. Appendix A identifies some of our model's limitations and a sensitivity analysis was also conducted to further understand the influence of each model variable (Appendix B). All equations and variables are available in the equation and variable lists (Appendix C) as well as in the python code associated with our model. Table A3 (Appendix D) provides a list of equipment that would likely be necessary for industrial ACBM production. The costs were broadly broken down into annual operating costs and capital expenditures then annualized.

2.1 Capital Expenditures of an ACBM plant

We accounted for the volume each myoblast/myosatellite cell (MSC) occupies with the operating constraint that the total cell volume cannot exceed bioreactor operating capacity for each batch. Cell volumes are variable, so a reported volume estimate of 5×10^{-15} m^3 $cell^{-1}$ was used [18]. Eukaryotic muscle cell density is approximately 1060 kg m^{-3} and was used to estimate mass of ACBM per batch [29]. The actual density of ACBM may be lower due to incorporation of bovine adipose cells or other sources of fat. A decrease or increase in batch time influences economic viability of ACBM production. The batch time is the sum of the cell growth phase and maturation time (equation 1). The cell concentration is considered a variable that can change with technological innovation. Using a given cell concentration, the mass of each batch of ACBM was determined using equations 2-4. The batch time was then used to calculate the annual ACBM batches per bioreactor and the number of bioreactors required to achieve the desired annual ACBM production mass (equations 5 and 6).

Cost estimates of food-grade bioreactors were calculate using a method which accounts for equipment scaling, installation, and inflation (equations 7 and 8) [30]. This method applies a set unit cost of \$50,000 m^{-3} for a food grade bioreactor and a common scaling factor of 0.6 [30]. To account for inflation and changes in cost over time the Chemical Engineering Plant Cost Index (CEPCI) values for heat exchangers and tanks were used to determine an adjusted value factor [31,32]. Adjusted value factor of 1.29 was determined dividing the recent CEPCI values with the values from when the set unit cost was referenced. The Lang factor is used to estimate cost associated with installation and piping. This factor can range from 1.35-2.75 for traditional food production operations and to up to 4.80 for fluids processing [33]. A Lang factor was estimated to be 2 for all scenarios. For new plant cost the Lang factor value should be increased by 1 [33]. This estimated the minimum capital expenditures for the required number of bioreactors which are necessary to meet the desired ACBM production mass. This method doesn't account for any other equipment which would likely be necessary for ACBM production (Table A3) besides the primary bioreactor systems.

2.2 Operating costs of an ACBM plant

The potential manufacturing cost of an ACBM plant can be broken into three categories: fixed manufacturing costs, variable capital costs and indirect (overhead) costs. All fixed manufacturing costs were estimated as a percentage of the fixed equipment costs except loan and equity interest (equation 9) [33]. These costs include equipment maintenance, insurance, taxes and royalties costs [33]. Indirect costs which are cost not related to amount of product processed, such as sales expenses and local taxes and are not accounted for in our model since these costs are outside of plant operation expenses and will vary company to company. Our model provides an estimate of several variable capital costs related to downstream ACBM production. Costs associated with general meat production such as

packaging material and facility lighting are not included. The variable costs estimated in our model include ingredients, raw materials, utilities and labor costs. Equation 10 accounts for all the operating costs associated with the model we have provided.

2.2.1. Ingredients and raw materials

A key material for animal serum-free ACBM production is the specialized media required for myoblasts/MSCs growth. We assume bovine myoblast/MSCs have been harvested from cattle and preserved in a manner which will allow for propagation in animal serum-free media. Our model examines the use of Essential 8, an animal free growth medium which contains over 50 ingredients including ascorbic acid 2-phosphate, sodium bicarbonate, sodium selenite, insulin, transferrin, fibroblast growth factor-2 (FGF-2), and transforming growth factor beta (TGF-b§) [18]. A report from the Good Food Institute provides an excellent breakdown of the individual components of Essential 8 media and the 2019 pricing of each media component [18]. Cell glucose consumption rates can vary based upon several factors including glucose concentration present in the growth medium and the metabolic pathways being utilized by the cell [34,35]. Glucose consumption rates have been reported to be between 2 to 20 nmol[1] million $cell^{-1}$ min^{-1} in human stem cells [35]. While there can be many limiting factors in a complex medium system; glucose consumption and the total number of cells in the bioreactor were used to estimate the media requirements and expense per batch. The starting glucose concentration is reported to be $1.78x10^{-2}$ mol L^{-1} [18]. Only media used in the main bioreactor was accounted for. An oxygen supply is also critical for aerobic cell culture and is also considered an operating expense for ACBM production. Equation 11 was used to determine total amount of myoblasts/MSCs in the bioreactor at a given time. During the growth phase, the glucose consumption rate changes as time changes and this was accounted for using equation 12. The total glucose required for the growth phase was determined by equation 13. The total glucose required per batch was determined by adding the total glucose used in the maturation and growth phase (equations 14 and 15).

The media requirement was then determined by examining the total amount of glucose in the Essential 8 media. To understand the volume requirement per batch, a charge was deemed the equivalent to the working volume of the bioreactor. This assumption was done to account for any innovations related to vascularization and does not account for the volume of the cells. The total media volume required per batch/year and total annual media costs were determined by equations 16-19.

An oxygen supply is critical for aerobic cell cultures and is also considered an operating expense for ACBM production. The oxygen levels in the bioreactor were assumed to be kept in a steady state concentration of 2% for optimal cell growth [27,36]. This is expressed by equation 20 [37]. The initial oxygen needed for the bioreactor system was determined by equation 21. The annual oxygen requirement was determined in the same manner as the media requirement and is calculated using equations 11 and 22-27.

2.2.2. Utility related expenses

Our model accounts for some bioreactor operating expenses. These should be viewed as theoretical minimum estimates based upon conventional thermodynamic equations. The energy requirements for heating the media, cooling the bioreactors and cooling of the ACBM mass leaving the bioreactor systems were estimated. The water/media was assumed to enter the facility at approximately 20 ˚C. The media is also assumed to have an isochoric specific heat of approximately water. The density of the media was assumed to be 1 kg L^{-1} and would be heated to 37 ˚C. The minimum energy required to heat the media was calculated using equation 28. The metabolic consumption of glucose and oxygen produces heat which must be removed from the system. Approximately 470 kJ of heat is released per mol of O_2 consumed during glucose combustion (equation 29) and this value was used to approximate cellular heat generation [37]. The minimum energy required to be removed from the system to ensure cell health was calculated using equation 30. The ACBM mass leaving the bioreactor must be cooled from 37 ˚C to 4 ˚C to ensure food safety standards are maintained [38]. The specific heat of ACBM is assumed to be the same as beef which is 2.24 kJ kg^{-1} $˚C^{-1}$ [39]. An estimation of energy used during the cooling process (equation 31) was made based on the efficiency of the heat exchanger system.

Energy costs can be variable depending upon the location, time of day and amount used. A yearly national grid average for industrial electricity and natural gas prices was obtained from the United States Energy Information

Administration (EIA) from 1999-2019 [40]. One thousand cubic feet of natural gas contains approximately 303.6 kWh of potential energy and the cost per kWh was determined using this value [41]. The average costs were normalized to January 2019 prices using the CPI inflation calculator (Table A4 and A5) [42]. To estimate the energy/electricity cost a comparison of the industrial price of natural gas and electricity was made from 1999-2019 (Figure A1). Equation 32 was derived from a linear relationship of the cost of electricity and natural gas (Figure A2). Equation 32 was then used to estimate energy/electricity costs from a public supplier. Natural gas was chosen since it is the most used source of energy for electricity production in the United States in 2019 [43]. The costs of energy/electricity produced via an onsite boiler-turbine system was estimated by equation 33. A steam pressure of 42.5 bar is assumed because it is used as a reference pressure for cost of steam production and is adequate for steam turbine electricity production [44,45]. Solar generation of electricity was considered as well and was estimated to have a negligible operating cost for the facility. The equipment costs for solar are not accounted for since this is a facility dependent item. Equation 34 estimates the minimum cost of energy at an ACBM production facility.

Our model assumes media will be produced onsite given the scale of the operation. All water used for media production is considered process water, however it should be noted that deionized water could be required due to the operational sensitivity of myoblasts/MSCs. Compressed air is a common utility in food production facilities; however, it is not estimated in this analysis due to being a site-specific consideration. Cost of sterile filtration of the water for media production is not accounted for. The spent media is considered wastewater and must be treated to comply with environmental regulations [46]. The wastewater is assumed to be treated by a filtration and biological oxidation step. Cost estimates have been made for process water and wastewater treatment and these estimates have been adjusted to January 2019 values to account for inflation (Table A6) [42,44]. It should be noted that this does not account for water used for sanitation or for losses during the production process. Equation 35 is used to estimate the annual process and wastewater costs.

2.2.3. Labor related expenses

Our scenarios assume that the ACBM production facility is operating 24 hours/day and year around. It is assumed the facility is fully staffed and no overtime is required. Each shift is assumed to be an 8-hour shift. The facility is also assumed to be in the United States in an area of standard income. The required production operators (required manpower) for the ACBM production facility per shift is estimated by amount and type of processing equipment in the facility (Table A3) [30,44]. This processing equipment could include centrifugal pumps, plate filters, media holding vessels, heat exchangers, bioreactor seed train, positive displacement pumps and bioreactors. In the four scenarios, this equipment was deemed site specific and only the main bioreactors were accounted for. The labor cost were determined using the mean hourly rate, \$13.68 (USD h^{-1}) for a meat packer [47]. The manpower requirement was one laborer per full-scale bioreactor and then the labor costs were estimated using a factorial method with a labor cost correction factor (equations 36-38) [44].

2.2.4. Finance related expenses

Our model accounts for the expenses related to equity recovery and debt using a standard finance calculation (equations 39-46) [48]. For all scenarios, the input variables were kept constant. Equations 39-46 convert the capital expenditures to an annual cost which is used to calculate the total annual minimum costs in conjunction with the annual operating costs (equation 47).

2.2.5. Sensitivity analysis

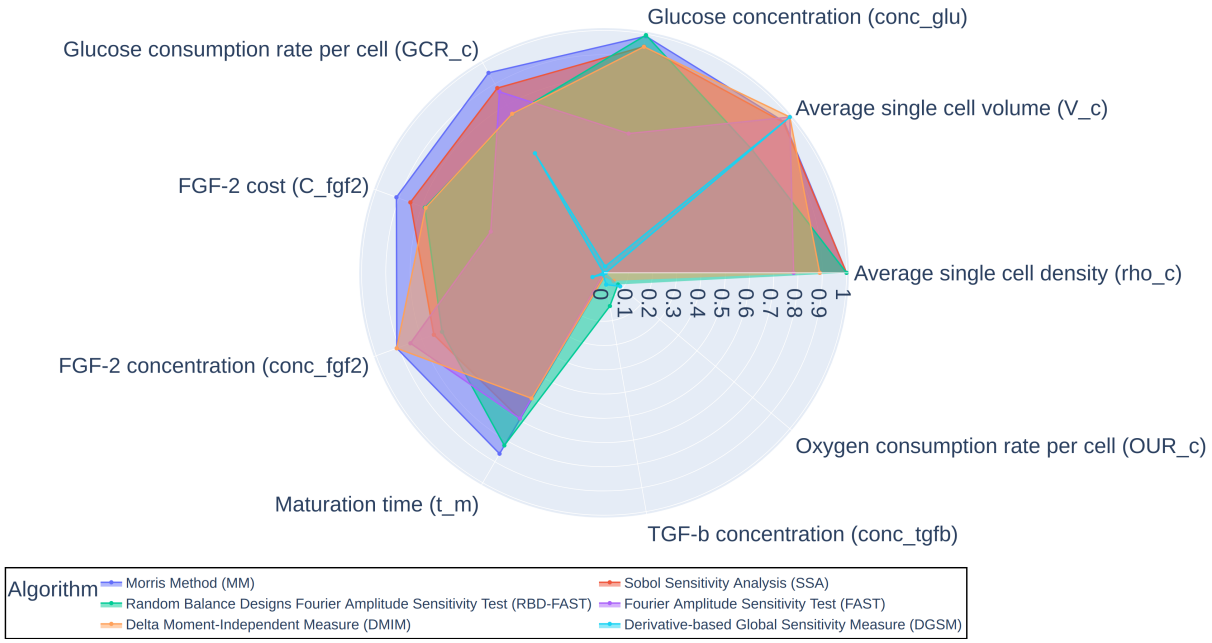
We performed a sensitivity analysis of the ACBM price model using 6 algorithms that use different approach to variance and rate of change to assess sensitivity: the Derivative-based Global Sensitivity Measure (DGSM), Delta Moment-Independent Measure (DMIM), Morris Method (MM), Sobol Sensitivity Analysis (SSA), Fourier Amplitude Sensitivity Test (FAST), and the Random Balance Designs Fourier Amplitude Sensitivity Test (RBD-FAST). We used the SALib Python package for this work [49]. Additional information regarding sensitivity analysis algorithms can be found in the Appendix B.

3. Results

Using cellular biology and chemical/process engineering conventions, we identified sixty-seven key variables that influence capital or/and annual operating costs (Tables A1a-A1f). The capital cost of a single 20 m^3 food-grade bioreactor was estimated to be US$778,000 [30]. We limit bioreactor size to 20 m^3 given the sensitivity of animal cells to elevated hydrostatic pressures as compared to fungal/bacterial cells which can be viable in >500 m^3 scale bioreactors [50]. The annual operating expenses include fixed manufacturing costs, media, oxygen, energy, process water, and wastewater treatment costs.

To understand the impact of each model variable on the estimated capital and annual operating expenses, we performed a robust sensitivity analysis (Figure 4 and Table A2). We applied six global sensitivity analysis algorithms (Derivative-based Global Sensitivity Measure, Delta Moment-Independent Measure, Morris Method, Sobol Sensitivity Analysis, Fourier Amplitude Sensitivity Analysis, Random Balance Designs-Fourier Amplitude Sensitivity Test) to identify the top nine factors that most influenced capital and annual operating expenses by consolidating the top 5 parameters across all six algorithms. These nine factors were then clustered into technological components (including maturation time, fibroblast growth factor 2 (FGF-2) concentration and costs, glucose concentration, glucose consumption rates, oxygen consumption rate and transforming growth factor beta (TGF-β)) and cell-based components (e.g. average cell volume and density).

Figure 4. ACBM sensitivity analysis of key model variables.



ACBM sensitivity analysis of key model variables. Each algorithm independently examined 67 parameters for sensitivity. The 5 parameters exhibiting the most sensitivity were selected from each algorithm. This resulted in 9 unique parameters visualized in the figure. The sensitivity measurements of the algorithms were scaled from 0 to 1 using minimum-maximum normalization except DGSM. The measurement of DGSM was first scaled by taking its

sixteenth root and then normalized from 0 to 1 by minimum-maximum. Abbreviations for each of the 9 unique parameters are provided for reference to the input variables in Data S1. Figured produced using Python.

The results from the sensitivity analysis then informed the specification of four technology development scenarios (Tables 1 and A1a- A1f). Scenario 1 represents a baseline scenario a based on existing ACBM production, including 2019 cost estimates for animal serum-free media and growth factors [18]. Scenario 4 was designed as a bookend scenario, where nearly all technical challenges are resolved, including reduced growth factor costs, increased MSC/myoblast tolerance to glucose concentrations, decreased MSC/myoblast doubling and maturation time, and reduced basal media costs [6,18,20,23]. Scenario 2 represents a mid-point scenario between Scenarios 1 and 4, and Scenario 3 adapts Scenario 2 by eliminating FGF-2 growth factor costs. To incorporate economic scalability, we also examined the capital and annual operating expenditures (Table 2) to produce enough ACBM to replace 1% of the United States beef market (121,000,000 kg) [15].

Table 1. Model scenario settings

Scenario	Achievable cell concentration (cells/ml)	FGF-2 conc. (g/L)	FGF-2 cost (USD/g)	Glucose conc. in basal media (mol/L)	Glucose consumption rate per cell (mol/ h cell)	Hours per doubling (h)	Maturation time (h)
1	$1.00x10^{7}$	$1.00x10^{-4}$	$2.05x10^{6}$	$1.78x10^{-2}$	$4.13x10^{-13}$	24.0	240
2	$9.5x10^{7}$	$5.00\ x10^{-5}$	$1.00x10^{6}$	$2.67x10^{-2}$	$2.07x10^{-13}$	16	156
3	$9.5x10^{7}$	$5.00\ x10^{-5}$	0	$2.67x10^{-2}$	$2.07x10^{-13}$	16	156
4	$2.00x10^{8}$	0	0	$3.56x10^{-2}$	$4.13x10^{-14}$	8	24

Table 2. Annualized expenditures with quantified drivers of capital and operating expenditures

Scenario	Total required bioreactors	Volume of media needed for annual production (L)	Minimum price of ACBM to meet annual capital and operating expenses (USD/kg)
1	**5205**	**$1.40x10^{11}$**	**$4.37x10^{5}$**
2	**360**	**$3.06x10^{10}$**	**$5.72x10^{4}$**
3	**360**	**$3.06x10^{10}$**	**$4.46x10^{4}$**
4	**50**	**$8.56x10^{8}$**	**1.95**

Scenario Description: Scenario 1 represents a baseline scenario which utilizes a 2019 baseline cost estimate of Essential 8 media from a Good Food Institute report [12]. Scenario 4 is a scenario where nearly all technical challenges are resolved. Scenario 2 represents a mid-point scenario between Scenarios 1 and 4, and Scenario 3 is identical to Scenario 2 except FGF-2 growth factor costs are eliminated.

The results of our calculations indicate that ACBM production will only approach economic viability as a commodity when the significant technical challenges are overcome as outlined in Scenario 4 (Table 1 and 2). In Scenario 1, the cost per kilogram remains exceedingly expensive at approximately US$400,000. Scenarios 2 and 3 illustrates the significant impact of reducing the cost of FGF-2, which reduces the operating cost of ACBM by an order of magnitude from Scenario 1. Only in Scenario 4 does ACBM approach commodity level prices at approximately US$2 per kg.

The cost of the bioreactor was the main driver of capital costs in the model. To displace the demand for beef in the U.S. by 1%, the scenarios ranged from requiring the deployment of 5205 to 50 bioreactors (20 m^3) at a total capital cost of 4 billion to 37 million U.S. dollars. The capital expenditures in scenario 3 remain the same as scenario 2 since eliminating the growth factor cost has no impact on the capital expenditures. Finally, it is important to reiterate that these costs are based on estimates for standard food-grade bioreactors and that more sophisticated bioreactors (i.e. single-use or novel perfusion systems) may substantially increase capital costs.

While capital expenditures are significant, the operating expenses (largely based upon cellular metabolism and media consumption) represent a substantial hurdle for the large-scale production of ACBM. Achieving the outcomes presented in Scenario 4 would require significant technological advancements on multiple fronts as specified by the model, where media costs are reduced from 376.80 US$/L to 0.24 US$/L, glucose/media consumption is reduced by an order of magnitude, and cell growth and maturation times are heavily decreased from 24h to 8h and 240h to 24h, respectively.

4. Discussion

The results of the scenario analysis clearly highlight and quantify the technological and economic challenges for ACBM to reach commercial viability. We suggest the following three areas of focus to reach techno-economic feasibility, which we will discuss further: cell selection or engineering to lower the media consumption rate, reducing or eliminating the cost of growth factors, and scaling up of perfusion bioreactors.

The analysis identified cell metabolism as a key limiting factor for the economic viability of ACBM, so understanding and potentially manipulating cellular metabolism represents a key area of innovation for driving down operating costs. The glucose consumption rate of cultured cells establishes the media requirements in our model, which is by far the largest operating expense for ACBM production. Scenario 1 was based upon reported human embryonic stem cells' glucose uptake rate. These cells were likely exhibiting a Warburg metabolism (aerobic glycolysis) based upon their lactate production rates [51,52]. This metabolic mode is common during cell proliferation; however, it is energetically less efficient than oxidative phosphorylation (i.e. production of 2 ATP vs. a theoretical 38 ATP per glucose molecule) [52]. Engineering and/or screening for cell lines which shift rapidly from a Warburg metabolism to a more glucose-efficient metabolism represents an opportunity to reduce the media consumption rate in line with Scenario 4.

In healthy cells, glucose uptake is stimulated by growth factors such as insulin, FGF, or/and TGF[51,53]. Our model highlights that growth factors are a major contributor to ACBM production expenses, with FGF-2 being particularly impactful. Thus, eliminating the need for FGF-2 would significantly reduce costs. One potential pathway for this solution would be to leverage the ability of cancer cells to increase glucose uptake rates and exhibit cell proliferation without the presence of growth factors [51]. Thus, cell lines could be engineered or identified to express oncogenes related to these traits. However, utilizing cultured cells that behave similar to cancer cells would likely be very challenging from both a regulatory perspective as well as for consumer acceptance. It should also be noted that is Essential 8 media is not generally recognized as safe (GRAS) for human consumption and ensuring cell culture media is composed of ingredients which are GRAS will be an additional regulatory/technical challenge.

Our model indicates that cellular metabolic requirements will require multiple changes of media per batch and higher cell concentrations [54,55]. The use of perfusion bioreactors could deliver these capabilities for ACBM production [56]. Concentrations of 2.0 x10^8 cells/ml have been reported for Chinese hamster ovary (CHO) cells in a lab-scale, disposable perfusion bioreactor system [56]. However, this is a profoundly different technology than the large-scale, continuously stirred bioreactors we assume in our model. To the authors' knowledge, a perfusion bioreactor system with a 20 m^3 working capacity is not currently in existence for myoblasts/MSC propagation.

ACBM has been presented as a potentially disruptive technology that can transform the global meat sector. However, our techno-economic analysis of this alternative meat production pathway suggests that the profitable mass production of products composed entirely of ACBM remains a significant challenge. Our model indicates that several technical challenges must be overcome before industrial scale-up is likely to be profitable. Media consumption rates

must be measured and optimized at the cellular level and the costs of growth factors must be significantly reduced or eliminated altogether.

While these factors indicate that ACBM may not be economically viable as a commodity for some time, it does not preclude the potential to enter the market place sooner as a minor ingredient which lends desirable organoleptic qualities to an otherwise plant-based product. Alternatively, there may be opportunity for viable competition in the specialty foods markets, where ACBM costs compare more favorably to such items as almas beluga caviar (US$10,000/kg), Atlantic bluefin tuna (US$6,500/kg), and foie gras (US$1,232/kg) [57].

5. Conclusions

Our model has highlighted some of the significant economic challenges which impede the techno-economic viability of ACBM, but it is not comprehensive. Given the uncertainty of ACBM production, our model and scenario analysis should be considered a starting point for those interested in the scalability of ACBM. Our scenario analysis is based upon the production of ACBM in the United States which influences factors such as energy and labor costs. The energy and labor costs were minor contributors to our limited model's operating expenses; however, these costs will likely increase on a fully scaled system.To enable further, and customizable, exploration of how advances in technology might inform ACBM production costs, we have developed an open-source, web-based version of our model that is publicly available at https://acbmcostcalculator.ucdavis.edu .

Supplementary Materials: The following are available online:

Data S1: Techno-economic analysis and sensitivity analysis python code for ACBM https://github.com/IBPA/IBPA-Collection-of-Reproducible-Code-and-Results/tree/master/2020_Artificial_Meat

Data S2: Techno-economic analysis web-based program for ACBM https://acbmcostcalculator.ucdavis.edu

Author Contributions: Conceptualization, Derrick Risner, Justin Siegel and Edward Spang; Data curation, Derrick Risner; Formal analysis, Derrick Risner and Fangzhou Li; Funding acquisition, Edward Spang; Investigation, Derrick Risner, Fangzhou Li and Edward Spang; Methodology, Derrick Risner, Fangzhou Li, Jason Fell, Sara Pace and Ilias Tagkopoulos; Project administration, Edward Spang; Resources, Justin Siegel and Ilias Tagkopoulos; Software, Fangzhou Li and Jason Fell; Supervision, Justin Siegel, Ilias Tagkopoulos and Edward Spang; Validation, Derrick Risner and Sara Pace; Visualization, Derrick Risner, Fangzhou Li, Jason Fell, Justin Siegel, Ilias Tagkopoulos and Edward Spang; Writing – original draft, Derrick Risner, Fangzhou Li and Jason Fell; Writing – review & editing, Derrick Risner, Sara Pace, Justin Siegel and Edward Spang.

All authors have read and agreed to the published version of the manuscript.

Funding: This was funded by the Innovation Institute for Food and Health at UC Davis. J.S. was supported by the University of California Davis, Innovation Institute for Food and Health at UC Davis, the National Institutes of Health (R01 GM 076324-11), the National Science Foundation (award nos. 1827246, 1805510, and 1627539), and the National Institute of Environmental Health Sciences of the National Institutes of Health (award no. P42ES004699). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, National Institute of Environmental Health Sciences, National Science Foundation, or UC Davis.

Acknowledgments: The team would be like to thank Andrew Holguin for his technical expertise in the creation of the online calculator.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Model Limitations

In human pluripotent stem cells, as the cells exit pluripotency and enter the initial differentiation phase a metabolic shift to mitochondrial OXP occurs [58,59]. A similar shift occurs as myoblasts fuse differentiate into myotubes [60]. As myoblasts differentiate into myotubes it has been reported that the metabolic rate is maintained despite a greater reliance on OXP pathway for ATP production[60,61]. However, it is not known if this metabolic rate will be maintained during the undefined scaffolding and maturation process. During this undefined scaffolding and maturation process, the myotubes diameter could potentially increase 20-fold [20,21,62]. Our model assumes glucose and oxygen uptake rate are maintained during this process; however, these values could change to meet the metabolic needs of the maturing myotubes. Once the myotubes mature, they rely upon OXP to meet their metabolic needs and this shift may require an adjustment to operation factors such as an increased or decreased media or oxygen supply.

Our model did not account for amino acid uptake rates due to glucose being the most consumed nutrient in cell culture, however amino acid (AA) metabolism should be a consideration for commercial scale up. An example of the importance of this consideration is that stem cell amino acid metabolism can vary species to species [63,64]. Bovine and mouse embryonic stem cells are sensitive to extrinsic deprivation of threonine, whereas human embryonic stem cells are not sensitive extrinsic deprivation of threonine, but require increased levels of methionine [64–66]. This extrinsic threonine requirement does not apply to other mouse or bovine cells which are proliferating[63]. This illustrates how these requirements can vary by species and by cell type.

Glutamine is utilized as both a nitrogen donor and energy substrate in proliferating myosatellite/myoblast cells [67,68]. Glutamine is the second most consumed nutrient in animal cell cultures and contributes to nucleic acid, protein and lipid production [69]. Glutamine concentration has been show to influence the myoblasts proliferation rate with 300 μM being reported as the optimal conditions for human myoblasts proliferation [68]. This indicates that amino acid levels in the media could potentially influence operating costs via increased or decreased doubling times. This would likely be cell line dependent and should again be a consideration for companies wishing to develop multiple products from different cell lines.

The volume of animal cells also plays an important factor in our modeling which accounts for the volume of each cell. Animal myoblasts cells volume are orders of magnitude larger than common prokaryotic or single cell fungi [70]. This places hard constraints on the number of cells a single bioreactor can produce per batch i.e. bioreactor with a working volume of 20 m^3 can only produce the number of cells whose total volume is 20m^3. This does not account for repulsive forces or for the media within bioreactor. While this was done to account for any innovations in vascularization it makes the model less conservative and should be a consideration for any company considering scale up. It also does not account for cellular volume increases during the unknown scaffolding and maturation phase. The diameter of the myotube can increase up to 20 times it's original size as contractile protein is formed [20,21,62]. This increase in size of the cells during maturation could make the bioreactor more efficient, however it was not included in our model due to the unspecified nature of the commercial process.

Figure 2 represents a potential upstream production system for ACBM, however the capital expenditures that were estimated by our model only estimate the cost of a series of 20,000 L continuous stirred bioreactors designated by letter A. We did not adjust the maximum bioreactor operating capacity of the bioreactors in any scenario due to fragility of animal cells which lack a cell wall and cannot withstand the hydrostatic pressures which yeast or prokaryotic organisms can [50]. Innovations in bioreactor design could potentially increase the maximum working capacity. An increase in bioreactor working capacity would potentially lower capital expenses and annual operating costs. However, this would initially increase the base cost ($50,000/$m^3$) of the bioreactor measured in our model. In a more detailed analysis as the metrics we have outlined are achieved, interest rate and learning curve equations could be applied to estimate capital and operating expenses in finer granularity. We also assume that the unknown

scaffolding and maturation process could be accomplished within the bioreactors. If a separate bioreactor or maturation vessel is needed this would also increase capital expenditures. We did not account for the other equipment since this will be a site-specific variable. The Lang factor is used to estimate actual cost of equipment by accounting for installation related expense. A Lang factor of 2 was chosen for all scenarios to represent a food/bioprocessing facility that could be easily configured to accommodate ACBM production. However, a Lang factor of 2 is considered to be low by general conventions for a brand new facility or novel technology; a Lang factor of 3 to 5 would be more appropriate [30]. We anticipated that once the ACBM is cooled it will be processed in a manner similar to other ground meat products. We also did not account for any additional ingredients being added to the product. Cellular propagation technology could potentially be applied for myoblasts/MSC propagation. Cytodex® 1 microcarriers have been employed for bovine myoblasts proliferation and achieved a cell concentration of approximately $9x10^6$ cells/ml [26]. Our model does not account for this technology or any additional propagation technology which may increase capital or operating expenses. It has also been reported that bovine muscle satellite cells have been cultured with hemoglobin and myoglobin[71]. Costs associated with additional ingredients or media supplementation have not been accounted for and could substantially increase the annual operating expenses.

Appendix B. Additional sensitivity analysis information

All sensitivity analysis calculations were conducted using the SALib Python package [49]. Regarding sampling techniques and parameters, Delta Moment-Independent Measure [72,73] and Random Balance Designs Fourier Amplitude Sensitivity Test [74–76] used 1000 samples generated using Latin hypercube sampling [77], where Random Balance Designs Fourier Amplitude Sensitivity Test used the inference number of 10. Sobol Sensitivity Analysis used 1000 samples generated using Saltelli sampling [78–80]. Morris Method was sampled with 1000 trajectories and 4 grid levels [81]. Fourier Amplitude Sensitivity Test used 1000 samples with the inference number of 4 [82]. Derivative-based Global Sensitivity Measure used 1000 samples with finite difference step size of 0.0001 [83]. The result of the sensitivity analysis is shown in Figure 4 and Table A2.

Appendix C. Variables and equations

Variables are listed in the order they appear in the equations.

t_b= time of batch (h)
t_{gf}= Time growth phase ends (h)
t_m= Time of maturation phase (h)
F_c= Final concentration of cells in bioreactor (cells L^{-1})
B_v= Bioreactor working volume (L)
N_c= Total number of cells in bioreactor (cells)
V_c= Volume of single cell (m^3 $cell^{-1}$)
V= Volume (m^3)
ρ_c= Density of muscle cell (kg m^3)
M_b= mass of ACBM produced per batch (kg $batch^{-1}$)
b_{BY}= Number of batches a single bioreactor can produce in year (batches $year^{-1}$)
M_{BY}= Mass of ACBM a bioreactor can produce in a year (kg $year^{-1}$)
M_{DY}= Desired annual mass of ABCM (kg)
B_T = Total number of bioreactors required to annual production goal
C_{eq}= Total equipment costs (USD)
C_F= Fixed equipment cost (USD)
f_{Aj}= Adjusted value factor for equipment j
C_{Uj} = Unit costs for equipment j
U_j= Base unit for equipment j
U_{aj}= Actual unit for equipment j
f_s= Scale factor for equipment j
f_L= Lang factor
f_{FM}= Fixed manufacturing cost factor
C_{FM}= Fixed manufacturing costs (USD)
C_{op}= Annual operating costs (USD)
C_{mY}= Total annual costs of media (USD)
C_{O_2Y}= Total annual costs of oxygen (USD)
E_{Hm} = Minimum energy required to heat media (kWh)
E_{BR} = Minimum energy required bioreactor heat removal (kWh)
E_{ACBMR}= Minimum annual energy required for ACBM heat removal (kWh)
C_L= Estimated annual labor costs (USD)
C_E= Cost of energy (cents kWh^{-1})
C_W= Annual process water and wastewater costs (USD)
c_t = Total number of cells at time (t)
c_o = Total number of cells present in inoculum (cells)
t_D Doubling time (h)

t = Time (h)
GCR_B = Glucose consumption rate within the bioreactor (mol h^{-1})
GCR_c= Glucose consumption rate per cell (mol h^{-1} $cell^{-1}$)
G_{Gg}=Total moles of glucose required for growth phase (mol)
G_{GM}= Total moles of glucose required for maturation phase (mol)
G_G= Total moles of glucose required per batch (mol)
m_{ch}= Total media charges per batch (charge)
M_{Gch}= Moles of glucose per charge (g)
V_b= Total volume of media required per batch (L)
V_{ch}= Volume of charge or bioreactor (L)
V_m = Total media volume per year (L $year^{-1}$)
b_y = Batches per year
C_{mL}=Cost of media per liter (USD L^{-1})
OUR_B = Oxygen uptake rate in bioreactor (mol s^{-1})
OTR_B = Oxygen transfer rate in bioreactor (mol s^{-1})
k = mass transfer coefficient (m s^{-1})
A = mean bubble specific interfacial surface area (m^2)
e_{con}= equilibrium concentration (mol m^{-3})
a_{con}= actual dissolved oxygen concentration (mol m^{-3})
O_2^i = Initial oxygen in required in the system (mol)
ρ_m = Density of media (kg L^{-1})
P_{O_2} = Percentage of oxygen (O_2) in media by weight (%)
O_2^{mol} = molar mass of O_2 (kg mol^{-1})
OUR_c= rate of oxygen consumption per cell mol $cell^{-1}$ h^{-1}
O_2^g = Total oxygen required for growth phase per batch (mol)
O_2^M=Total oxygen required for maturation phase per batch (mol)
O_2^b= Total oxygen used per ACBM batch (mol)
O_2 = Total amount of oxygen required per year (mol)
$C_{O_{2Y}}$ = Total annual costs of oxygen (USD)
C_{O_2} = Cost of oxygen (USD mol^{-1})
M_{mY}=Mass of media used per year (kg)
ΔT = Temperature difference (˚C)
W_{C_v}= Specific heat of water at constant volume (kWh kg^{-1} $˚C^{-1}$)
$\in_{Hm}$ = Energy efficiency of heating system (%)
O_2 = Oxygen required annually (mol)
h = Heat released per mol of oxygen consumed (kWh mol^{-1})
$\in_{BR}$ = Energy efficiency of bioreactor cooling system (%)
$ACBM_{C_v}$ = Specific heat of ACBM (kWh kg^{-1} $˚C^{-1}$)
$\in_{ACBMR}$ = Energy efficiency of ACBM cooling system (%)
C_{EP} = Cost of electricity from a public supplier (USD kWh^{-1})
C_{NG}= Cost of natural gas (USD 1000 ft^{-3})
C_{bT} = Cost of energy from onsite boiler-turbine system (USD kWh^{-1})
C_{NGP} = natural gas price (USD kWh^{-1})
ϵ_{bT} = boiler-turbine system efficiency (%)
f_{EP} = percentage of electricity produced by from a public supplier (%)
f_{bT} = percentage of energy produced by on site boiler-turbine system (%)
C_{PW} = Process water costs (USD m^{-3})
C_{WF} = Wastewater filtration costs (USD m^{-3})
C_{BO}= Biological oxidation of wastewater costs (USD m^{-3})

P = required manpower (production workers)
P_j = production worker required for single piece of equipment
j = Individual piece of equipment
N = All downstream equipment used in downstream ACBM production
f_{lab} = Labor cost correction factor
f_C = Country effect
f_{Sca} = Supervising and clerical assistance
f_T = Advanced technological and automating
f_Q = Skilled and qualified level of the personnel
f_B = Social benefits
f_O = Overtime work
C_{Lab} = Estimated annual labor costs (USD)
t_y = Annual operating time (h)
C_L = Production worker hourly rate (USD h^{-1})
EQ_r= Equity ratio
C_D= Total debt costs (USD)
D_r= debt ratio (%)
C_{TEQ}= Total equity costs (USD)
f_{CRD}= Capital recovery factor for debt
f_{CREQ}= Capital recovery factor for equity
D_p= Annual debt payment (USD)
EQ_p= Annual equity recovery (USD)
C_{cap}= Minimum annual cost of capital expenditures (USD)
C_{total} = Total minimum annual costs (USD)

Equation 1. Time of batch

$$t_b = t_{gf} + t_m$$

Equation 2. Total number of cells in a single bioreactor after maturation

$$N_c = F_c \, B_V$$

Equation 3. Total volume occupied by cells

$$V = N_c \, V_c$$

Equation 4. Cell mass in bioreactor per batch

$$M_b = V \, \rho_c$$

Equation 5. Annual ACBM production per bioreactor

$$M_{BY} = M_b \, b_{BY}$$

Equation 6. Bioreactors needed to match desired annual beef production

$$B_T = \frac{M_{DY}}{M_{BY}}$$

Equation 7. Equipment costs equation

$$C_{eq} = \sum_j f_{Aj}\, C_{Uj} \left(\frac{U_{aj}}{U_j}\right)^{f_s}$$

Equation 8. Fixed equipment costs

$$C_F = f_L\, C_{eq}$$

Equation 9. Fixed manufacturing costs

$$C_{FM} = f_{FM}\, C_F$$

Equation 10. Minimum annual operating costs

$$C_{op} = C_{FM} + C_{mY} + C_{O_2Y} + C_E\, E_{Hm} + C_E\, E_{BR} + C_E\, E_{ACBMR} + C_{Lab} + C_W$$

Equation 11. Cells in bioreactor during growth phase

$$c_t = 2^{\frac{t}{t_D}} c_o$$

Equation 12. Glucose consumption rate during growth phase

$$\frac{dGCR_B}{dt} = GCR_c \times c_t$$

Equation 13. Total glucose required for growth phase per ACBM batch

$$G_{Gg} = \int_{t=0}^{t=t_{gf}} GCR_B\, dt$$

Equation 14. Total glucose required for maturation phase per ACBM batch

$$G_{GM} = GCR_B \times t_m$$

Equation 15. Total glucose required per batch

$$M_G = G_{Gg} + G_{GM}$$

Equation 16. Total required media charges per batch

$$m_{ch} = G_G / G_{Gch}$$

Equation 17. Total media volume required per batch

$$V_b = m_{ch} V_{ch}$$

Equation 18. Total media volume per year

$$V_m = V_b b_y$$

Equation 19. Total annual costs of media

$$C_{mY} = V_m C_{mL}$$

Equation 20. Oxygen uptake rate

$$OUR_B = OTR_B = kA(e_{con} - a_{con})$$

Equation 21. Initial oxygen in the for the system

$$O_2^i = \frac{V_b \times \rho_m \times P_{O_2}}{O_2^{mol}}$$

Equation 22. Oxygen uptake rate changing with time

$$\frac{dOUR_B}{dt} = OUR_c \times c$$

Equation 23. Total oxygen required for growth phase per ACBM batch

$$O_2^g = \int_{t=0}^{t=t_{gf}} OUR_B \, dt$$

Equation 24. Total oxygen required for maturation phase per ACBM batch

$$O_2^M = OUR_B \times t_m$$

Equation 25. Total oxygen required per ACBM batch

$$O_2^b = O_2^i + O_2^g + O_2^M$$

Equation 26. Total amount of oxygen required per year

$$O_2 = O_2^b b_y$$

Equation 27. Total annual costs of oxygen

$$C_{O_{2Y}} = O_2 C_{O_2}$$

Equation 28. Estimation of energy to heat media to required temperature

$$E_{Hm} = \frac{M_{mY} \times \Delta T \times W_{C_v}}{\in_{Hm}}$$

Equation 29. Glucose combustion reaction

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6CO_2 + 6\ H_2O + \text{heat}$$

Equation 30. Estimation of energy usage for bioreactor cooling per ACBM batch

$$E_{BR} = \frac{O_2 \times h}{\in_{BR}}$$

Equation 31. Estimation of annual energy usage for cooling of ACBM

$$E_{ACBMR} = \frac{M_{DY} \times \Delta T \times ACBM_{C_v}}{\in_{ACBMR}}$$

Equation 32. Cost of energy per kWh from public supplier

$$C_{EP} = 0.0969C_{NG} + 6.78$$

Equation 33. Cost of self-generated electric/energy per kWh from a boiler-turbine system

$$C_{bT} = \frac{C_{NGP}}{\epsilon_{bT}}$$

Equation 34. Cost of energy per kWh

$$C_E = f_{EP}C_{EP} + f_{bT}C_{bT}$$

Equation 35. Annual process water and wastewater costs

$$C_W = V_m\, C_{PW} + V_m\, C_{WF} + V_m\, C_{BO}$$

Equation 36. Required manpower for operation

$$P = \sum_{j}^{N} P_j$$

Equation 37. Labor cost correction factor

$$f_{lab} = f_C f_{Sca} f_T f_Q f_B f_O$$

Equation 38. Estimated annual labor costs

$$C_{Lab} = t_y f_{lab} C_L P$$

Equation 39. Equity ratio

$$EQ_r = 100\% - D_r$$

Equation 40. Total debt costs

$$C_D = C_F D_r$$

Equation 41. Total equity costs

$$C_{TEQ} = EQ_r C_F$$

Equation 42. Capital recovery factor for debt

$$f_{CRD} = I_D(1 + I_D)^{Le} / ((1 + I_D)^{Le-1})$$

Equation 43. Capital recovery factor for equity

$$f_{CREQ} = I_{EQ}(1 + I_{EQ})^{Le} / ((1 + I_{EQ})^{Le-1})$$

Equation 44. Annual debt payment

$$D_p = f_{CRD} C_D$$

Equation 45. Annual equity recovery

$$EQ_p = f_{CREq} C_{TEq}$$

Equation 46. Minimum annual cost of capital expenditures

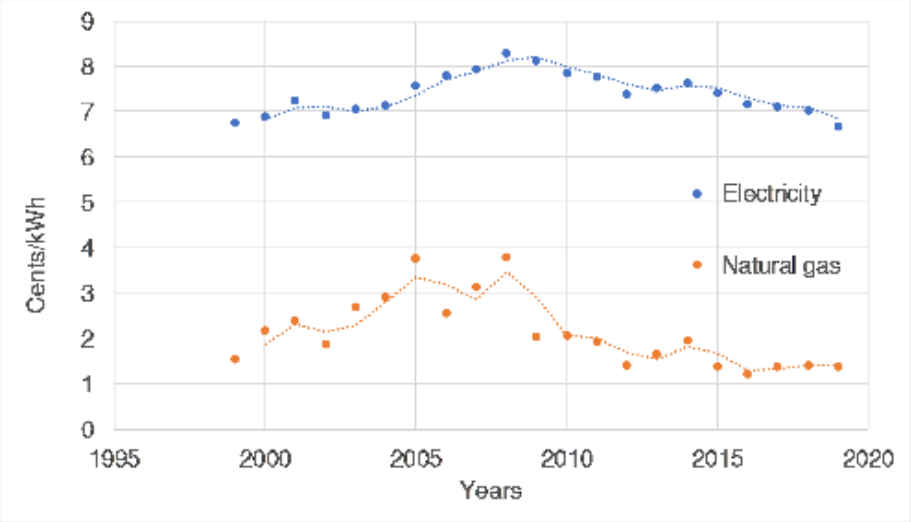
$$C_{cap} = D_p + Eq_p$$

Equation 47. Total minimum annual cost

$$C_{total} = C_{cap} + C_{op}$$

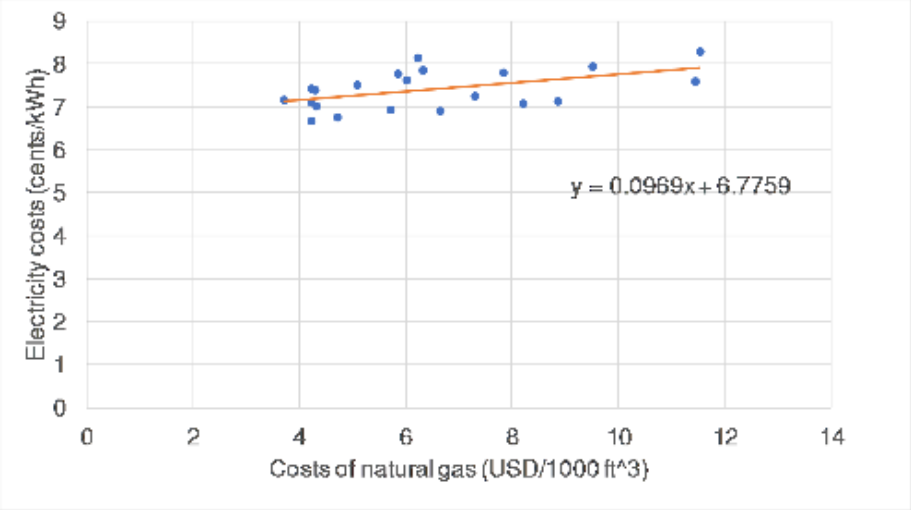
Appendix D. Additional tables and figures

Figure A1. Costs comparison of the average United States industrial electricity and natural gas (USD kWh^{-1}) 1999-2019



Costs comparison of the average United States industrial electricity and natural gas (USD kWh^{-1}) 1999-2019. Information was obtained from the United States EIA and average costs were normalized to January 2019 US currency[40,42].

Figure A2. Linear relationship between electricity and natural gas cost.



Linear relationship between electricity and natural gas cost. This relationship was used to determine equation 32. Information was obtained from the United States EIA and average costs were normalized to January 2019 US currency[40,42]. Figure produced using Microsoft Excel.

Table A1a. Model variable inputs: Operations

Scenarios	Inoculum concentration (cells/ml)	Inoculum bioreactor volume (L)	Seed bioreactor volume (L)	Seed bioreactor (cell/ml)	Bioreactor volume (m^3)	Desired and achievable cell concentration (cell/ml)	Desired mass of meat produced (kg)
1	$1.00x10^7$	2.00	$2.00x10^2$	$1.00x10^7$	$2.00x10^1$	$1.00x10^7$	$1.21x10^8$
2	$9.50x10^7$	2.00	$2.00x10^2$	$9.50x10^7$	$2.00x10^1$	$9.50x10^7$	$1.21x10^8$
3	$9.50x10^7$	2.00	$2.00x10^2$	$9.50x10^7$	$2.00x10^1$	$9.50x10^7$	$1.21x10^8$
4	$2.00x10^8$	2.00	$2.00x10^2$	$2.00x10^8$	$2.00x10^1$	$2.00x10^8$	$1.21x10^8$

Table A1a. Model variable inputs: Operations continued 1

Scenarios	Adjusted value factor for bioreactor	Lang factor	Maturation time (h)	Annual operating time (h)	Bioreactor scale factor	Fixed manufacturing costs factor	Bioreactor unit costs (USD/m^3)
1	1.29	2.00	240.00	8,760.00	0.60	0.15	$5.00x10^4$
2	1.29	2.00	156.00	8,760.00	0.60	0.15	$5.00x10^4$
3	1.29	2.00	156.00	8,760.00	0.60	0.15	$5.00x10^4$
4	1.29	2.00	24.00	8,760.00	0.60	0.15	$5.00x10^4$

Table A1b. Model variable inputs: Cell attributes

Scenarios	Average single cell volume (m^3/ cell)	Average single cell density (kg/m^3)	Hours per doubling (h)	Glucose consumption rate per cell (mol/h cell)	Rate of oxygen consumption per cell (mol/h cell)
1	$5.00x10^{-15}$	$1.06x10^3$	24	$4.13x10^{-13}$	1.80E-14
2	$5.00x10^{-15}$	$1.06x10^3$	16	$2.07x10^{-13}$	1.80E-14
3	$5.00x10^{-15}$	$1.06x10^3$	16	$2.07x10^{-13}$	1.80E-14
4	$5.00x10^{-15}$	$1.06x10^3$	8	$4.13x10^{-14}$	1.80E-14

Table A1c. Model variable inputs: Media

Scenarios	Basal media (USD/l)	Ascorbic acid 2-phosphate (g/L)	Ascorbic acid 2-phosphate (USD/g)	NAHCO3 (g/L)	NAHCO3 (USD/g)	Sodium selenite (g/L)	Sodium selenite (USD/g)
1	3.12	$6.40x10^{-2}$	7.84	$5.43x10^{-1}$	0.01	$1.40x10^{-5}$	0.10
2	3.12	$6.40x10^{-2}$	7.84	$5.43x10^{-1}$	0.01	$1.40x10^{-5}$	0.10
3	3.12	$6.40x10^{-2}$	7.84	$5.43x10^{-1}$	0.01	$1.40x10^{-5}$	0.10
4	0.24	$6.40x10^{-2}$	0.00	$5.43x10^{-1}$	0.00	$1.40x10^{-5}$	0.00

Table A1c. Model variable inputs: Media continued 1

Scenarios	Insulin (g/L)	Insulin (USD/g)	Transferrin (g/L)	Transferrin (USD/g)	FGF-2 (g/L)	FGF-2 (USD/g)	TGF-b§ (g/L)	TGF-b§ (USD/g)
1	$1.94x10^{2}$	340.00	$1.07x10^{2}$	400.00	$1.00x10^{-4}$	$2.01x10^{6}$	$2.00x10^{-6}$	$8.09x10^{7}$
2	$1.94x10^{2}$	340.00	$1.07x10^{2}$	400.00	$5.00x10^{-5}$	$1.00x10^{6}$	$2.00x10^{-6}$	$8.09x10^{7}$
3	$1.94x10^{2}$	340.00	$1.07x10^{2}$	400.00	$5.00x10^{-5}$	0.00	$2.00x10^{-6}$	$8.09x10^{7}$
4	$1.94x10^{2}$	0.00	$1.07x10^{2}$	0.00	0.00	0.00	$2.00x10^{-6}$	$0.00

Table A1c. Model variable inputs: Media continued 2

Scenarios	Percentage of oxygen in initial charge (w/w)	Oxygen (USD/ton)	Glucose (mol/l)	Density of media (kg/l)
1	2.00	$4.00x10^{1}$	$1.78x10^{-2}$	1.00
2	2.00	$4.00x10^{1}$	$2.67x10^{-2}$	1.00
3	2.00	$4.00x10^{1}$	$2.67x10^{-2}$	1.00
4	2.00	$4.00x10^{1}$	$3.56x10^{-2}$	1.00

Table A1d. Model variable inputs: Utility

Scenarios	Boiler energy efficiency (%)	Percentage of electricity self-generated (%)	Temperature of water/media entering facility (˚C)	Desired Temperature of media entering bioreactor (˚C)	Specific heat of water (kWh/ kg (˚C))	Energy efficiency of media heating system (%)	Heat released per mol of oxygen consumed (kWh)	Energy efficiency of bioreactor cooling system (%)
1	85	50	20	37	$1.16x10^{-3}$	100	$1.30x10^{-1}$	100
2	85	50	20	37	$1.16x10^{-3}$	100	$1.30x10^{-1}$	100
3	85	50	20	37	$1.16x10^{-3}$	100	$1.30x10^{-1}$	100
4	85	50	20	37	$1.16x10^{-3}$	100	$1.30x10^{-1}$	100

Table A1d. Model variable inputs: Utility continued

Scenarios	Specific heat of ACBM (kWh/kg ˚C)	Temperature of ACBM in bioreactor (˚C)	Temperature of cooled ACBM (˚C)	Energy efficiency of ACBM cooling system (%)	natural gas cost (dollars per 1000 ft^3)	Natural gas (cents per kWh)	Process water cost (USD/m^3)	Wastewater filtration treatment costs (USD/m^3)	Biological oxidation of wastewater costs (USD/m^3)
1	$6.22x10^{-4}$	37	4	100	4.17	1.42	0.63	0.51	0.57
2	$6.22x10^{-4}$	37	4	100	4.17	$1.42	0.63	0.51	0.57
3	$6.22x10^{-4}$	37	4	100	4.17	$1.42	0.63	0.51	0.57
4	$6.22x10^{-4}$	37	4	100	4.17	$1.42	0.63	0.51	0.57

Table A1e. Model variable inputs: Labor

Scenarios	Production worker hourly rate (USD/h)	Country effect	Supervising and clerical assistance	Advanced technology and automating	Skilled and qualified level of the personnel	Social benefits	Overtime work	Bioreactors labor factor
1	13.68	1.00	1.20	0.80	1.50	1.40	1.25	1.00
2	13.68	1.00	1.20	0.80	1.50	1.40	1.25	1.00
3	13.68	1.00	1.20	0.80	1.50	1.40	1.25	1.00
4	13.68	1.00	1.20	0.80	1.50	1.40	1.25	1.00

Table A1f. Model variable inputs: Finance

Scenarios	Debt ratio (%)	Interest rate on Debt (%/y)	Economic life (y)	Interest cost of equity (%/y)
1	90	5	20.00	15
2	90	5	20.00	15
3	90	5	20.00	15
4	90	5	20.00	15

Model variable inputs. Inputs without unit in parentheses are unitless.

Table A2. Sensitivity analysis numerical results

Algorithm	Average single cell density (rho_c)	Average single cell volume (V_c)	Glucose concentration (conc_glu)	Glucose consumption rate per cell (GCR_c)	FGF-2 cost (C_fgf2)	FGF-2 concentration (conc_fgf2)	Maturation time (t_m)	TGF-b concentration (conc_tgfb)	Oxygen consumption rate per cell (OUR_c)
DGSM	$6.83x10^{3}$	$1.00x10^{0}$	$2.70x10^{-2}$	$5.70x10^{-1}$	$2.40\ x10^{-3}$	$5.07x10^{-2}$	$8.03x10^{-3}$	$4.93x10^{-2}$	$8.68x10^{-2}$
SSA	$1.00x10^{0}$	$9.66x10^{-1}$	$9.48x10^{-1}$	$8.80x10^{-1}$	$8.50x10^{-1}$	$7.47x10^{-1}$	$6.95x10^{-1}$	$2.16x10^{-3}$	$1.69x10^{-3}$
DMIM	$8.90x10^{-1}$	$1.00x10^{0}$	$9.47x10^{-1}$	$7.58x10^{-1}$	$7.83x10^{-1}$	$9.10x10^{-1}$	$5.98x10^{-1}$	$1.37x10^{-2}$	$5.13x10^{-2}$
FAST	$7.82x10^{-1}$	$1.00x10^{0}$	$5.83x10^{-1}$	$8.63x10^{-1}$	$4.97x10^{-1}$	$8.50x10^{-1}$	$6.94x10^{-1}$	$1.59x10^{-4}$	$1.93x10^{-6}$
MM	$1.00x10^{0}$	$9.70x10^{-1}$	$9.91x10^{-1}$	$9.53x10^{-1}$	$9.11x10^{-1}$	$9.09x10^{-1}$	$8.62x10^{-1}$	$1.44x10^{-2}$	$1.44x10^{-8}$
RBD-FAST	$1.00x10^{0}$	$7.94x10^{-1}$	$9.96x10^{-1}$	$7.54x10^{-1}$	$7.86x10^{-1}$	$7.11x10^{-1}$	$8.22x10^{-1}$	$1.39x10^{-1}$	$7.48x10^{-2}$

Sensitivity analysis numerical results. DGSM = Derivative-based Global Sensitivity Measure, SSA = Sobol Sensitivity Analysis, DMIM = Delta Moment-Independent Measure, FAST = Fourier Amplitude Sensitivity Analysis MM = Morris Method and RBD-FAST = Random Balance Designs-Fourier Amplitude Sensitivity Test. The 5 parameters exhibiting the most sensitivity were selected from each algorithm. This resulted in 9 unique parameters listed in the table. This analysis was performed using peer reviewed open source SALib Python package for this work [49].

Table A3. Potential industrial scale equipment for ACBM production.

Equipment	Unit	Unit costs ($1000's)	Scale index	Production Operators required (P)	Adjusted value factor (f_{Aj})	Accounted for in equipment cost analysis
Centrifugal pumps	Power (kW)	5	0.60	0.1	1.42	-
Plate filters	Area (m^2)	3	0.75	1.0	1.64	-
Media holding vessel	Volume (m^3)	10	0.50	0.2	1.29	-
Heat exchanger	Area (m^2)	3	0.65	0.5	1.29	-
Inoculum bioreactor	Volume (m^3)	50	0.60	1.0	1.29	-
Seed bioreactor	Volume (m^3)	50	0.60	1.0	1.29	-
Bioreactors	Volume (m^3)	50	0.60	1.0	1.29	+
Positive displacement pump	Power (kW)	5	0.60	0.1	1.42	-

Potential industrial scale equipment for ACBM production. Created using information from *Food Plant Economics* and CEPI [31,32,44].

Table A4. Annual United States national industrial grid electricity costs 1999-2019

Year	Average nominal consumer cost per year (cents kWh^{-1})	Inflation adjusted cost (cents kWh^{-1})
1999	4.42	6.77
2000	4.63	6.9
2001	5.04	7.25
2002	4.88	6.94
2003	5.11	7.08
2004	5.25	7.14
2005	5.72	7.59
2006	6.15	7.81
2007	6.39	7.95
2008	6.95	8.29
2009	6.83	8.14
2010	6.76	7.85
2011	6.81	7.78
2012	6.66	7.4
2013	6.88	7.52
2014	7.09	7.63
2015	6.90	7.43
2016	6.75	7.17
2017	6.87	7.12
2018	6.92	7.03

Annual United States industrial national grid electricity costs 1999-2019. Information was obtained from the United States EIA and average costs were normalized to January 2019 US currency[40,42].

Table A5. Annual United States national industrial natural gas costs 1999-2019

Year	Average nominal cost per year (USD thousand cubic feet^{-1})	Inflation adjusted cost (cents kWh^{-1})
1999	3.08	1.55
2000	4.45	2.19
2001	5.08	2.40
2002	4.02	1.88
2003	5.91	2.70
2004	6.51	2.92
2005	8.67	3.77
2006	7.82	2.58
2007	7.65	3.13
2008	9.66	3.79
2009	5.23	2.05
2010	5.44	2.08
2011	5.12	1.93
2012	3.85	1.41
2013	4.64	1.67
2014	5.58	1.98
2015	3.91	1.39
2016	3.49	1.22
2017	4.08	1.39
2018	4.17	1.42

Annual United States national average natural gas costs 1999-2019. Information was obtained from the United States EIA and average costs were normalized to January 2019 US currency[40,42].

Table A6. Cost of process and wastewater treatment

Utility	Cost (USD m^{-3})
Process water	0.63
Wastewater filtration treatment	0.51
Biological oxidation of wastewater	0.57

Cost of process and wastewater treatment. Cost were reported in *Food Plant Economics* and were adjusted to account for inflation reported in January 2019 US currency [42,44].

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Chapter 3. Techno-Economic Assessments of Cellular Agriculture

Book chapter pending publication: Cellular Agriculture: Technical and Scientific Foundations

Chapter: Techno-Economic Assessments of Cellular Agriculture

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Brief abstract:
The difficulty of scaling new technology has been a challenge since the advent of mass manufacture. Techno-economic assessment (TEA) has been employed in multiple industries to understand the potential impact and scalability of emerging technologies. This chapter examines the three major technoeconomic assessments of animal cell-based meat which have been conducted at the time of this writing. The scope and assumptions which are made by each TEA are identified and compared. Capital and operating expenditures are compared separately and summarized to provide an informative analysis of each TEA. The results and key assumptions which influence the outcomes of each TEA are then contrasted to give the reader an informed perspective of the economic outlook for animal cell-based meat products.
Keywords: Technoeconomic assessment, economic analysis, animal cell-based meat, cultivated meat, cultured meat, cellular agriculture, bioreactor

Key Objectives

- Understand the basic concept of techno-economic modeling
- Be able to identify the scope of the modeled production system for each animal cell-based meat (ACBM) techno-economic assessment (TEA)
- Be able to recognize the key assumptions for capital and operating expenditures for each ACBM TEA
- Be able to compare the results of each TEA based upon system scope and underlying assumptions

Section 1.0 Introduction

The concept of *in vitro* meat production is appealing for multiple reasons. It has the potential to address concerns over animal welfare, provide an additional source of protein as the population increases, improve health, and generate economic opportunity. Current popular interest in the technology can be traced to a 2013 public demonstration of a 140 gram "animal cell-based meat (ACBM) hamburger," even though the price tag of that single burger at the time was in excess of 270,000 USD (Kupferschmidt, 2013). At the time of this publication, investment in this potential meat production technology exceeded 1 billion United States dollars (USD) and it has been forecast by analysts that the cost would dramatically decrease and reach cost parity with conventionally-produced meat within 10-30 years (Suhlmann et al., 2019; Tubb and Seba, 2019; Turi, 2021).

This technological forecasting and investment in ACBM companies has led to both increased interest and increased skepticism from the scientific community, especially relating to the economic and technical feasibility of mass produced ACBM products (Cohen et al., 2022; Fassler, 2021). When examining an emerging technology such as ACBM, technoeconomic assessment (TEA) is commonly utilized to assess the commercial viability of producing and selling a product at scale. TEAs generally examine both the fixed capital expenditures and operating expenses of a technology (Maroulis and Saravacos, 2007c).

Fixed capital includes:

- Purchased equipment
- Installation
- Piping
- Instrumentation and control
- Electrical work
- Buildings
- Site Improvement
- Land
- Off-site facilities
- Engineering costs
- Construction costs
- Start-up
- Contractor fees
- Contingency

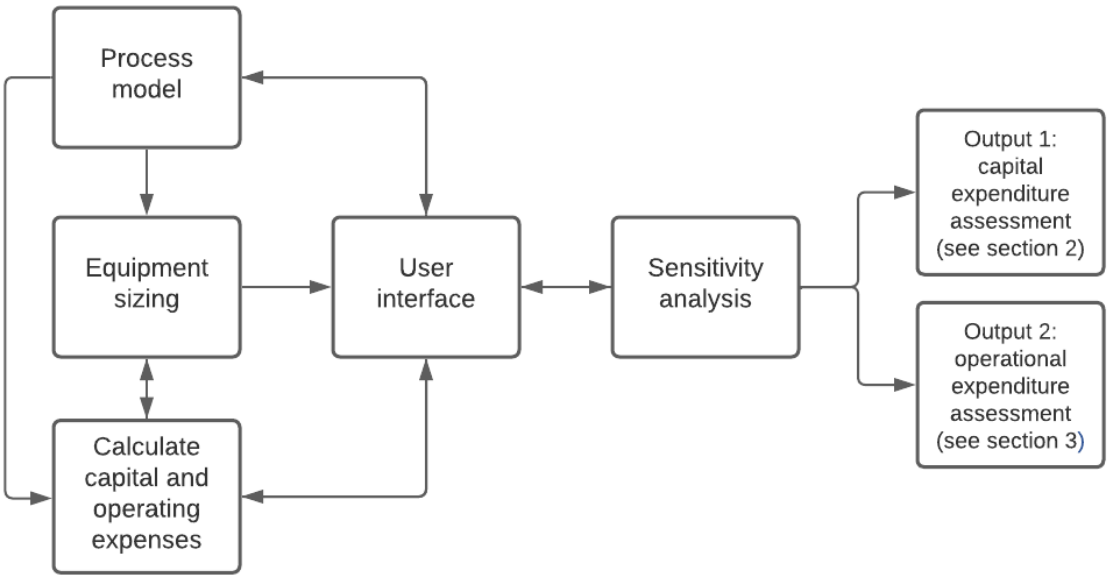
Operating expenses or manufacturing costs are broken down into three categories:

- Fixed manufacturing costs includes maintenance, depreciation, insurance, taxes, and royalties.
- Variable manufacturing costs includes raw materials, consumables, packaging, waste disposal, utilities, laboratory QA/QC, and labor.
- Indirect manufacturing costs (overhead) includes sales expense and other general expenses.

The fixed capital costs associated with a technology can be annualized so that a minimum product cost or cost of goods sold (COGS) can be determined. This allows for the interest accrued from debt and equity financing to be accounted for as well.

TEA also combines process modeling and design with economic evaluation to analyze the costs, risks, uncertainties, alternative designs and timeframes of emerging technology(McNulty et al., 2021; National Renewable Energy Laboratory, 2022; United States Department of Energy, 2022). Conducting a TEA enables the estimation of both the fixed capital expenditures and operating expenses of a technology (Maroulis and Saravacos, 2007c), but is also used to inform technical design, quantify environmental impacts, identify potential health and safety hazards, identify potential research needs, improve the value proposition, and guide investment decisions (Alam et al., 2018; Budzinski et al., 2019; Burk, 2018). These assessments often begin with building a technoeconomic model of a process that consists of iterative steps (Figure 1).

Figure 1. Steps for developing a technoeconomic model



This figure represents a common processed utilized for technoeconomic modeling. This is an iterative process which ideally becomes more accurate with each iteration.

At the time of writing, three TEAs of large-scale ACBM bioprocessing systems have been published and are publicly accessible.

Section 2.0 Existing ACBM TEAs capital expenditures

The first peer-reviewed TEA of ACBM (UC Davis TEA) was a collaborative effort from researchers at the University of California-Davis and only examines the main production bioreactor system for fixed capital expenditures(Risner et al., 2020). The second TEA (Humbird TEA) was conducted by David Humbird for Open Philanthropy and examines two different bioreactor systems (fed batch and perfusion) with associated buildings and equipment for commercial operation at scale(Humbird, 2021, 2020). The third TEA (CE Delft TEA) was conducted by CE Delft for the Good Food Institute and examines a dual bioreactor system (batch and perfusion) with a partial set of auxiliary equipment required for scale-up (Odegard et al., 2021). CE Delft's TEA is publicly available but has not undergone a traditional scientific peer-review process. These TEAs each examine different bioreactor configurations/systems and facilities, it may be useful for the reader to have a basic understanding bioreactor design and operations.

Section 2.1 UC Davis TEA capital expenditures:

The UC Davis TEA limited its capital expenditure estimates to only the production bioreactors (Risner et al., 2020). The TEA assumed the use of a stirred tank bioreactor with a working volume of 20

m^3. This volume is in line with current pharmaceutical technology and similar volumes are utilized in the other two TEAs of ACBM (Humbird, 2021, 2020; Odegard et al., 2021). The UC Davis TEA estimated annual production volume equivalent to 1% of beef produced in the United States in 2018 (121,000,000 kg). The model utilized this production volume and animal cell properties (volume, density, doubling time, batch time, achievable cell concentration) to determine the total number of 20 m^3 bioreactors needed.

UC Davis used a scenario-based approach to explore how potential technological advancements might influence both capital and operating expenses. The first scenario, which was designed to represent the current level of technology at the time of the study found that >5,200 production scale bioreactors would be required to meet annual production of 1% of annual beef production. Global mammalian cell culture capacity in 2021 was 11.75 million liters and the first scenario would require >104 million additional liters of mammalian cell culture capacity (Langer and Rader, 2021). The fourth scenario, which represents a highly aspirational scenario where all core technical challenges are solved and physical limits are pushed to their boundaries, estimated that 50 bioreactors would be needed to produce the target annual production quantity. Scenarios 2 and 3 represented mid-point pathways between scenarios 1 and 4 and indicated that 360 bioreactors would be necessary to meet annual production. Cellular metabolism which plays an important role in each TEA was adjusted in each scenario ranging from a Warburg type metabolism (Warburg, 1956)in scenario 1 to more efficient metabolism which may utilize oxidative phosphorylation as the cell's primary metabolic pathway for adenosine triphosphate production in scenario 4.

Once the required number of bioreactors was determined, a standard method for determining equipment costs was applied to determine total capital expenditures (Maroulis and Saravacos, 2007a). The resultant estimated cost for each 20 m^3 bioreactor was reported to be 778,000 USD including installation. It should be noted that this estimate is for a basic food grade bioreactor and use of higher quality steel will increase the cost estimate by many multiples (e.g., the use of stainless steel 310 increases cost estimates by an order of magnitude as compared to food grade carbon steel.) (Maroulis and Saravacos, 2007a). After standard equity and debt financing equations were utilized to determine an annualized capital cost (California Biomass Collaborative, 2016). The study results suggested total capital costs to range from 4 billion (scenario 1) to 37 million USD (scenario 4) with the capital costs of scenarios 2 and 3 being estimated at 280 million USD. It is important to note that UC Davis TEA only accounted for production bioreactors and installation (lang factor 2) and did not account for the entire upstream (e.g. seed train, media preparation/sterilization) and downstream (e.g. cell recovery/concentration) production system. Further, the authors used equipment estimates based on food-grade bioreactors as opposed to pharmaceutical-grade bioreactors.

Finally, in an effort to increase transparency and access to the study, the UC Davis TEA was adapted into a user-friendly web-based calculator where core model assumptions for capital/operating expenditures could be adjusted and allow users to explore their own scenarios. ACBM Cost calculator: https://acbmcostcalculator.ucdavis.edu

Section 2.2 Humbird TEA capital expenditures

The Humbird TEA examined the capital costs of a large-scale ACBM production facility with the greatest level of detail as compared to the UC Davis and CE Delft TEAs. Two potential ACBM

production systems were assessed and the capital costs for a fed-batch and a perfusion production facility were analyzed separately(Humbird, 2021). The following capital costs were accounted for with each system: production bioreactors, seed bioreactors, perfusion equipment (when applicable), media prep, dewatering, pressure swing adsorption oxygen generator, clean-in-place system, production clean room, laboratory clean room and other equipment and buildings. The fed-batch system utilized 20 m^3 fed-batch stirred production bioreactors and the perfusion system utilized 2 m^3 perfusion production bioreactors. Annual production was 100,000,000 kg of ACBM/year to understand scaled-up economics and set global demand for media component costs.

The metabolic inefficiency of wild type cells which utilize a Warburg metabolism during proliferation was acknowledged, however the two scenarios assume cells are "metabolically enhanced" to allow for a more efficient metabolism(Humbird, 2021). This enhanced metabolism would reduce catabolic inhibition allowing for greater biomass concentration to be achieved (110 g /L vs. 7 g /L wet basis in the fed-batch system and 195 g/L vs. 20 g/L wet basis in the perfusion system). These order of magnitude increases in biomass concentrations are utilized in both production scenarios.

Aspen cost capital estimates (ACCE) were utilized to estimate capital costs for the production bioreactors. Other capital costs were estimated using a combination of ACCE, SuperPro Designer® and correlations from literature (Couper et al., 2012). A 20 m^3 stirred tank bioreactor was estimated to cost 1.5 million USD including installation (lang factor 3.5), whereas each 2 m^3 perfusion bioreactor was estimated to be 865,000 USD. The ACBM production facility in the fed-batch case contained 24 x 20 m^3 production bioreactors which produces 6.8 million kg of ACBM/year. The perfusion case study estimates that 96 x 2m^3 perfusion production bioreactors would be needed to produce a comparable mass of ACBM annually. More than 14 ACBM production facilities would be needed to produce ACBM at the scale of the assumed global demand for growth medium components (100 million kg wet mass of ACBM/year). Achieving this scale would require 54,000 individual batches per year. Table 1 and 2 show the breakdown of capital costs when applied to the total industry. Also, it should be that a capital charge factor (15%) is utilized to account for financing of the capital costs.

The Humbird TEA estimated that the total capital investment required for the annual production of 100 million kg of ACBM is 4.8-9.5 billion USD. The Humbird TEA case studies assumed a significant optimization of cellular metabolism to achieve biomass concentrations which are an order of magnitude greater than what is initially assumed possible. This increased biomass concentration (calculated to be $3.7x10^7$ cells/ml and $6.6x10^7$ cells/ml for the fed batch and perfusion systems, respectively) is within an order of magnitude of cell concentrations ($1.0\text{-}9.5x10^7$ cells/ml) assumed in the UC Davis TEA's scenarios one, two, and three.

Table 1. Direct costs for upstream production of 100,000,000 kg of ACBM for Humbird TEA case studies

Equipment/buildings	**Fed-batch (1,000,000 USD)**	**Perfusion (1,000,000 USD)**
Production bioreactors	500	1203
Seed Bioreactors	338	130

Perfusion equipment	n/a	1290
Media preparation equipment	250	594
Dewatering equipment	59	29
Pressure swing adsorption oxygen generator	309	275
Clean in place equipment	147	130
Other equipment	324	623
Production clean room	588	710
Laboratory clean room	58	43
Other buildings	74	188
Total direct costs	2,647	5,215

Table 2. Capital costs for upstream production of 100,000,000 kg of ACBM for Humbird TEA case studies

Capital costs	**Fed-batch (million USD)**	**Perfusion (million USD)**
Total direct costs	2,647	5,215
Engineering and construction	1,574	3,130
Fees and contingencies	632	1,246
Total capital investment	4,853	9,591

Section 2.3 CE Delft TEA capital expenditures

The CE Delft TEA is a mid-point in between the UC Davis and Humbird TEA in terms of the scope of capital cost assessment. The CE Delft TEA examines eight different scenarios where input assumptions are altered relating to investment payback time, decrease in production time and increase in cell volume. It should also be noted that each scenario builds upon the previous scenario in the CE Delft TEA. The described ACBM production system is partially derived from a Good Food Institute (GFI) analysis of cell culture medium costs for ACBM (Specht, 2019).

The total required capital investment is maintained throughout scenarios 1-6, however scenarios 7-8 represent potential scenarios of decreased total required capital investment. The production facility is reported to have a main production bioreactor which acts as the cell proliferation vessel and four perfusion bioreactors where cells are seeded to differentiate/mature then be harvested. One hundred thirty of these production facilities are reported to produce 10,000,000 kg of ACBM/year. The equipment pricing information utilized in the CE Delft TEA was based on conversations with members of the ACBM industry. Initial required capital investment was reported for 10,000,000 kg of ACBM/year,

however Table 3 linearly adjusts the required capital investment for scenarios 1-6 to 100,000,000 kg of ACBM/year for ease of comparison across the three TEAs.

Table 3. Capital costs for upstream production of 100,000,000 kg of ACBM for CE Delft TEA scenarios 1-6

Equipment	**Pieces of equipment**	**Equipment costs (USD/piece of equipment)**	**Lang factor**	**Total costs (million USD)**
Perfusion reactor, 2,000 L	5,200*	600,000	n/a	3,120
Stirred tank reactor 10,000 L	1,300	325,000	3.5	1,479
Stirred tank reactor 50 L	1,070	90,000	2.2	212
Storage and mixing tank 60,000 L	150	175,000	3.5	92
Clean-in-place system	10	3,500,000	2.2	77
Total capital investment				4,982

*The reported number was 430, however as the described system indicates that four perfusion bioreactors would be utilized for each stirred tank system and 130 x 4= 520. We have utilized 520 perfusion units in our calculations.

Scenario 6 examines how an increase in investor payback time decreases the annual payment of capital costs (4 years to 30 years). An actual decrease in total capital expenses does not occur. Interest rates were also not applied to capital costs calculations. In scenario seven, a 25% reduction in production run time is assumed. The shorter production run time decreases equipment sizes and lowers investment costs to 3.65 billion USD for 100,000,000 kg of ACBM produced annually. Scenario 8 examines how an increase in cell volume would decrease capital investment due to a greater volume of ACBM potentially being produced from the same cell number density (cells/ml). This could potentially increase bioreactor productivity and was reported to reduce the investment costs to 3.20 billion dollars for 100,000,000 kg of ACBM produced annually.

The CE Delft TEA examined several potential capital expenses; however, it is not as extensive as the Humbird TEA. The assumption of utilizing a bioreactor seed train which increases from a 50 L working bioreactor to a 10,000 L bioreactor (a 200 fold increase) requires further justification. For animal cell culture, generally a 4-5-fold volume increase is utilized in the seed train. A 10-100 fold increase volume is ideal for industrial yeast and bacteria fermentations and a 10-fold increase has been utilized for animal cell culture (Junker, 2004; Yang et al., 2007). This would require a minimum of one additional

bioreactor to transfer from a 500 L seed bioreactor to a 10,000 L production bioreactor, assuming that a 20-fold volume increase is deemed acceptable. If the 4-fold volume increase is applied this would require three additional bioreactors (200 L, 800 L and 3,200 L) and thereby significantly increase the needed capital investment to achieve 100,000,000 kg of ACBM/year of ACBM production.

Section 2.4 Capital investment summary

Each of the TEAs have the commonality of examining a proposed ACBM production system which is operated in a batch fashion and utilizes steel bioreactors. Each TEA examines capital expenses from a near term perspective in which production would occur utilizing existing large scale cell proliferation technology. The TEAs examine how the use of bioreactors can be utilized for cell proliferation with a similar initial total capital investment range (3 to 9 billion USD). These similarities help with ease of comparison across the TEAs, but it is also important to note the differences in the assumptions made in each TEA. These assumptions can influence the capital expenditure estimates across the various scenarios for each TEA.

The cell concentration is largely similar for each TEA, however it is a scenario dependent input for the UC Davis TEA. The UC Davis TEA's first scenario utilizes a concentration of $1.0x10^7$ cells/ml with an increase in concentration to $9.5x10^7$ cells/ml in scenarios 2 and 3 and an increase of $2.0x10^8$ cells/ml which approaches the volume constraints of the bioreactor in the fourth scenario. The Humbird TEA cell concentrations fall within an order of magnitude of the first three UC Davis TEA scenarios with approximated cell concentrations of ~$3.7x10^7$ cells/ml and ~$6.6x10^7$ cells/ml for the fed batch and perfusion systems, respectively. The CE Delft TEA maintains a cell concentration of $5.0x10^7$ throughout its scenarios, however an assumed decrease in production time and increase in cell volume decreases capital expenses in scenario seven and eight. Understanding the difference in cell concentration is vital since it plays an important role in estimating total capital expenditures i.e., a lower cell concentration means it is necessary to invest in more production bioreactors to obtain the same amount of product.

Each TEA examined capital expenses at different granularities and utilized different scenarios. The UC Davis TEA provides estimates based upon four scenarios and only examines the production bioreactors for the system. It presents the results of the analysis as four potential scenarios ranging from the current technological level to a highly aspirational "best case" scenario that the Authors consider to be rather unlikely. The Humbird TEA examines two case studies (batch-fed and perfusion bioreactor systems) with the inclusion of a full production facility. Each case study operates under the assumption that significant innovation has greatly increased cellular metabolic efficiency allowing for an increased cell concentration. The CE Delft TEA outlined a partial production facility system which included both stirred tank bioreactors and perfusion bioreactors utilized for cell proliferation and differentiation/maturation, respectively. CE Delft TEA's unique financing assumption which extends payback time from 4 to 30 years and does not account for investor profit or interest in scenarios 6-8 also influences the impact of the capital costs on each kilogram product produced. Each ACBM TEA examined a different level of annual production, however for ease of comparison of each TEA we have linearly converted the values to 100,000,000 kg of ACBM/year. Table 4 outlines the estimated capital investment required for each described ACBM production system to produce 100,000,000 kg of ACBM/year.

Table 4. Estimated capital investment for UC-Davis, Humbird and CE Delft TEAs for the annual production of 100,000,000 kg of ACBM

TEA	Capital estimated	Reported estimated capital costs in million USD (High)	Reported estimated capital costs in million USD (Low)
UC Davis	Production bioreactors	3,348	32*
Humbird	Full upstream production system with centrifuge	9,591	4,853
CE Delft	Partial upstream production system	4,982	3,200**
Average estimated capital investment	-	5,974	2,695

*This represents scenario 4 which is an extremely unlikely scenario due to physical constraints and limits

**Accounts for lower number of perfusion bioreactors (430 vs. 520)

These results indicate that capital expenditures will be a significant economic challenge for ACBM industry especially with the retail price for beef being ~$10/kg(United States Department of Agriculture, 2022). These capital costs represent an important hurdle for the industry especially if the goal is to develop commodity-type products.

Section 3.0 Existing ACBM TEAs operating expenditures

Each of the existing ACBM TEAs takes a different approach for evaluating operating expenses, e.g., growth medium, labor, utilities, waste treatment and other consumables. However, the models also share many common assumptions, including the assumption that animal products such as fetal bovine serum (FBS) will not be utilized for the industrial production of ACBM. A common finding for all three ACBM TEAs was that the growth medium is currently the major cost driver.

Section 3.1 UC Davis TEA estimated operating expenditures

The UC Davis TEA is limited to the production bioreactors but includes costs associated with the growth medium, oxygen, energy, water, waste treatment, and labor. The growth medium used in the TEA was Essential 8^{TM}. Essential 8^{TM} is an animal-serum-free, chemically-defined growth medium utilized in embryonic/pluripotent stem cell research and has been suggested for use as a growth medium for industrial ACBM production (Chen et al., 2011; Specht, 2019). Essential 8^{TM} contains low concentrations of presently expensive growth factors which influence the animal cell's proliferation and differentiation processes.

The required media volume was determined by a cellular glucose consumption rate and is assumed to increase as the cell concentration (cells/mL) increases. The differentiation/maturation stage

was modeled and also contributes to the overall growth medium usage. To produce 122,000,000 kg of ACBM/year (1% of annual USA beef production), it was reported to take 1.40×10^{11} to 1.56×10^{8} liters of media annually. The oxygen cost was estimated by utilizing a method similar to the media consumption method (on a per cell basis). The scenarios presented in the UC Davis TEA highlight how overall expenditures can be reduced by changing key model inputs (See Table 5).

Table 5. UC Davis initial model scenarios

Scenario	Achievable cell concentration (cells/ml)	FGF-2[1] conc. (g/L)	FGF-2 cost (USD/g)	Glucose concentration in basal media (mol/L)	Glucose consumption rate per cell (mol/ h cell)	Hours per doubling (h)	Maturation time (h)
1	1.00×10^{7}	1.00×10^{-4}	2.05×10^{6}	1.78×10^{-2}	4.13×10^{-13}	24	240
2	9.5×10^{7}	5.00×10^{-5}	1.00×10^{6}	2.67×10^{-2}	2.07×10^{-13}	16	156
3	9.5×10^{7}	5.00×10^{-5}	0	2.67×10^{-2}	2.07×10^{-13}	16	156
4	2.00×10^{8}	0	0	3.56×10^{-2}	4.13×10^{-14}	8	24

Table adapted from the article Preliminary techno-economic assessment of animal cell-based meat(Risner et al., 2020)

[1]Fibroblast growth factor 2 (FGF-2)

The energy calculation utilized a few basic thermodynamic calculations: heating of media entering the bioreactor, heat removal from the bioreactor, and cooling of ACBM leaving the bioreactor. The cost related to the energy usage was then determined by adapting a method from *Food Plant Economics* (Maroulis and Saravacos, 2007b). Only the volume of media was considered for process water use and waste treatment via filtration and biological oxidation. Published costs, adjusted for inflation, were utilized to calculate the process water usage and wastewater treatment (Maroulis and Saravacos, 2007b). Labor costs were conservatively estimated by assuming one operator per bioreactor and by utilizing a factorial cost escalation method described in *Food Plant Economics* (Maroulis and Saravacos, 2007b). Fixed manufacturing costs were estimated as 15% of the total capital costs. The total reported operating expenditures to produce 122,000,000 kg of ACBM/year ranged from 5.3×10^{13} to 2.4×10^{8} USD (Table 6). The high operating expense in scenario 1 can largely be attributed to growth medium costs >350 USD/L and the significant growth medium volume requirement (1,147 L/kg ACBM).

Table 6. Operating expenses to produce 120,000,000 kg of ACBM annually for each UC Davis TEA scenario (millions USD)

Operating Expenses	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**
Media	52,898,422	6,931,864	5,397,339	209
Labor	1,567	107	107	15
Energy	673	147	147	4
Fixed manufacturing costs	606	41	41	6
Non-electricity utility	240	52	52	1
Oxygen	112	25	25	1
Total	52,901,620	6,932,236	5,397,711	236

The UC Davis TEA only examined a portion of the operating expenditures and estimated media usage based upon glucose consumption on a per cell basis. It did not account for potential inhibitory metabolite production or another substrate being the limiting factor in cellular expansion. It was assumed that once the glucose in the growth medium was consumed that fresh Essential 8^{TM} would be used to supply the cells with additional nutrients. This could potentially lead to an over-estimation of the media requirement if the cells are metabolically efficient, and they only produce minimal amounts of inhibitory compounds like lactate or ammonia. It should be noted that the cost estimate of 0.24 USD/L for the growth medium in scenario 4 was taken from a non-profit report and should be considered a highly optimistic value (Specht, 2019).

Section 3.2 Humbird TEA estimated operating expenditures

The Humbird TEA provides a more complete look at the operating expenditures, but the assumptions utilized in the two case studies should be carefully considered. As for the capital cost evaluation, a production scale of 100,000,000 kg of ACBM/ year is assumed. The growth medium represents a substantial cost in both the fed-batch and perfusion cases, making up 59% and 41% of the total production cost, respectively. The Humbird TEA breaks growth medium costs into two broad categories: macronutrients and micronutrients. Macronutrients include glucose, amino acids and a plant protein hydrolysate that acts as an additional source of amino acids. It should be noted that a plant protein hydrolysate is not commonly used as a primary source of amino acids for stem cell culture. Micronutrients included in the Humbird TEA are insulin, transferrin, fibroblast growth factor, and transforming growth factor β. These micronutrients are assumed to drop in price as scale increases using a

log-log demand versus price correlation based on data for a variety of recombinant protein products. The micronutrients which make up the bulk of the cost today were estimated to be only 5-8% of the production costs in the case studies. The case studies also assume that the micronutrients are not degraded or consumed over time which may be an incorrect assumption according to the author (Humbird, 2021, 2020).

Labor estimates are developed from literature and utilize the author's own method (Humbird, 2020; Reisman, 2019). The utility costs include energy and water usage. The main contributor to energy costs is clean room power usage. The water estimates include water used for media production and steam-in-place systems with estimates from literature (Clean in place systems explained, 2018; Gsell et al., 2019; Pereira Chilima et al., 2020). Maintenance and insurance were accounted for annually as percentages of total capital investment (5% and 4%, respectively). Table 7 provides a breakdown in annual operating expenditures for 100,000,000 kg annual ACBM production facility.

Table 7. Annual Operating expenditures for upstream production of 100,000,000 kg of ACBM for Humbird TEA case studies

Operating expenses	**Fed-batch (million USD)**	**Perfusion (million USD)**
Macronutrients	1,900	1,800
Micronutrients	300	300
Consumables	100	500
Utilities	100	100
Labor	100	200
Total operating expenses	2,500	2,900

Each case study operates on the same assumption that cellular metabolism has become more efficient and fewer inhibitory metabolites are produced. This reduction in inhibitory metabolite production allows an order of magnitude an increase in cell density (g FW/L) when compared with wild-type cell lines. As stated by the Humbird TEA, this will require significant technical advancement. Another key assumption is substantial decrease in the cost of the micronutrients which is justified by the scale of production. Additional micronutrients such as vitamins, salts and minerals are not accounted for and would likely be necessary for animal cell growth. It is also assumed that additional micronutrients are not needed as the cell concentration increases. It is acknowledged by the author that this may not be the case and micronutrient deficiency could limit cell proliferation.

Section 3.3 CE Delft TEA estimated operating expenditures

The CE Delft TEA operating expenditures include material inputs, staffing for plant operation, wastewater treatment and maintenance. The material inputs include culture medium inputs, electricity, heat and other inputs such as chemicals, filters, scaffolds, vials and ultrapure water. The inventory data for the CE Delft TEA included cell density, cell volume, production time, quantity of growth medium and

medium composition. These data were obtained via input from fifteen ACBM companies. Data related to wastewater treatment and energy use were estimated using the authors' judgement and were cross-checked with literature and consultants from industry. Price data included prices for energy, medium ingredients, investment, maintenance, staffing, and wastewater treatment. These values were obtained from a mix of sources including the World Energy Outlook, the Alibaba online marketplace, engineering consultants, and CE Delft calculations. The growth medium is the major cost driver across all three baseline scenarios.

It is initially reported to require 41,300 L of growth medium to produce 3,080 kg of ACBM with a cell concentration of $5x10^7$ cells/mL and average cell volume being 3,500 μm^3/cell. Table 8 provides a breakdown of the composition of the growth medium, and mass of each ingredient utilized to produce one kg of ACBM. There is a >90% reduction from the high cost/use scenario and low cost/use scenario for several growth medium ingredients including glucose, recombinant protein, buffering agents, vitamins and growth factors. This reduction is significant and relies on an increase in metabolic efficiency.

Table 8. CE Delft baseline scenarios: grams of growth medium ingredient per kilogram of ACBM produced

Ingredient	**High-cost (g of ingredient/kg of ACBM)**	**Mid-cost (g of ingredient/kg of ACBM)**	**Low-cost (g of ingredient/kg of ACBM)**
Amino acids from hydrolysate	300.0	237.0	187.5
Amino acids from conventional production	100.0	79.0	62.5
Glucose	396.0	75.5	14.0
Pyruvate	4.0	2.0	1.0
Recombinant proteins	50.0	7.1	1.0
Salts	160.0	80.0	40.0
Buffering agent	100.0	31.6	10.0
Vitamins	20.0	2.0	0.2
Growth factor	0.001	$3.20x10^{-4}$	$1x10^{-4}$
Water	40,000.0	12,649.0	7,500.0
Total (g)	**41,130.0**	**13,163.5**	**7,816.2**
Total (L)	**41.7**	**13.4**	**8.0**

Scenarios four and five further examine potential methods to lower the cost of the growth medium. Each of these scenarios incorporates the assumptions from scenario three (the low-cost scenario). Scenario four assumes a 1,000-fold decrease in growth factor price. It is assumed that these growth factors can be produced in a manner similar to food-grade enzymes. Scenario four decreases the cost of the growth medium to under 100 USD/kg of ACBM. Scenario five retains the assumptions from scenarios three and four and explores the potential reduction in recombinant protein use. According to CE Delft TEA, albumin is a major cost driver and thus its price/use must be decreased. Citing an undisclosed industry source, a 100-fold decrease in cost is assumed for recombinant proteins such as albumin and insulin. This would indicate that albumin and insulin could be produced and purified for $0.4/kg and $1.55/kg, respectively. This reduces the growth medium costs to approximately one USD per kg of ACBM produced. The sources for other operating expenditures such as utilities and consumables are supplied, but raw data for calculations are not publicly available. Scenarios seven and eight which decrease production time and increase cell volume also reduce some utility costs as well as decrease capital expenses. The labor requirement is estimated to be 200 full time employees to produce 10,000,000 kg of ACBM annually. The maintenance costs are estimated to be 5% of the bare equipment cost.

The CE Delft TEA examines eight scenarios and understanding the underlying scenario assumptions is important for critical analysis of the reported results. The first major assumption is the use of a geometric mean to calculate ingredient prices and cellular metabolic requirements for scenario two (mid-cost). If scenario 2 was calculated utilizing the arithmetic mean, then scenario 2 would be 11,286 vs. 1,708 USD/per kg of ACBM. The use of a plant hydrolysate as a primary source of amino acid is also assumed. While an interesting concept, plant protein hydrolysate is not commonly used as a primary source of amino acids for stem cell culture. The increases in cellular metabolic efficiency with the simultaneous reduction of the price of key nutrients is also assumed for scenario two and three. Tables 8 and 9 compares each scenario's cellular metabolic requirements and the reported ingredient prices.

Table 9. CE Delft baseline scenarios: Ingredient prices

Ingredient	**High-cost scenario (USD/kg of ingredient)**	**Mid-cost scenario (USD/kg of ingredient)**	**Low-cost scenario (USD/kg of ingredient)**
Amino acids from hydrolysate	3.50	2.65	2.00
Amino acids from conventional production	50.00	11.18	2.50
Glucose	0.70	0.53	0.41
Pyruvate	100.00	10.00	1.0
Recombinant proteins	400,000.00	198,919.58	98,922.50
Salts	2.10	0.46	0.10
Buffering agent	55.00	35.57	23.00
Vitamins	60.00	20.49	7.00
Growth factor	2,391,176,470.59	890,151,808.52	331,372,549.02
Water	0.01	0.01	0.01

The percentage of price decrease from the high-cost scenario and the low-cost scenario indicates that multiple ingredients (conventionally produced amino acids, pyruvate, and salts) will decrease by over 90%. Decreases of this magnitude may indicate that it is assumed industries producing these ingredients have increased in scale and that they can be bought as food-grade ingredients (Specht, 2019). Table 10 provides a comparison of the total cost per kg of ACBM for each baseline scenario and several key ingredient costs (pyruvate, recombinant protein, salts, vitamins and growth factors) have been reduced >98% in comparison to high costs scenario.

Table 10. CE Delft baseline scenarios: Total costs ingredient per kg of ACBM produced

Ingredient	**High-cost scenario (USD/kg of ACBM)**	**Mid-cost scenario (USD/kg of ACBM)**	**Low-cost scenario (USD/kg of ACBM)**
Amino acids from hydrolysate	1.05	0.63	0.38
Amino acids from conventional production	5.00	0.88	0.16
Glucose	0.28	0.04	0.01
Pyruvate	0.40	0.02	0.00
Recombinant proteins	20,000.00	1,406.57	98.92
Salts	0.34	0.04	0.00
Buffering agent	5.50	1.12	0.23
Vitamins	1.20	0.04	0.00
Growth factor	2,391.18	281.49	33.14
Water	0.40	0.13	0.08
Total	**22,405**	**1,691**	**133**

It is important to remember that order of magnitude cost reductions is assumed for growth factors and recombinant proteins for scenarios four and five. While these assumptions lead to intriguing results, comparison with industrial food-grade enzymatic production may not be appropriate if these proteins are highly purified. The assumption of utilizing food-grade technology and ingredients for animal cell production has yet to be proven out as well.

3.4 Operating expenditures summary

The growth medium was identified as the major operating expense in all three TEAs of ACBM. The UC Davis TEA explored different scenarios which varied the volume and price of Essential 8™ growth medium. The media requirement in the UC Davis TEA was based on cellular glucose consumption rate and varied depending upon cell concentration. The Humbird TEA concluded that wild-type cell metabolism was too inefficient due to catabolic repression and utilized a more efficient metabolic reaction to examine the operating expenditures of two case studies. The Humbird TEA concluded that even with some key assumptions (substantial increases in cellular metabolic efficiency, use of a plant hydrolysate as a partial source of bulk amino acids, and reduction in micronutrient/growth factor costs) that ACBM would be too costly to produce as a commodity product. The CE Delft TEA explored scenarios which reduced growth medium costs significantly (<1 USD/L) but these scenarios

required an expanding series of assumptions to be true for these orders of magnitude reductions in price to occur. They also assume a substantial order of magnitude increase in cellular metabolic efficiency. Table 11 provides minimum and maximum operating expenditures for each TEA for the annual production of 100,000,000 kg of ACBM.

Table 11. Maximum and minimum reported annual operating expenses (USD) for the production of 100,000,000 kg of ACBM.

UC Davis TEA		**Humbird TEA**		**CE Deflt***	
Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
$4.37x10^{13}$	$1.92x10^{8}$	$4.20x10^{10}$	$3.68x10^{9}$	$2.24x10^{12}$	$5.36x10^{8}$

*Each facility is assumed to produce 10,000,000 kg of ACBM/year and ten identical production facilities are assumed to be constructed. These values were estimated visually from graphs within the CE Delft report.

Section 4.0: TEA results summary and conclusion

Each TEA explored different production systems and made multiple assumptions about the production process, facility design, as well as equipment, material, consumables and energy costs. The peer-reviewed ACBM TEAs (UC Davis and Humbird TEAs) have also released the underlying model and calculations to the public. The CE Delft TEA utilizes data obtained from industry partners and has not released all underlying model calculations likely due to confidentiality agreements. The assumptions in the scenarios were previously described in each TEA and should be carefully considered when reviewing the results.

Table 12 shows cost per kilogram of ACBM for a production system with the capacity to produce 100,000,000 kg of ACB annually for each TEA. It should be noted that at the time of this writing the average price of beef is ~$5/kg and it is currently the most expensive commodity meat.

Table 12. Reported maximum and minimum reported cost of ACBM per kilogram (USD/kg)

UC Davis TEA		Humbird TEA		CE Deflt	
Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
437,179	1.95	51	37	22,423	6.43

The UC Davis TEA maximum cost of ACBM is largely attributed to the high costs associated with utilizing pharmaceutical grade growth medium. The minimum cost of ACBM scenario reduces the growth medium cost to 0.24 USD/L; this is a >99% reduction in the growth medium costs. This minimum cost also assumes cell concentration reaches the actual physical limits in terms of bioreactor volume. The UC Davis TEA authors also stated that scenario 4 is a highly unlikely scenario. The Humbird TEA developed two case studies examining a fed-batch system and a perfusion system. The fed-batch system was found to be more economically viable with production costs estimated to be approximately 27% less than the perfusion system. Both case studies assumed an “enhanced” cell metabolism that prevented catabolic inhibition at uneconomic cell densities. The CE Delft TEA maximum cost of ACBM is from scenario one which assumes high growth medium usage and higher priced ingredients. The CE Delft minimum cost of

ACBM is the price point where all possible price reductions have been achieved. CE Delft ACBM TEA does not discuss the technical challenges or likelihood of occurrence for each scenario. The UC Davis and Humbird TEA highlight some of the technical challenges related to ACBM production and provide insight for areas of focus for researchers, namely increasing cellular metabolic efficiency, media costs reduction and bioreactor design.

Additionally, it will be important to evaluate the environmental, safety and health impacts of potential ACBM production facilities. As the environmental impacts are evaluated, it may be prudent to identify valorization opportunities for the spent growth medium and other waste streams. Food safety issues which are unique to animal cells should also be considered and these facilities may be at risk for adventitious viral which could potentially make the facility inoperable for an extended period or permanently. These TEAs provide an initial guide for additional studies which may account for food safety related challenges, waste stream valorization and examine the process mass intensity of potential ACBM production facilities.

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Chapter 4. A techno-economic model of continuous mycoprotein production: A journey to price parity with beef protein

Journal article formatted for and awaiting submittal: Frontiers in sustainable food systems

A techno-economic model of continuous mycoprotein production: A journey to price parity with beef protein

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Keywords: mycoprotein, techno-economic, model, bioreactor, airlift, continuous, protein.

Abstract

Predicted famines due to population increase created an interest in the development of protein alternatives during the 1950s. Currently, a renewed interest in protein alternatives has developed as a potential strategy to decrease the environmental impact of protein production and meet the global demand for protein as the population increases. *Fusarium venenatum* A3/5/3, the organism used for mycoprotein production has been commercially available since the 1980s, however new fungal protein companies are currently interested in scaling up their own fungal protein production facilities. To aid those interested in scaling a mycoprotein production facility, we created an economic model with over 340 inputs which examines the continuous production of mycoprotein utilizing airlift bioreactors. Utilizing a sensitivity analysis, we identified key inputs and developed a user-friendly excel model which can be manipulated to create custom scenarios for interested stakeholders. Our findings indicate that mycoprotein may be cost competitive with beef on a protein basis given the current prices of beef. The findings also indicate that

mycoprotein may not be an economically feasible alternative for other types of commodity meats (chicken or pork) or for inexpensive products (pet food) which utilize offal or meat products not traditionally consumed in the modern western diet.

Introduction

Modern interest in protein alternatives began in the late 1950's when it was predicted that a worldwide shortage of high-protein foods would occur in the 1980s (Moore et al., 2021). In response, the Rank Hovis McDougall Research Centre began a project in 1964 to convert waste starch from cereal processing into a high-protein food (Finnigan, 2011). While the predicted protein shortage did not occur due to the Green Revolution, in 1969, the fungal strain *Fusarium venenatum* A3/5/3 was identified and selected as the organism which would convert glucose and ammonia into a protein-rich biomass called mycoprotein (Moore et al., 2021). After ten years of safety testing (1970-1980) and a review of a 2 million word, 26-volume food safety report submitted to United Kingdom's Ministry of Agriculture, Fisheries and Food, the first mycoprotein product was sold to the public in 1985. The product was branded as Quorn® and initially sold as a health food given its low fat (2.9%) and high fiber (5.1%) characteristics (Moore et al., 2021). Quorn® became first available in the United States in 2002 after approval by the United States' Food and Drug Administration (Moore et al., 2021). Nearly twenty years after US regulatory approval of mycoprotein, a renewed interest in alternative protein sources has emerged, harkening back to the original concerns of the late 1950s and 1960s.

Looking forward, global demand for meat is expected to continually increase as global incomes rise and the global population increases (Food and Agriculture Organization of the United Nation (FAO), 2019; United Nations, 2017). The projected increase in meat production has raised concerns about the anticipated environmental impacts, such as increased greenhouse gas (GHG) emissions and land, water,

and energy resource consumption (Food and Agriculture Organization of the United Nation (FAO), 2018; Olivier and Peters, 2020). These environmental concerns, as well as concerns related to animal welfare and human health, have driven interest in meat alternatives which are "food products that have the organoleptic qualities of meat, but whose origin is not from slaughtered animals" (Risner et al., 2020). The sum of these convergent trends has prompted a renewed interest in meat alternatives from scientists, non-profit groups, companies, governments, and investors. In addition to this expected demand for meat or meat-like products, analysts have predicted a substantial (60-70%) displacement of conventional ground beef with meat alternatives with in 10-20 years (Suhlmann et al., 2019; Tubb and Seba, 2019).

Meat alternatives can be broadly categorized into three groups: plant-based products, animal cell-based meat, and microbial-based products. The total commercial sector of meat alternatives has received $11.1 billion in capital investment since 2010 with 73% of the investment being raised since 2020 (Good Food Institute, 2022). The fermentation category of meat alternatives, including microbial cell proteins such as mycoprotein, received $1.7 billion in investment in 2021 (Good Food Institute, 2022). This level of investment suggests the need for a flexible technoeconomic model that examines the scaling of core production technologies and incorporates different biological factors and limitations, such as the specific growth rate of an organism. Other technoeconomic assessments (TEAs) have examined mycoprotein production from different perspectives including integration into a multi-product biorefinery and the use of agriculture waste streams as a fermentation substrate (Bulkan et al., 2020; Ritchie et al., 2017; Upcraft et al., 2021). However, our model provides the additional function of flexible scenario analyses to enable identification of potential innovations in fungal meat alternatives research or production.

Materials and Methods

To understand the economic potential of mycoprotein and a processed, Quorn®-like product (PQP), we developed a TEA model utilizing process and chemical engineering methodology. The modeled system is a continuous fermentation system operating at capacity that accounts for the time

requirements for the initial growth phase as well as sanitation/cleaning periods. PQP is then processed utilizing a reported process for meat-like texture development. All variables and equations are available in appendix A and the excel model has been supplied in the supplemental material. The annual costs were divided into annualized capital costs and annual operating expenditures.

Capital expenditures for mycoprotein production facility

Mycoprotein is currently commercially produced utilizing aerobic airlift bioreactors operated in a continuous fashion (Finnigan, 2011; Moore et al., 2021). In addition to the primary bioreactor system, an RNA reduction system is utilized to reduce the RNA content of the mycoprotein, a centrifuge is used to dewater the mycoprotein, and a vacuum chiller is used to quickly reduce the temperature of mycoprotein to storage temperature (See figure 4.1).

Commercial airlift bioreactors utilized for mycoprotein production have a reported working volume of 155 m^3 and can produce approximately 2 metric tons of consumable mycoprotein per hour (Derbyshire and Ayoob, 2019). These reactors operate in a continuous fashion (for approximately 1000 h) and a concentration of 10-15 g/L of biomass (wet basis) is maintained in the reactor while it is continuously harvested (Moore et al., 2021). To understand the required fermentation capacity of the system; a mass-balance of the mycoprotein production system was conducted (equations 1-4, 12-16). This includes accounting for the heat induced RNA reduction that causes a ~30% loss of solids from the final mycoprotein product (Moore et al., 2021). This processing step is necessary because it reduces the RNA content of mycoprotein from ~8% (w/w) to ~1% (w/w) which is approximately the RNA content of mammalian liver and within the World Health Organization's upper limit of 2% (w/w) RNA content for food products (Finnigan, 2011). The concern is in regard to the breakdown of nucleic acid in humans leading to excess uric acid in the bloodstream, which can cause gout and renal stones (Moore et al., 2021; Ragab et al., 2017). After the RNA reduction, mycoprotein water content is reduced to approximately 76-70% (w/w) (Finnigan, 2011; Moore et al., 2021).

Airlift bioreactors

Once the mass of harvestable mycoprotein per liter of growth medium was determined, the necessary fermentation capacity was calculated utilizing a user-defined hourly production goal and a reported specific growth rate of *F. venenatum* ATCC PTA-2684 (equations 17-18). The maximum dilution rate of the airlift bioreactor cannot exceed the maximum specific growth rate, otherwise the rate of biomass withdrawal exceeds the rate of biomass production and the cells will be washed out of the system. The necessary fermentation capacity and maximum bioreactor working volume was utilized to determine the number of airlift bioreactors needed to reach the hourly production goal during continuous operation (equations 19-22). Equipment cost estimates were then applied to the system utilizing a method described in *Food Plant Economics* (equations 23-26) (Maroulis and Saravacos, 2007a, 2007c). The *Food Plant Economics* method for capital costs estimation was utilized throughout the model unless otherwise stated (Maroulis and Saravacos, 2007a, 2007c). Each airlift bioreactor system was outfitted with an individual RNA reduction vessel and a centrifuge. The quantity of vacuum chilling units is determined by model inputs.

RNA reduction vessels and centrifuge

The RNA reduction vessel heats the mycoprotein in suspension for 15-30 minutes. The RNA reduction vessels working volume was determined utilizing the airlift bioreactor's working volume and an RNA reduction factor (equation 28). The RNA reduction factor was estimated at ~10% of the airlift bioreactor working volume based upon the specific growth/withdrawal rate during the continuous phase of mycoprotein production and the hold time in the reactor. However, this factor is adjustable in the model to accommodate variable processing scenarios. The centrifuge processing rates were determined utilizing equations 29-30. Once the processing rate was determined, the capital expenditures were estimated utilizing our model's standard method (Maroulis and Saravacos, 2007a, 2007c).

Vacuum cooling unit

After centrifugation, the mycoprotein is chilled utilizing a vacuum cooling unit before being shipped to a facility that would further process it into consumer products. Vacuum chilling is considered to have high capital cost but is an economically viable cooling process given its ability to rapidly cool products and its low manpower requirements. Equations 31 and 32 provide the capital cost estimation method for the vacuum cooling unit which utilizes a USD/kg-day costing unit and accounts for inflation, Lang factor, and material composition costs. The capital costs related to onsite storage of mycoprotein are not accounted for and the mycoprotein is transported to a PQP production facility at no cost in our limited model.

Capital expenditures for PQP production facility

After being dewatered and cooled, mycoprotein can be further processed to develop a fibrous, meat-like texture. A series of processing steps are utilized for the development of the final PQP (figure 4.2). The capital expenditures for each processing step were estimated utilizing the *Food Plant Economics* methodology and other literature sources as needed (Maroulis and Saravacos, 2007a, 2007c).

Mixer

The mycoprotein is considered to have an appearance and texture similar to bread dough but lacks its elasticity (Finnigan, 2011). Mixing tanks associated with breadmaking were utilized to estimate the capital expenditures for the mixing process. We utilized mixing tanks to estimate the unit cost (75,000 USD) and a base equipment sizing unit of 1,000 kg per hour (kg/h) was utilized.

Former

After the mixing of mycoprotein and other PQP minor ingredients, the mass is discharged into common food processing equipment which utilizes pressure to shape the PQP into blocks (Finnigan,

2011). Bread forming equipment was utilized to estimate the unit costs (60,000 USD) at a base equipment sizing unit of 3,000 kg/h (Maroulis and Saravacos, 2007a).

Steam cooker

Once formed into blocks, steam is utilized to raise the block's internal temperature to 90 ˚C. The steam cooking could be achieved using a variety of systems; however, a steam blanching/cooking system was utilized to estimate the unit costs (200,000 USD) at a base equipment sizing unit of 5,000 kg/h (Maroulis and Saravacos, 2007a).

Chiller

The initial freezing occurs over 30 minutes and reduces the temperature to -10 ˚C. Freezing can be achieved utilizing a variety of freezing technologies, however we utilized a belt freezer to estimate the unit costs (250,000 USD) at base equipment sizing unit of 2,000 kg/h (Maroulis and Saravacos, 2007a).

Size reduction equipment

A cutter or grinder can be utilized depending upon the final desired PQP geometry. We utilized an estimate for a generic size reduction unit with an estimated unit costs ($10,000 USD) at a base equipment sizing unit of 1 kg/s (Maroulis and Saravacos, 2007a).

Frozen aging process

The freezer volume was determined utilizing a required storage time and the hourly production rate (equations 34-36). Capital expenditures were then estimated utilizing estimates from the FAO, accounting for inflation (equation 37)(Johnston et al., 1994).

Total capital costs of mycoprotein and PQP

Once the individual capital expenditures were determined, the total capital cost for each production process was calculated utilizing equations 33 and 38. Final reported PQP costs include

mycoprotein capital costs. It should be noted that only items in the model are included in the capital expenses (see supplemental material for adjustable model).

Operating expenditures

Manufacturing costs for mycoprotein and PQP can be broken into three categories: fixed manufacturing, variable capital costs, and indirect (overhead) costs. Fixed manufacturing costs are estimated as a percentage of the annual capital expenditure payment except loan and equity interest, which is accounted for separately. These fixed manufacturing cost include equipment maintenance, insurance, taxes and royalty costs (Maroulis and Saravacos, 2007d). Indirect costs are unrelated to amount of product processed, such as sales expenses and local taxes, and are not accounted for in our model since these expenditures are outside of processing facility expenses and vary firm to firm. Our model estimates several variable capital expenditures for mycoprotein and PQP production, however, the model should be considered a limited model. Costs associated with general food production such as lighting, pumping, conveyor belts, packaging or transport are not included in the model. In this technoeconomic model, additional ingredients can be added to PQP production but in the current scenarios no additional ingredients have been considered. The estimated variable costs include growth medium components, other raw materials, some utilities (some energy, process water and wastewater processing) and labor costs.

Growth medium for mycoprotein production

Growth medium usage was accounted for during the growth phase and continuous production phase (equations 39-42). The exact growth medium composition for commercial mycoprotein production was not available to the authors. To estimate the minimum glucose required, we utilize a reported protein content of mycoprotein and a reported glucose-to-protein conversion rate to determine annual cost for glucose minimum (38 g/L) (equations 43-47) (Moore et al., 2021). It should be noted that the initial goal of direct conversion of starch to biomass was found to be a rate limiting step during process development,

so a highly refined glucose syrup is utilized as the carbon source (Whittaker et al., 2020). Annual expenses related to oxygen production were estimated utilizing reported industrial values and fungal oxygen consumption rates (equations 48-50) (Humbird et al., 2017; Rossi et al., 2017). Ammonia usage was estimated via the protein content. Once the annual protein production was determined, a protein estimation factor taken from the Kjeldahl method was used to convert the known protein mass to the estimated nitrogen mass (Mæhre et al., 2018). Once converted to nitrogen, another factor utilizing the molecular weights of nitrogen and ammonia was used to estimate the minimum mass of ammonia needed for annual production (equations 51-53). These calculations were used to estimate the minimum cost of glucose and ammonia.

It has been reported that other minor growth medium components are utilized for mycoprotein production, however the composition of these minor ingredients has not been publicly reported (Harrison and Johnson, 2018). Vogel's growth medium has been utilized in literature and was utilized to identify/quantify potential growth medium components (Hossenini et al., 2009; "Vogels," n.d.; Wiebe et al., 1994). The prices for laboratory grade growth medium components were obtained from a scientific supply site (Merck KGaA, 2022). The total annual costs of other growth medium components were then determined utilizing equation 54. One important note on the model is that growth medium components are supplied in excess during commercial operation to maintain a maximum specific growth rate and prevent mycotoxin formation (Moore et al., 2021). Additional ingredients can be added to the excel model for additional scenario development (see supplemental material).

Utilities

Energy costs were estimated utilizing a method which accounts for self-produced energy and energy sourced from a public supplier (equations 55-57) (Maroulis and Saravacos, 2007b; Risner et al., 2020). Energy estimates for mycoprotein production include sterilization and cooling of the growth medium before the entering airlift bioreactor (equations 58-59), heating of the growth medium during

RNA reduction (equation 60), cooling of the mycoprotein via vacuum chilling (equation 61), and compressed air energy estimates (equation 62) (U.S. Department of energy, 2004). Equations 63 and 64 were then utilized to estimate minimum energy costs in the mycoprotein production facility.

Minimum energy requirements for PQP production were estimated using several methods. The steam cooking and chilling of PQP was estimated using standard thermodynamic calculations (equations 65-66). The energy requirement for the size reduction equipment was estimated utilizing the semiempirical Bond law (equations 67-68) (Maroulis and Saravacos, 2007a) To obtain the proper meat-like texture, PQP is freezer-aged for approximately 2 weeks (Moore et al., 2021). To account for annual freezer energy, the freezer storage was estimated, and annual energy usage determined via Specific Electricity Consumption (SEC) number estimate (equation 69) (Prakash and Singh, 2008). The PQP processing plant compressed air was estimated via a compressed air factor (equation 70). The minimum energy usage and costs for a PQP were estimated utilizing equations 71-72.

The minimum process water utilized was the volume of growth medium utilized in a year. This does not include water used for sanitation or cleaning. The wastewater filtration and biological oxidation treatment volumes were determined utilizing volume/mass of media removed per kilogram of mycoprotein. The costs of the process water and wastewater treatment were obtained from literature (Maroulis and Saravacos, 2007b; Risner et al., 2020). Water usage for PQP is not accounted for and the required process and wastewater costs should be viewed as minimum costs.

Our model assumes that the production facility operates 24 h/day all year. We assume that the facilities are fully staffed, each shift is an 8-h shift and there is no overtime required. The facilities are assumed to be in a generic, standard income portion of the United States. The required manpower for each shift is estimated utilizing a standard method which assigns a manpower requirement by the amount and type of processing equipment in the facility (equation 74) (Maroulis and Saravacos, 2007b). The

labor costs for each facility were determined by using a mean hourly rate of 20 USD/h and a labor cost correction factor (equations 74-76). This allowed for the estimation of total labor costs at each facility.

Total annual expenditures with financing and fixed operating costs

The minimum annual operating expenses for the mycoprotein and PQP production facilities were estimated using equations 77-78. We utilize standard financing equations with a 20-year economic life to account for expenses related to equity recovery and debt for both the mycoprotein and PQP production facilities (equations 79-88) (California Biomass Collabortive, 2016). These equations annualize the capital expenditures and allow for a total minimum annual cost to be determined for each production facility. After annualization of the capital expenditures, fixed annual operating costs were accounted for as a percentage (3%) of the annualized capital expenditures.

Sensitivity analysis

We performed a sensitivity analysis of the mycoprotein and PQP production cost model using a standard one-factor-at-a-time (OAT) approach (Saltelli et al., 2008). We individually changed each input by ±25% and recorded its impact on the model's output variables. We then converted the input back to the original value and repeated this for each input variable. This allowed for identification of impactful input variables which allowed for a streamlining of the model user interface and helped to inform our scenario design. Results for the sensitivity analysis can be found in Appendix B.

Results

We identified >340 input variables which influence the capital and/or operating expenses for our limited model system. The capital costs in our base scenario (2000 kg/h) for mycoprotein was ~108 million USD. The main airlift bioreactor accounted for 66.7% of the capital costs. The reported maximum working volume (155 m^3) of the airlift bioreactor was utilized in each scenario, however this volume is adjustable in the model (Moore et al., 2021). The capital costs of a single 155 m^3 airlift bioreactor constructed with

304 stainless steel was estimated to be ~42 million USD and an additional bioreactor used to meet the production goal was estimated to be ~29 million USD. The estimated capital costs of the RNA reduction vessels, centrifuges and vacuum chillers is 18, 13, and 4 million USD, respectively (Figure 4.3).

Total capital expenses for PQP were an order of magnitude lower than for mycoprotein production, estimated to be ~13.8 million USD. The PQP chiller was responsible for over 44% of the PQP capital costs. Figure 4.4 indicates estimated capital costs for the processing equipment utilized for PQP production. The size reduction equipment was by far the least impactful estimated capital costs. It should also be noted that land purchase is not accounted in any capital expenditure, and this has the potential to increase total capital expenditures.

The annual operating expenses included debt/financing, growth medium, oxygen, energy, process water, wastewater treatment and labor expenses. An OAT sensitivity analysis was conducted to identify model inputs which were most impactful to the cost of mycoprotein production (Appendix B). The results of the sensitivity analysis were then utilized to develop a more limited interface with the inputs categorized into four broad input categories (general bioreactor operations, organism characteristics, continuous airlift bioreactor parameters, and growth medium characteristics). The sensitivity analysis informed the specification of three alternative scenarios relative to a baseline scenario. Scenario 1 is the baseline scenario and can be viewed in the excel model in the supplemental material. All scenarios maintained the baseline settings except where noted. Scenario 2 doubled the capital costs of the mycoprotein production equipment before financing. Scenario 3 decreased the costs of biotin and zinc sulfate heptahydrate to mass produced, food-grade prices of $0.373/g and $0.0018/g, respectively. Scenario 4 doubled the required glucose amount due to glucose being maintained in excess to maximize the specific growth rate during commercial production (Finnigan, 2011; Moore et al., 2021). A reported production rate of 2,000 kg mycoprotein/h was chosen for each scenario; however, this rate is user-defined within the model (Moore et al., 2021). Results of our baseline model estimate that mycoprotein

can be produced for $3.55/kg and PQP can be produced for $4.03/kg. The protein content of mycoprotein has been reported as approximately 11-12% (w/w) which indicates protein production costs are approximately $29.56/kg (Derbyshire and Delange, 2021; Moore et al., 2021). Figure 4.5 provides a cost comparison across the scenarios. Protein production costs were highest in Scenario 4 where the glucose concentration was doubled. Decreasing the cost of biotin and zinc sulfate heptahydrate, key minor component cost drivers in scenario 3 reduced protein costs by 22%.

USDA reported costs of choice beef was $6.56/kg as net farm value in March 2022, whereas our base model for mycoprotein production is $3.55/kg and $4.03/kg for PQP (United States Department of Agriculture, 2022). These values initially seem economically favorable for mycoprotein but when examined on a protein basis the difference is less significant with mycoprotein being $29.56/kg of protein and beef being $29.95/kg of protein (Derbyshire and Delange, 2021; United States Department of Agriculture, 2022). It can also be noted that in March 2021, the cost of choice beef was reported to be $5.53/kg or $25.25/kg of protein. Boiler chickens were reported by the USDA to be $1.95/kg at wholesale (United States Department of Agriculture, 2022). Approximately 71% (excluding skin and bones) of a chicken carcass is usable meat (Orr et al., 1984) which would give an approximate cost of $2.74/kg. Chicken breast has been reported have 28.4 g of protein per 100 grams, leading to an estimated of protein sourced from chicken of approximately $9.64/kg (Derbyshire and Delange, 2021). Our model indicates that mycoprotein may be produced as an economic alternative to beef protein but will not be an economic alternative for inexpensive products such as chicken or offal utilized in pet food production.

Discussion

Our technoeconomic model found that mycoprotein protein production utilizing a continuous production system was economically comparable to farm-raised beef protein production. However, if compared on a calorie basis, one kilogram of stewed beef mince has 2,090 kcal vs. one kilogram of

mycoprotein which has 850 kcal. This difference in caloric density indicates that ~2.5 times more mycoprotein would need to be consumed to achieve the same caloric intake as stewed beef; and subsequently, stewed beef would also represent a significantly less expensive option in terms of available calories(Derbyshire and Delange, 2021) . Chicken breast meat is reported to contain 1,600 kcal/kg and chicken appears to be the more economical choice as a protein source when compared to mycoprotein or beef. If only examined from a nutrient density viewpoint, an edible insect like the mopane caterpillar (*Imbrasia belina*) which is reported to contain 4,090 kcal/kg and 352 g of protein/kg may be of interest to food production stakeholders (Payne et al., 2016). While outside the scope of this TEA, techno-economic modeling of industrialized insect protein production would be necessary for a direct comparison of an insect protein source and mycoprotein.

The mycoprotein production system we modeled was a production system that operates continuously for ~1,000 h. The use of continuous airlift bioreactors allows for a five-fold increase in productivity when compared to a series of separate batch fermentations (Finnigan, 2011). It should be noted that batch fermentations are a norm in the commodity food/beverage fermentation industry (wine, beer, cheese), however cell biomass production is generally not the goal. Commercial yeast (*Saccharomyces cerevisiae*) biomass production mostly utilizes batch production; however, it is an important minor ingredient in baking, brewing, winemaking, etc.… not as a meat replacement. This difference in end use is illustrated by a difference in the global markets for meat and commercial yeast, ~USD 2.3 trillion and ~USD 7 billion, respectively (Thomas, 2021; Wood, 2021). This indicates that a fungal protein meat replacement, such as mycoprotein needs to be produced in the most efficient manner possible. The results of our model indicate that mycoprotein protein production is approximately equivalent to beef protein in economic terms, but only when the 5-fold productivity benefit of continuous production is achieved.

Continuous airlift bioreactor technology is not a new technology. The development of the world's largest aerobic fermenter (1,500 m^3) occurred in the 1970's. Operation began in 1979 and the bioreactor was decommissioned in 1987 due to economic and technical challenges (Humbird, 2020). This bioreactor was developed to produce an animal feed soy protein replacement, Pruteen from *Methylophilus methylotrophus,* a methane utilizing bacteria (Vasey and Powellf, 1984). Technical issues related to foaming and sterility required a systems control redesign that, when coupled with other economic issues, caused Pruteen to be sold at double the price of the soy protein it intended to replace in 1983 (Humbird, 2020). These factors led to the Pruteen plant decommissioning. However, the same technology was then utilized to scale up Quorn® production in the 1990s, albeit at an order of magnitude in reduced scale (155m^3) as compared to the Pruteen production fermenter (Moore et al., 2021). While our technoeconomic model is adjustable for the scale-up of the continuous, airlift bioreactor fermentation system, it is likely that any order of magnitude increase in the scale of this system would likely require supplementation or innovation of the core technology.

Reduction in growth medium costs is an evident area where operating expenses can be reduced. It has been reported that near laboratory-grade minor ingredients are utilized for mycoprotein production (Harrison and Johnson, 2018). Scenario 3 was designed to examine the cost impacts of utilizing research grade minor ingredients (biotin and zinc sulfate heptahydrate) versus food-grade/lower purity ingredients. Biotin and zinc sulfate heptahydrate were adjusted to food-grade costs since they were most economically impactful minor ingredients in our model. The total cost reduction in scenario 3 was 22%, however, the feasibility of reducing the purity of the ingredients is not clear. A reduction in the purity could result in an increased risk for a contamination event which could negatively impact mycoprotein producers in a variety of ways, including: lost production time, costs for investigations, costs of decontamination, decreased product quality, potential food safety issues, and lost revenue (Blackwell, 2017). This indicates

that it would be prudent to explore risks associated with utilizing lower purity ingredients relative to the potential cost savings.

Our techno-economic model indicated that there were several organism/product specific attributes that were impactful to capital and operating costs. The amount of solids in a kilogram of mycoprotein influences the total required fermentation capacity, which in turn, is an input into multiple capital expense calculations, annual growth media usage, and oxygen use calculations. This influence derives from our detailed mass balance calculations (See Appendix A or the supplemental material). Meanwhile, the protein content of the final mycoprotein product influences the determination of the minimum glucose/ammonia requirements as well as the thermodynamic calculations as the proportion of protein affects the specific heat of mycoprotein. Other expected organism related characteristics, such as specific growth rate and protein yield (g protein/ g glucose), influenced the operating expenditures. These identified input variables can be utilized to guide research questions related to increasing protein production or identifying other viable organisms for use in a continuous airlift bioreactor system.

Conclusion

Our technoeconomic model indicates that mycoprotein and PQP can currently economically compete with beef when examined on a protein basis. However, this is for general choice cuts of beef and does not necessarily include cheaper ground products or green/red offal that is often used for pet food production or sold internationally. Our technoeconomic model highlights the importance of utilizing a continuous fermentation system (as opposed to a batch system) to achieve cost parity with beef protein. Potential reductions in cost can be achieved through advances in organism-specific parameters, such as protein content, achievable concentration (g/L), and specific growth rate. The customizable technoeconomic model we have provided can be utilized to explore multiple scenarios beyond those provided in this paper, including custom combinations of minor growth medium components, multiple combinations of

materials used for bioreactor construction, different specific organism parameters, and many other scenarios given the full menu of >340 input variables (See supplemental material).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization, D.R., C.J. and E.S.S.; Data curation, D.R.; Formal analysis, D.R.; Funding acquisition, E.S.S and D.R.; Investigation, D.R.; Methodology, D.R. and E.S.S.; Project administration, E.S.S. and C.J.; Resources, E.S.S. and C.J.; Supervision, E.S.S. and C.J.; Validation, D.R., K.A.M. and E.S.S.; Visualization, D.R.; Writing-Original draft, D.R. and Writing-review and editing, D.R., K.A.M. and E.S.S.

Acknowledgments

Authors would like to thank the Mars Advanced Research Institute for funding the product.

Appendix A. Variables and equations

Variables

M_{GM*} = Mass of unprocessed growth medium in one kilogram of mycoprotein and unprocessed growth medium (kg)

M_{mp*} = Mass of mycoprotein in one kilogram of mycoprotein and unprocessed growth medium (kg)

M_{mp} = Mass of mycoprotein solids in one kilogram of RNA reduced growth medium and mycoprotein (kg)

M_{GM} = Mass of growth medium in one kilogram of RNA reduced growth medium and mycoprotein (kg)

$R_{\%}$ = Percentage of mycoprotein solids lost during RNA treatment

M_{GMr} = Mass of growth medium removed from final mycoprotein product (kg)

S_{mp} = Solids in one kilogram of final mycoprotein product (kg)

cp_{mp} = Estimated specific heat of mycoprotein (kJ/kg K)

$c_{mp\%}$ = Percentage of carbohydrates in mycoprotein on a wet weight basis

$p_{mp\%}$ = Percentage of protein in mycoprotein on a wet weight basis

$f_{mp\%}$ = Percentage of fat in mycoprotein on a wet weight basis

$a_{mp\%}$ = Percentage of ash in mycoprotein on a wet weight basis

$w_{mp\%}$ = Percentage of moisture in mycoprotein on a wet weight basis

t_{PC} = Total production cycle time (h)

t_g = Growth cycle time (h)

t_c = Continuous production time (h)

t_s = Sanitation time (h)

n_c = Quantity of fermentation cycles completed annual

t_{op} = Annual total operation time (h)

n_c^* = n_c rounded towards -∞ (h)

t_{fc} = Annual time for full cycles (h)

t_{ac} = Annual time in full cycle continuous production (h)

t_{ptc} = Time in partial cycle continuous production (if < 0 then 0) (h)

t_{tc} = Total annual time in continuous production (h)

V_{GM+mp} = Volume of 1 kilogram of unprocessed growth medium and mycoprotein (l)

p_{mp} = Density of the mycoprotein (kg/l)

p_{GM} = Density of unprocessed growth medium (kg/l)

L = liter

M_{mps} = Mycoprotein solids in the media before RNA reduction (kg)

M_{hmp} = Kilograms of harvestable mycoprotein (solids + remaining media) per liter (kg/L)

V_E = Liters extracted per hour to reach hourly production (l/h)

M_{mpt} = Desired kilograms of mycoprotein produced per hour (kg/h)

V_{fc} = Total needed fermentation capacity (liters) to reach hourly production

μ = Specific growth rate of organism (h^{-1})

Q_{ABmax} = Quantity of max capacity tanks needed (l)

V_{OAB} = Working volume of the other airlift bioreactor needed (l)

f_{Aj} = Inflation correction factor

$CEPCI$ = Chemical Engineering Plant Cost Index

f_{MCj} = Material composition factor

P_{cs} = Percentage of equipment composition (carbon steel)

r_{cs} = Relative price of carbon steel

P_{las} = Percentage of equipment composition (low alloy steel Cr-Mo)

r_{las} = Relative price of low alloy steel Cr-Mo

P_{ns} = Percentage of equipment composition (nickel steel (9%))

r_{ns} = Relative price of nickel steel (9%)

P_{304} = Percentage of equipment composition (stainless steel 304)

r_{304} = Relative price of stainless steel 304

P_{321} = Percentage of equipment composition (stainless steel 321)

r_{321} = Relative price of stainless steel 321

P_{316} = Percentage of equipment composition (stainless steel 316)

r_{316} = Relative price of stainless steel 316

P_{310} = Percentage of equipment composition (stainless steel 310)

r_{310} = Relative price of stainless steel 310

P_{hns} = Percentage of equipment composition (high nickel stainless steel)

r_{hns} = Relative price of high nickel stainless steel

P_{Cu} = Percentage of equipment composition (copper)

r_{Cu} = Relative price of copper

P_{Al} = Percentage of equipment composition (aluminum)

r_{Al} = Relative price of aluminum

P_{Ni} = Percentage of equipment composition (nickel)

r_{Ni} = Relative price of nickel

P_{m} = Percentage of equipment composition (monel)

r_{m} = Relative price of monel

P_{Ti} = Percentage of equipment composition (titanium)

r_{Ti} = Relative price of titanium

C_{eq} = Equipment costs (USD)

C_{Uj} = Unit cost (USD)

U_{aj} = Equipment sizing unit

U_{j} = Base equipment sizing unit

f_{s} = Scale factor

C_{F} = Fixed equipment costs (USD)

f_{L} = Lang factor

C_{FM} = Fixed manufacturing costs (USD)

f_{FM} = Fixed manufacturing cost factor

V_{RNA} = RNA reduction vessel working volume

f_{RNA} = RNA reduction factor estimated as percentage of airlift bioreactor working capacity

M_{CFmax} = Max scale centrifuge processing load (kg/s)

$M_{CFother}$ = Other centrifuge processing load (kg/s)

$M_{mp\ chilled}$ = Total mycoprotein mass chilled per day (kg/day)

C_{FVC} = Fixed equipment costs for vacuum chiller (USD)

$U_{VC\#}$ = Quantity of vacuum chiller units

f_{AVC} = Inflation factor for vacuum chiller

f_{LVC} = Lang factor for vacuum chiller

f_{MCVC} = Material composition factor for vacuum chiller

C_{VC} = Cost for vacuum chilling unit (USD/kg-day)

M_F = Mass of product stored at one time per freezer unit (kg)

t_{fstore} = Time in freezer storage for texture development (h)

N_F = Number of freezer units

V_P = Minimum volume of product stored in freezer space per unit (m^3)

V_F = Minimum volume of freezer space per unit (m^3)

P_{Vp} = Percentage of minimum volume of freezer space needed for storage (must be >100%)

$C_{Ffreezer}$= Fixed equipment costs for storage freezers (USD)

f_{AF} = Storage freezer inflation factor

C_{SFU} = Storage freezer unit costs (USD/m^3)

C_{FQ} = Fixed equipment costs for Quorn production (USD)

C_{Fmix} = Fixed equipment costs for mixer (USD)

C_{Fform} = Fixed equipment costs for former (USD)

C_{Fcook} = Fixed equipment costs for steam cooker (USD)

C_{Fchill} = Fixed equipment costs for chiller (USD)

C_{Fsize} = Fixed equipment costs for size reduction equipment (USD)

G_m = Media needed for each growth phase (l)

G_{Am} = Annual media requirement for growth phase (l)

V_{oc} = Volume of mycoprotein and media removed and replaced during an hour of continuous operation (l)

V_m = Total volume of media needed for first year of production (l)

M_{Amp} = Annual mass of mycoprotein produced (kg)

M_{Apro} = Annual mass of protein produced from mycoprotein (kg)

M_{mGLU} = Minimum mass of glucose utilized for mycoprotein production (kg)

G_{con} = Glucose consumed per kilogram of protein produced by F. venenatum

G_l = Glucose concentration in growth medium (g/l)

G_{add} = Glucose additional glucose in a liter of growth medium (g/l)

C_{GLU} = Annual glucose costs (USD)

$G_{\$}$ = Price of glucose per mass unit (USD/g)

$O_{2\ con}$ = Oxygen consumed during each hour of continuous operation (O_2 g/ L h)

$O_{2\ cell}$ = Oxygen consumed per gram of cells each hour (O_2 g/cell g h)

O_2 = Minimum annual oxygen utilized during continuous operation (g/year)

C_{O2} = Minimum annual oxygen costs (USD)

$O_{2\$}$ = Cost of oxygen per mass unit (USD/g)

$O_{2\ own\$}$ = Cost of oxygen production and ownership (USD/year m^3)

NH_3 = Minimum annual ammonia mass (g)

f_{proN} = Conversion factor to convert protein to nitrogen

f_{Nammo} = Conversion factor to convert nitrogen to ammonia

$NH_{3\ L}$ = Total ammonia per liter of growth medium (g/l)

$NH_{3\ add}$ = Additional ammonia per liter of growth medium (g/l)

C_{NH3} = Minimum annual ammonia cost (USD/year)

$NH_{3\,\$}$ = Cost of ammonia per mass unit (USD/g)

C_{com} = Total cost of other growth medium components (USD)

C_i = Cost of other growth medium component (USD/g)

M_i = Mass of other component in growth medium (g/L)

C_{EP} = Cost of energy per kWh from public supplier (USD/kWh)

C_{NG} = Cost of natural gas (USD/1000 m^3)

C_{bT} = Cost of self-generated electric/energy per kWh from a boiler-turbine system (USD/kWh)

C_{NGP} = Natural gas cost (USD/kwH)

ϵ_{bT} = Boiler energy efficiency

C_E = Cost of energy per kWh (USD/kWh)

f_{EP} = Fraction of energy produced from public supplier

f_{bT} = Fraction of energy from a boiler turbine system

E_{Sm} = Estimation of annual energy used to sterilize growth medium (kJ)

M_{Am} = Mass of growth medium utilized annually (kg)

ΔT = Change in temperature (C°)

W_{C_v} = Isobaric specific heat of water (kJ/kg K)

$\in_{Sm}$ = Energy efficiency of sterilization system

E_{Cm} = Estimation of annual energy used to cool growth medium entering bioreactor (kJ)

$\in_{Cm}$ = Energy efficiency of cooling system

E_{RNAm} = Estimation of annual energy used to heat growth medium during RNA reduction step (kJ)

$\in_{RNAm}$ = Energy efficiency of RNA reduction system

E_{VCmp} = Estimation of annual energy used to cool mycoprotein via vacuum chilling (kJ)

$\in_{VCmp}$ = Energy efficiency of vacuum chiller

E_{airmp} = Estimation of annual energy utilized for plant compressed air production (mycoprotein) (MJ)

f_{airmp} = Compressed air energy factor (mycoprotein)

E_{Tmp} = Estimated minimum energy in mycoprotein plant (kJ)

C_{Emp} = Estimated minimum energy costs in mycoprotein plant (kJ)

E_{SCQ} = Estimated minimum annual energy usage for steam cooking of Quorn (kJ)

$\in_{SCQ}$ = Energy efficiency of steam cooker

M_{AQ} = Mass of Quorn produced annually (kg)

cp_Q = Estimated specific heat of Quorn (kJ/kg K)

E_{CQ} = Estimated minimum annual energy usage for chilling of Quorn (kJ)

$\in_{CQ}$ = Energy efficiency of chiller

E_{BQ} = Energy required size reduction equipment (kW)

k_d = size reduction coefficient

w = Work index (kJ/kg)

D_2 = Output particle size (m)

D_1 = Input particle size (m)

E_{ASR} = Annual size reduction equipment energy estimates (kJ)

E_F = Annual Freezer energy estimates (kJ)

SEC = Annual Electricity Consumption (kWh)/ Storage Volume (m^3)

E_{airQ} = Estimation of annual energy utilized for plant compressed air production (Quorn) (kJ)

f_{airQ} = Compressed air energy factor (Quorn)

E_{TQ} = Estimated minimum energy in Quorn production plant (kj/year)

C_{EQ} = Estimated energy costs of Quorn production (USD)

C_W = Annual process water and wastewater costs (USD/year)

C_{PW} = Process water costs (USD/m^3)

C_{WF} = Wastewater filtration costs (USD/m^3)

C_{BO} = Biological oxidation of wastewater costs (USD/m^3)

P = Required manpower for operation

P_j = Typical manpower requirement for process/equipment

j = Individual process/ piece of equipment

f_{lab} = Labor cost correction factor

f_C = Country effect factor

f_{Sca} = Supervising and clerical assistance factor

f_T = Advanced technological and automating factor

f_Q = Skilled and qualified level of the personnel

f_B = Social benefit factor

f_O = Overtime work factor

C_{Lab} = Estimated annual labor costs

C_L = Production worker hourly rate

C_{opmp} = Minimum operating expenditures for mycoprotein production (USD/y)

C_{Wmp} = Annual process water and wastewater costs for mycoprotein (USD/y)

C_{Labmp} = Estimated annual labor costs for mycoprotein production (USD/y)

C_{opQ} = Minimum operating expenditures for Quorn production (USD/y)

C_{FOpmp} = Annual fixed operating costs for mycoprotein production other than financing costs (USD/y)

C_{mp} = Cost of mycoprotein (USD/y)

C_{comQ} = Cost of other components in Quorn product (USD/y)

C_{WQ} = Annual process water and wastewater costs for Quorn production (USD/y)

C_{LabQ} = Estimated annual labor costs for Quorn production (USD/y)

C_{FOpQ} = Annual fixed operating costs for Quorn production other than financing costs (USD/y)

EQ_r =Equity ratio

D_r = Debt ratio

C_D = Total debt costs (USD)

Equations

Equation 1. Mass of unprocessed growth medium in one kilogram of unprocessed growth medium and mycoprotein

$$M_{GM*} = 1 - M_{mp*}$$

Equation 2. Mass of mycoprotein solids in one kilogram of RNA reduced growth medium and mycoprotein

$$M_{mp} = M_{mp*} \times (1 - R_{\%})$$

Equation 3. Mass of growth medium in one kilogram of RNA reduced growth medium and mycoprotein

$$M_{GM} = 1 - M_{mp}$$

Equation 4. Mass Media removed per kilogram of finished mycoprotein

$$M_{GMr} = \frac{S_{mp}}{M_{mp}} M_{GM} - (1 - S_{mp})$$

Equation 5. Estimated specific heat of mycoprotein

$$cp_{mp} = 1.424\, c_{mp\%} + 1.54\, p_{mp\%} + 1.675 f_{mp\%} + 0.837 a_{mp\%} + 4.187 w_{mp\%}$$

Equation 6. Total production cycle time

$$t_{PC} = t_g + t_c + t_s$$

Equation 7. Cycles completed annually

$$n_c = \frac{t_{op}}{t_{PC}}$$

Equation 8. Time of full cycles

$$t_{fc} = n_c^* \times t_{PC}$$

Equation 9. Annual time in continuous production for full cycles

$$t_{ac} = n_c^* \times t_c$$

Equation 10. Time in continuous production for the partial cycle

$$t_{ptc} = (n_c - n_c^*)t_{PC} - t_g - t_s$$

Equation 11. Total annual time in continuous production

$$t_{tc} = t_{ac} + t_{ptc}$$

Equation 12. Volume of 1 kilogram of unprocessed growth medium and mycoprotein

$$V_{GM+mp} = \frac{M_{mp*}}{p_{mp}} + \frac{M_{GM*}}{p_{GM}}$$

Equation 13. Mass of unprocessed growth medium and mycoprotein per liter

$$M_{GM+mp} = \frac{1\ L}{V_{GM+mp}}$$

Equation 14. Mycoprotein Solids in the growth medium before RNA reduction

$$M_{mps*} = M_{GM+mp} \times M_{mp*}$$

Equation 15. Mycoprotein solids in growth medium after RNA reduction

$$M_{mps} = M_{mps*} \times (1 - R_{\%})$$

Equation 16. Kilograms of harvestable mycoprotein (solids + remaining media) per liter

$$M_{hmp} = \frac{M_{mps}}{S_{mp}}$$

Equation 17. Liters extracted per hour to reach hourly production goal

$$V_E = \frac{M_{mpt}}{M_{hmp}}$$

Equation 18. Total needed fermentation capacity (liters) to reach hourly production

$$V_{fc} = \frac{V_E}{\mu}$$

Equation 19. Percentage of max airlift bioreactor capacity

$$V_{max\%} = \frac{V_{fc}}{V_{max}}$$

Equation 20. Quantity of max capacity tanks needed

$$Q_{ABmax} = V_{max\%} \ (convert\ to\ whole\ number\ and\ round\ towards - \infty)$$

Equation 21. Working volume of the other airlift bioreactor needed

$$V_{OAB} = (V_{max\%} - Q_{ABmax}) V_{max}$$

Equation 22. Total quantity of airlift bioreactors needed

$$Q_{AB} = Q_{ABmax} + 1$$

Equation 23. Inflation correction factor calculation

$$f_{Aj} = \frac{CEPCI\ (current)}{CEPCI\ (reference)}$$

Equation 24. Material composition factor

$$f_{MCj} = P_{cs}r_{cs} + P_{las}r_{las} + P_{ns}r_{ns} + P_{304}r_{304} + P_{321}r_{321} + P_{316}r_{316} + P_{310}r_{310} + P_{hns}r_{hns} + P_{Cu}r_{Cu} + P_{Al}r_{Al} + P_{Ni}r_{Ni} + P_{mo}r_{mo} + P_{Ti}r_{Ti}$$

Equation 25. Equipment costs equation

$$C_{eq} = \sum_{j} f_{Aj}\, f_{MCj} C_{Uj} \left(\frac{U_{aj}}{U_j}\right)^{f_s}$$

Equation 26. Fixed equipment costs

$$C_F = f_L\, C_{eq}$$

Equation 27. Fixed manufacturing costs

$$C_{FM} = f_{FM}\, C_F$$

Equation 28. RNA reduction vessel working volume

$$V_{RNA} = V_{AB}\, f_{RNA}$$

Equation 29. Max scale centrifuge processing load

$$M_{CFmax} = \left(\frac{V_{max}}{V_{fc}}\right) V_E\, p_{mp+GM}$$

Equation 30. Other centrifuge processing load

$$M_{CFother} = \left(\frac{V_{OAB}}{V_{fc}}\right) V_E p_{mp+GM} \;\; if\ less\ than\ one\ round\ towards\ \infty$$

Equation 31. Total mycoprotein mass chilled per day

$$M_{mp\ chilled} = M_{mpt} \times 24$$

Equation 32. Fixed equipment costs for vacuum chiller

$$C_{FVC} = U_{VC\#} f_{AVC} f_{LVC} f_{MCVC} M_{mp\ chilled} C_{VC}$$

Equation 33. Total fixed equipment costs for mycoprotein production

$$C_{Fmp} = C_{FAB} + C_{FRNA} + C_{FC} + C_{FVC}$$

Equation 34. Mass of product stored at one time per freezer unit

$$M_F = \frac{M_{mpt} t_{fstore}}{N_F}$$

Equation 35. Minimum volume of product stored in freezer space per unit

$$V_P = \frac{M_F}{p_{mp}}$$

Equation 36. Minimum volume of freezer space per unit

$$V_F = V_P P_{Vp}$$

Equation 37. Fixed equipment costs for storage freezers

$$C_{Ffreezer} = f_{AF} N_F V_F C_{SFU}$$

Equation 38. Fixed equipment costs for Quorn production

$$C_{FQ} = C_{Fmix} + C_{Fform} + C_{Fcook} + C_{Fchill} + C_{Fsize} + C_{Ffreezer}$$

Equation 39. Media needed for each growth phase

$$G_m = V_{fc}$$

Equation 40. Annual media requirement for growth phase

$$G_{Am} = G_m n_c^*$$

Equation 41. Volume of mycoprotein and media removed and replaced during an hour of continuous operation

$$V_{oc} = G_m \mu$$

Equation 42. Total volume of media needed for first year of production

$$V_m = G_{Am} + V_{oc} t_{tc}$$

Equation 43. Annual mass of mycoprotein produced

$$M_{Amp} = M_{mpt} t_{tc}$$

Equation 44. Annual mass of protein produced from mycoprotein

$$M_{Apro} = p_{mp\%} M_{Amp}$$

Equation 45. Minimum mass of glucose utilized for annual mycoprotein production

$$M_{mGLU} = G_{con} M_{Apro}$$

Equation 46. Glucose per liter of growth medium

$$G_l = \frac{M_{mGLU}}{V_m} + G_{add}$$

Equation 47. Annual glucose costs

$$C_{GLU} = G_l V_m G_\$$$

Equation 48. Oxygen consumed during each hour of continuous operation (O_2 g/ L h)

$$O_{2\,con} = O_{2\,cell} M_{mp*}$$

Equation 49. Minimum annual oxygen utilized during continuous operation

$$O_2 = O_{2\,con} t_{tc} V_{fc}$$

Equation 50. Minimum annual oxygen costs

$$C_{O2} = O_2 O_{2\$} + V_{fc}\, O_{2\,own\$}$$

Equation 51. Minimum annual ammonia mass

$$NH_3 = M_{Apro} f_{proN} f_{Nammo}$$

Equation 52. Ammonia utilized per liter

$$NH_{3\,L} = \frac{NH_3}{V_m} + NH_{3\,add}$$

Equation 53. Minimum annual ammonia cost

$$C_{NH3} = NH_{3\,L} V_m NH_{3\,\$}$$

Equation 54. Total annual costs of other growth medium components

$$C_{com} = V_m \sum_i C_i M_i$$

Equation 55. Cost of energy per kWh from public supplier

$$C_{EP} = 0.0969 C_{NG} + 6.78$$

Equation 56. Cost of self-generated electric/energy per kWh from a boiler-turbine system

$$C_{bT} = \frac{C_{NGP}}{\epsilon_{bT}}$$

Equation 57. Cost of energy per kWh

$$C_E = f_{EP} C_{EP} + f_{bT} C_{bT}$$

Equation 58. Estimation of annual energy used to sterilize growth medium

$$E_{Sm} = \frac{M_{Am} \times \Delta T \times W_{C_v}}{\in_{Sm}}$$

Equation 59. Estimation of annual energy used to cool growth medium entering bioreactor

$$E_{Cm} = \frac{M_{Am} \times \Delta T \times W_{C_v}}{\in_{Cm}}$$

Equation 60. Estimation of annual energy used to heat growth medium during RNA reduction step

$$E_{RNAm} = \frac{M_{Am} \times \Delta T \times W_{C_v}}{\in_{RNAm}}$$

Equation 61. Estimation of annual energy used to cool mycoprotein via vacuum chilling

$$E_{VCmp} = \frac{M_{Amp} \times \Delta T \times cp_{mp}}{\in_{VCmp}}$$

Equation 62. Estimation of annual energy utilized for plant compressed air production (mycoprotein)

$$E_{airmp} = \left(E_{Sm} + E_{Cm} + E_{RNAm} + E_{VCmp}\right) f_{airmp}$$

Equation 63. Estimated minimum energy in mycoprotein plant

$$E_{Tmp} = E_{Sm} + E_{Cm} + E_{RNAm} + E_{VCmp} + E_{airmp}$$

Equation 64. Estimated minimum energy costs in mycoprotein plant

$$C_{Emp} = C_E E_{Tmp}$$

Equation 65. Estimated minimum annual energy usage for steam cooking of PQP

$$E_{SCQ} = \frac{M_{AQ} \times \Delta T \times cp_Q}{\in_{SCQ}}$$

Equation 66. Estimated minimum annual energy usage for chilling of PQP

$$E_{CQ} = \frac{M_{AQ} \times \Delta T \times cp_Q}{\in_{CQ}}$$

Equation 67. Bond law for size reduction equipment energy estimates

$$E_{BQ} = k_d w M_{mpt} \left(\frac{1}{\sqrt{D_2}} - \frac{1}{\sqrt{D_1}}\right)$$

Note: M_{mpt} is in kg/s for this equation

Equation 68. Annual size reduction equipment energy estimates

$$E_{ASR} = E_{BQ} t_{tc}$$

Equation 69. Annual Freezer energy estimates

$$E_F = SEC\ V_F$$

Equation 70. Estimation of annual energy utilized for plant compressed air production (PQP)

$$E_{airQ} = (E_{SCQ} + E_{CQ} + E_{ASR} + E_F) f_{airQ}$$

Equation 71. Estimated minimum energy in PQP production plant

$$E_{TQ} = E_{SCQ} + E_{CQ} + E_{ASR} + E_F + E_{airQ}$$

Equation 72. Estimated energy costs of PQP production

$$C_{EQ} = C_E E_{TQ}$$

Equation 73. Annual process water and wastewater costs

$$C_W = V_m\, C_{PW} + V_m\, C_{WF} + V_m\, C_{BO}$$

Equation 74. Required manpower for operation

$$P = \sum_{j=1}^{N} P_j$$

Equation 75. Labor cost correction factor

$$f_{lab} = f_C f_{Sca} f_T f_Q f_B f_O$$

Equation 76. Estimated annual labor costs

$$C_{Lab} = t_{op} f_{lab} C_L P$$

Equation 77. Minimum operating expenditures for mycoprotein

$$C_{opmp} = C_{GLU} + C_{O2} + C_{NH3} + C_{com} + C_{Emp} + C_{Wmp} + C_{Labmp} + C_{FOpmp}$$

Equation 78. Minimum operating expenditures for Quorn production

$$C_{opQ} = C_{mp} + C_{comQ} + C_{EQ} + C_{WQ} + C_{LabQ} + C_{FOpQ}$$

Equation 79. Equity ratio

$$EQ_r = 100\% - D_r$$

Equation 80. Total debt costs

$$C_D = C_F D_r$$

Equation 81. Total equity costs

$$C_{TEQ} = EQ_r \, C_F$$

Equation 82. Capital recovery factor for debt

$$f_{CRD} = I_D(1 + I_D)^{L_e} / \ ((1 + I_D)^{L_e - 1})$$

Equation 83. Capital recovery factor for equity

$$f_{CREQ} = I_{EQ}(1 + I_{EQ})^{L_e} / \ ((1 + I_{EQ})^{L_e - 1})$$

Equation 86. Annual debt payment

$$D_p = f_{CRD} C_D$$

Equation 87. Annual equity recovery

$$EQ_p = f_{CREq} C_{TEq}$$

Equation 88. Minimum annual cost of capital expenditures

$$C_{cap} = D_p + EQ_p$$

Equation 89. Total minimum annual cost

$$C_{total} = C_{cap} + C_{op}$$

Appendix B. Sensitivity analysis results

An OAT sensitivity analysis was conducted in the manner described in methods section. Input variables which caused a change greater than 3% from the base price were identified. These input variables were broadly categorized as into capital and operating expenditures (Figure 4.6 and 4.7). The results of the analysis were then used to inform our scenario design.

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Figures

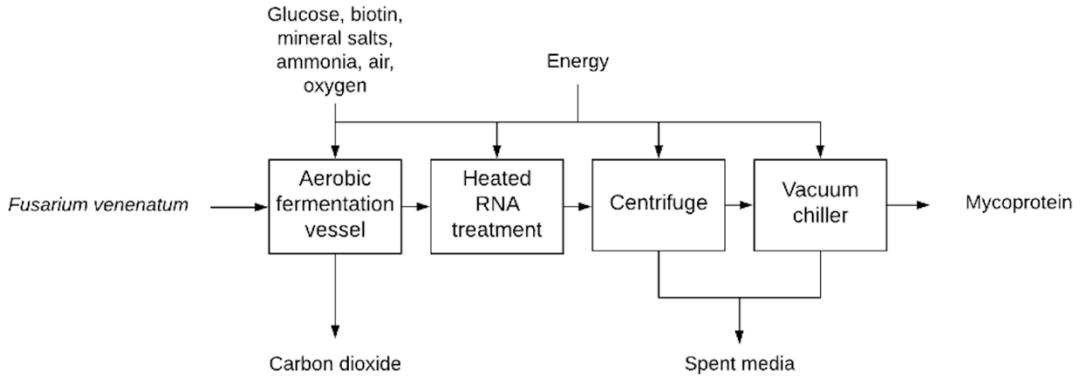


Figure 4.1 Mycoprotein production overview

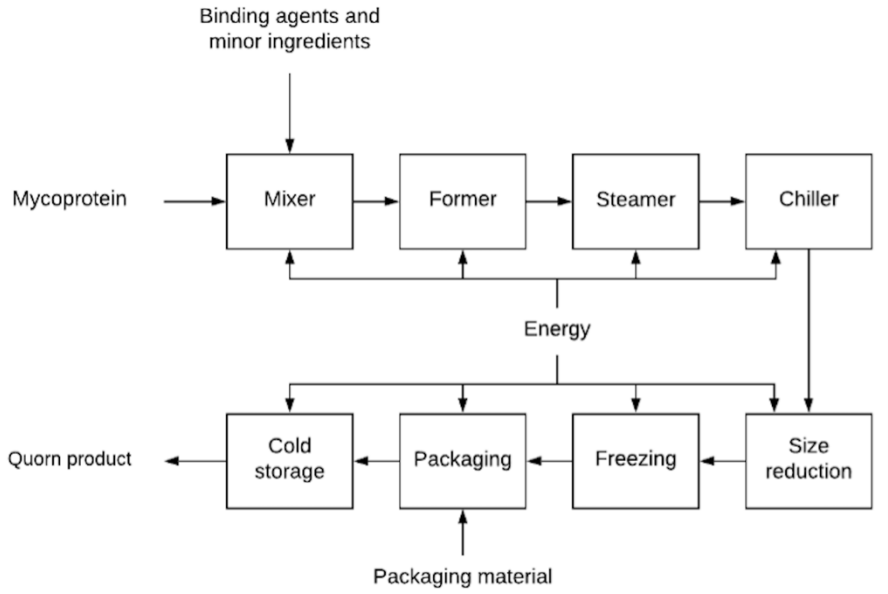


Figure 4.2 Simplified PQP production process

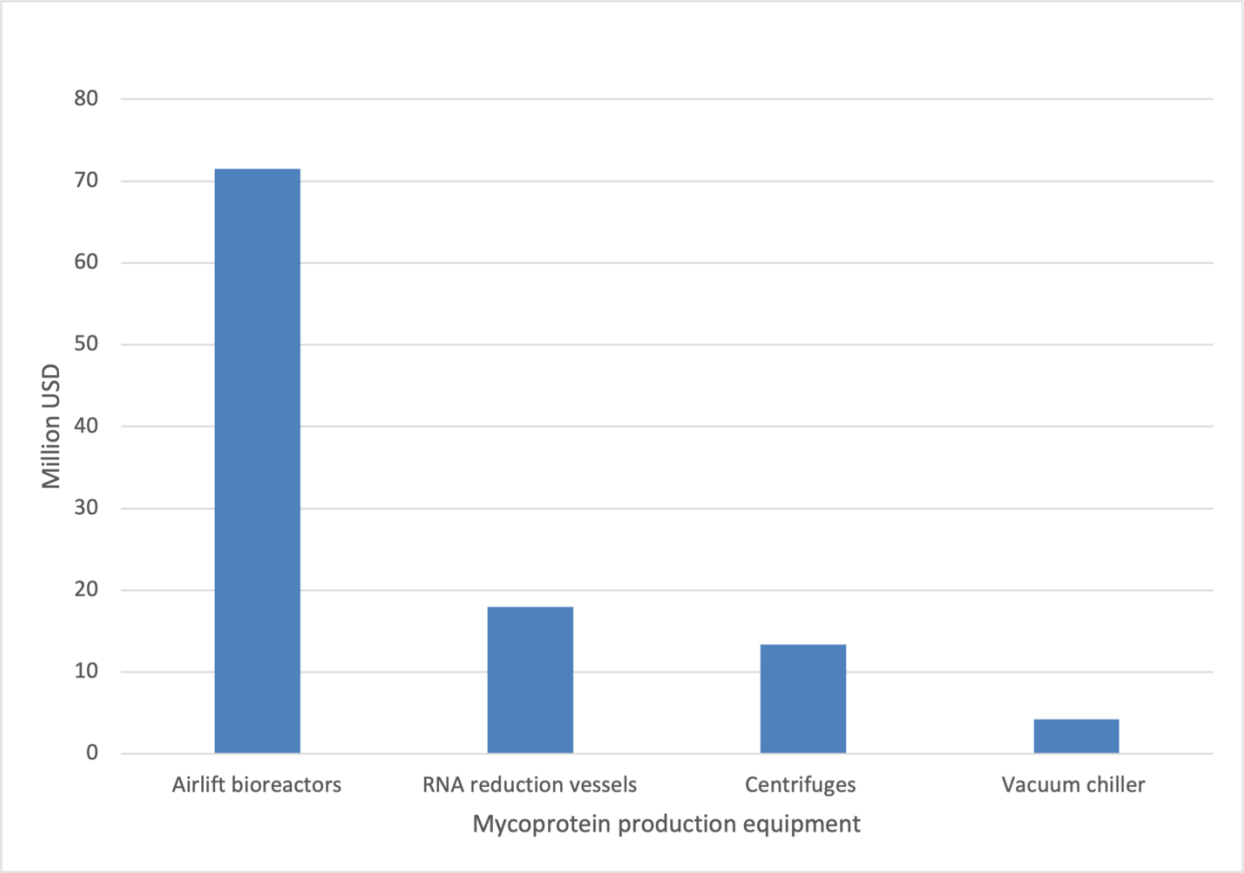
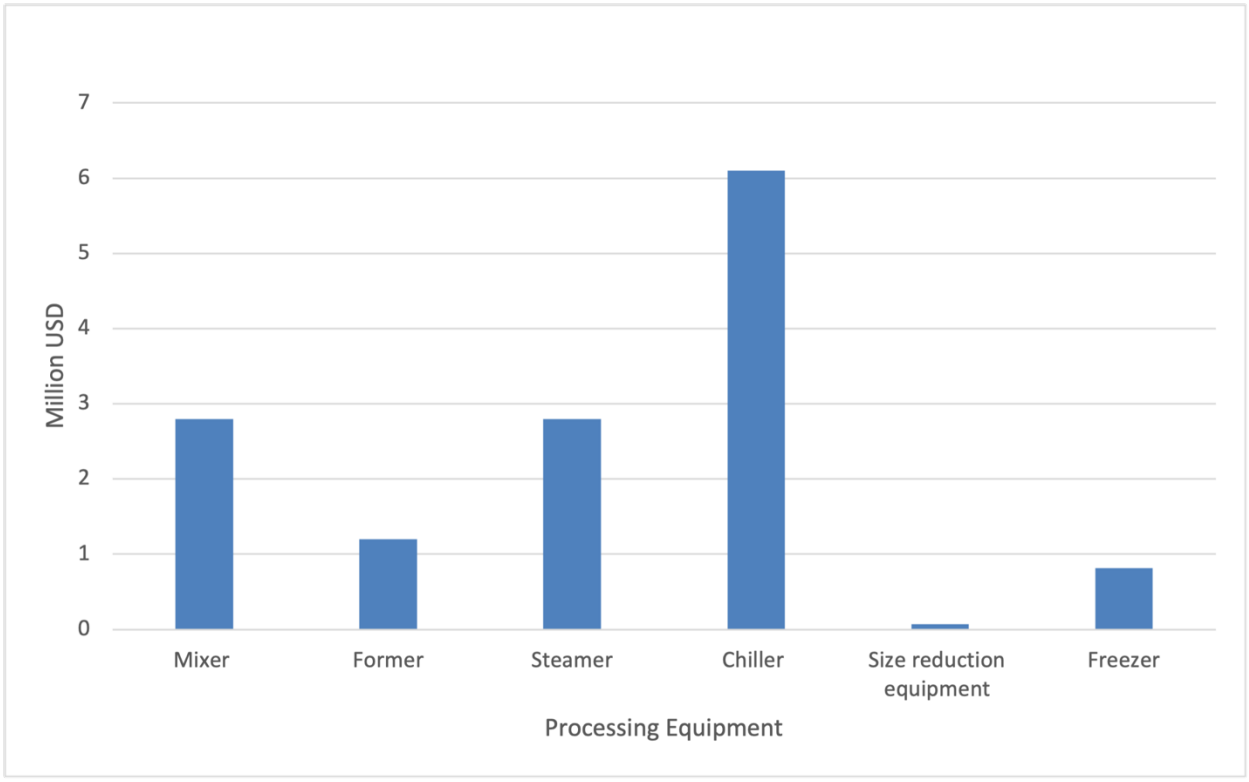


Figure 4.3 Capital costs before financing for mycoprotein for base scenario


7
6
5
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3
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1
0
Million USD
Mixer
Former
Steamer
Chiller
Size reduction equipment
Freezer
Processing Equipment

Figure 4.4 Capital costs before financing for Processed Quorn-like Product for base scenario

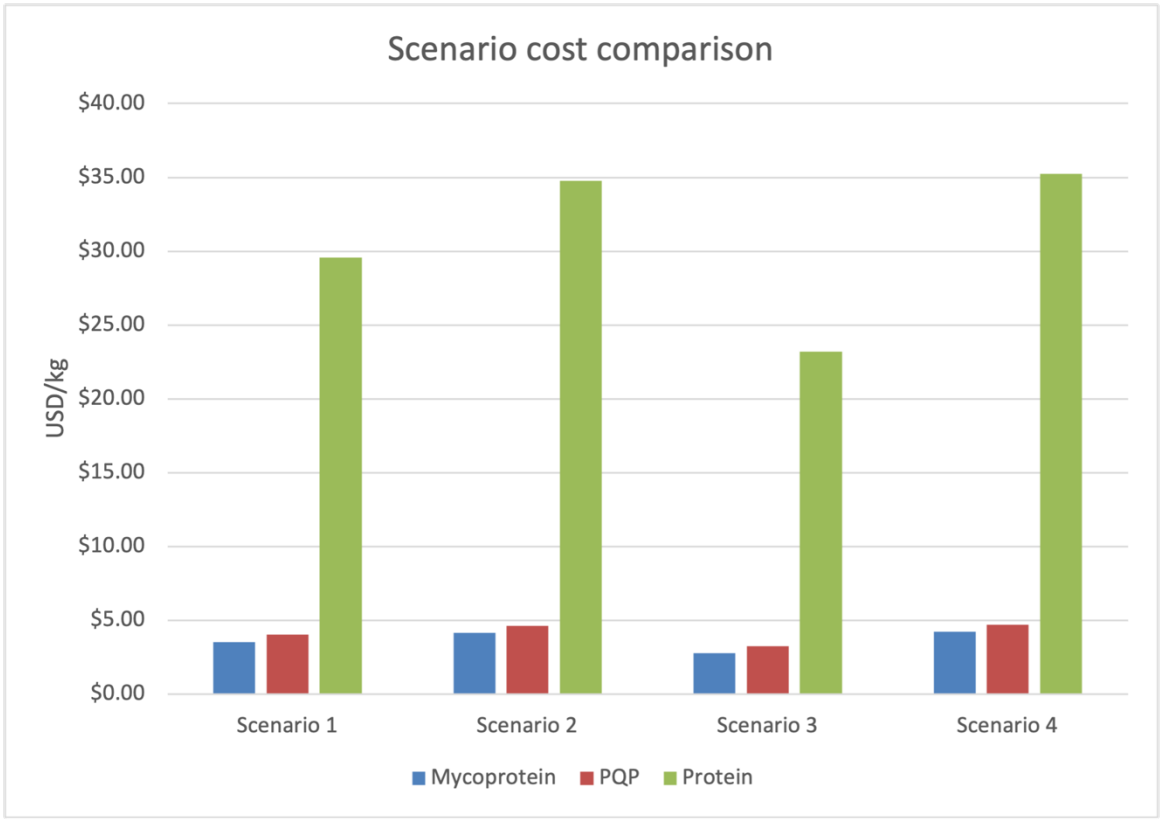

Figure 4.5 Annualized cost for each scenario

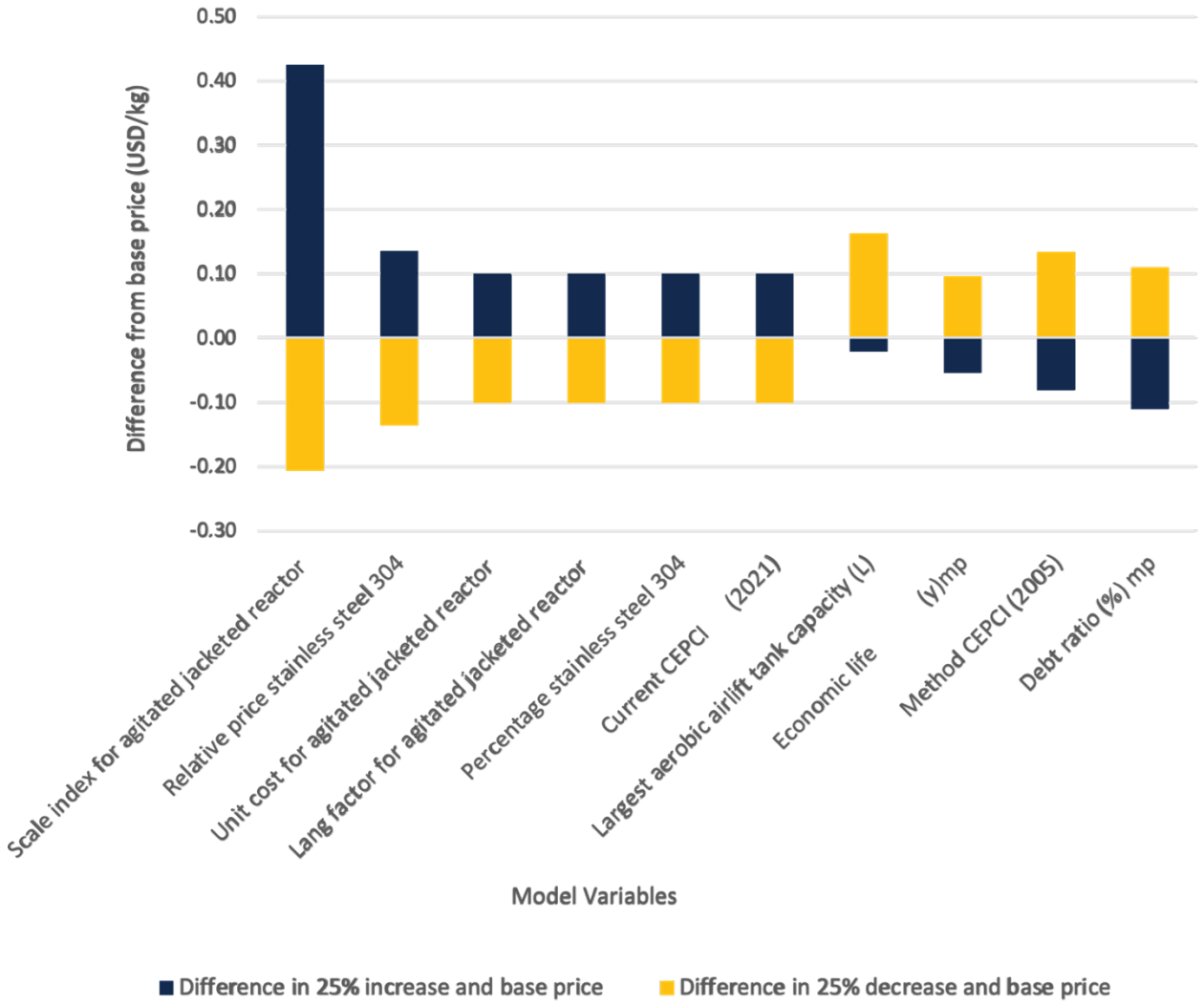

Figure 4.6 Sensitivity analysis results for capital expenditures of mycoprotein production

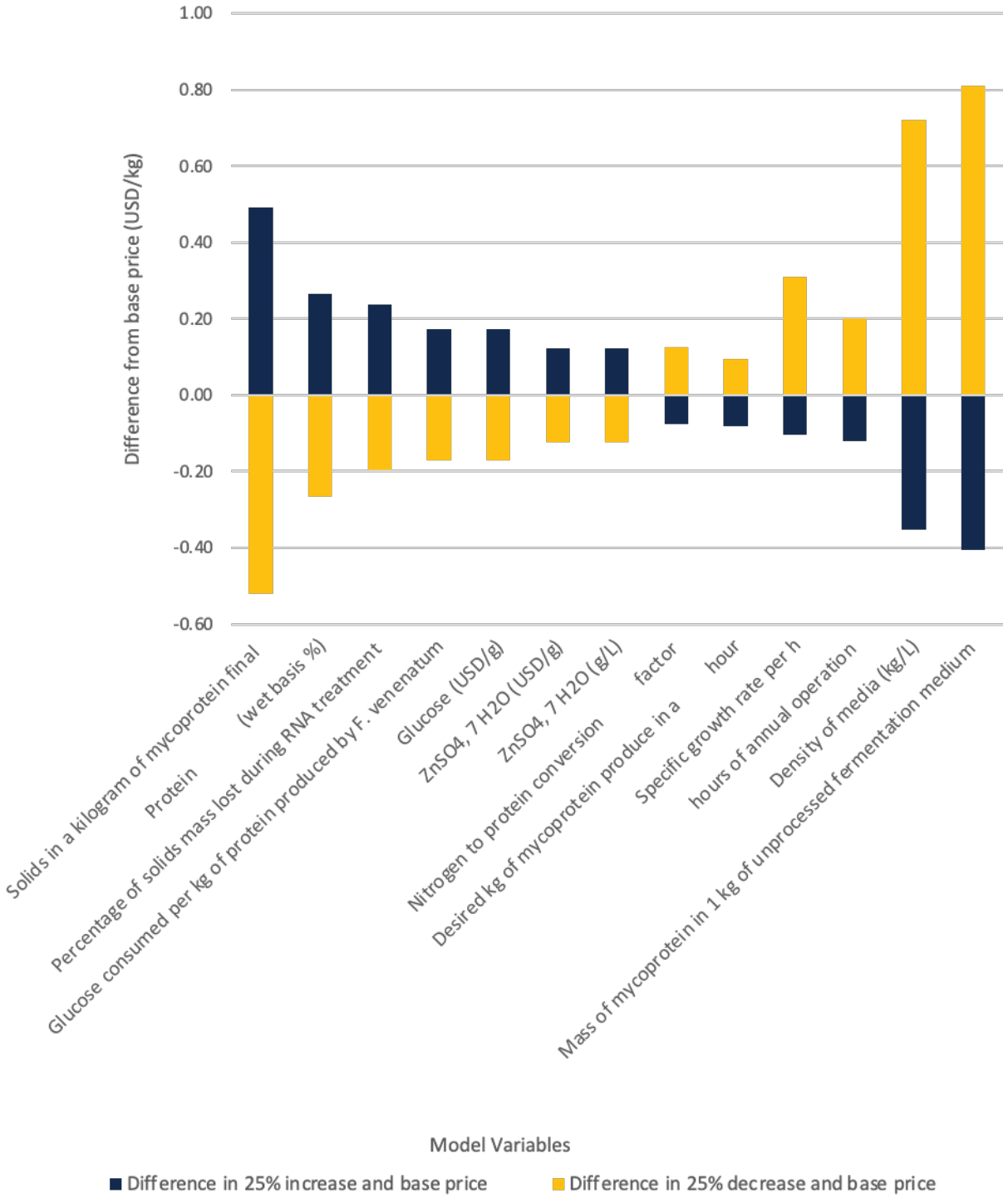

Figure 4.7 Sensitivity analysis for operating expenditures for mycoprotein production

Chapter 5. Cradle to production gate life cycle assessment of cultured meat growth media: A comparison of Essential 8 and Beefy-9

Journal article formatted for and awaiting submittal: Nature Foods

Cradle to production gate life cycle assessment of cultured meat growth media: A comparison of Essential 8™ and Beefy-9

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Abstract

There is an increasing interest in use of biotechnology as a means of sustainable manufacture; however, pharmaceutical biotechnology is resource and energy intensive. Recent interest in animal cell-based meat (ACBM) has prompted scientific and engineering questions about the economic and environmental viability of these proposed ACBM products. This study provides an environmental assessment of two proposed growth mediums (Essential 8™ and Beefy-9) for ACBM production. The study found that the addition of antibiotics/antimycotics (10,000 μg/mL) to the growth media increased the environmental metrics such as the cumulative energy demand and global warming potential by two orders of magnitude. To account for additional processing for animal cell culture a scenario analysis was conducted to assess the potential environmental impacts of growth medium component refinement. The study indicates that the refinement of the growth medium components may undermine the sustainability of future ACBM products.

Introduction

Biotechnology has historically been utilized to preserve and enhance food properties and is currently responsible for many pharmaceutical and bioindustrial achievements. The modern bio-based economy includes not just food and animal feed, but also bio-based chemicals, materials, health products, and bio-based fuel (Lange et al., 2021). Bio-based economies have been prescribed as sustainable and as a potential means to achieve the United Nations' Sustainable Development Goals (SDG) (Lange et al., 2021; United Nations, 2015). More specifically, in response to SDG 2 (End hunger, achieve food security and improved nutrition, and promote sustainable agriculture), bioprocessing technology utilizing bioreactors has been proposed for food/protein/meat production (Moritz et al., 2015; Post, 2012; E. A. Specht et al., 2018; L. Specht, 2019). Additionally, there has been sizable financial investment (>1 billion USD) in companies which aim to utilize bioreactor-based technology to produce animal cell-based meat or "cultured meat" (Risner et al., 2020; Turi, 2021).

In contrast, critics have raised concerns that a strong focus on developing a bio-based economy may actually hinder the achievement of some of the ecological SDGs (Fritsche & Iriarte, 2014; Heimann, 2019). For example, while pharmaceutical technology has produced many positive impacts for human health, it has been reported that the global pharmaceutical industry has an emission intensity 55% higher than the automotive industry (Belkhir & Elmeligi, 2019; Buxbaum et al., 2020). It has also been reported that the production of active pharmaceutical ingredients has a cumulative energy demand twenty-times greater than bulk chemical production (Wernet et al., 2010). These results indicate that additional efforts to critically examine of proposed biotechnological solutions for the SDGs are required to inform decision-making and investment in this space.

Previous efforts to quantify the environmental impact of cultured meat have been based on forward-looking projections and do not entirely account for all the inputs and processes required for animal cell culture (Mattick et al., 2015; Tuomisto et al., 2014; Tuomisto & Teixeira de Mattos, 2011). A

gap analysis of existing life cycle assessments (LCAs) of cultured meat specifically identified the need for a more robust environmental assessment of the animal cell growth media (Carus et al., 2019). Previous work also indicates that the volume of media required for industrial cultured meat production is a limiting economic factor (Risner et al., 2020). This indicates that quantification of the embedded resources within the animal cell growth media is necessary to evaluate the environmental impact of this potential food production technology.

Growth media for animal cell culture can vary in composition but can be broadly categorized as either "complex" or "defined" growth media. Complex media is inherently variable, containing components which are not completely chemically defined such as fetal bovine serum. This can introduce unknown factors which can affect animal cell proliferation and differentiation. The utilization of growth media containing animal-based components would also largely be contradictory to the "spirit" of the cultured meat products, especially in terms of the technology addressing the issue of animal welfare in conventional meat production systems. In contrast, defined media is chemically defined with set concentrations of proteins, amino acids, sugars, vitamins, minerals, salts, among other constituents.

Essential 8^{TM} (E8) is a defined growth medium which has been utilized and promoted as a viable growth medium for stem cells and animal cell-based meat production (Chen et al., 2011; Kolkmann et al., 2020; L. Specht, 2019; Verbruggen et al., 2018). The E8 growth medium was originally designed for researchers studying human induced pluripotent stem cells and embryonic stem cells. E8 was formulated as a consistent, defined medium to improve experiment reproducibility, but was not originally designed as a growth medium for industrial cell biomass production (Chen et al., 2011).

E8 is largely composed of Dulbecco's Modified Eagle Medium/Hams' F12 (DMEM/F12) basal medium, which is widely used for animal cell culture along with 7 other ingredients, including: 2-phospho-L-ascorbic acid trisodium salt, insulin, transferrin, sodium selenite, fibroblast growth factor-2 (FGF-2), transforming growth factor beta (TGF-β), and additional sodium bicarbonate. DMEM/F12 is

also the base for the recently developed animal cell growth medium, Beefy-9 (B9) (Andrew Stout et al., 2021). In addition to DMEM/F12, B9 contains the same components as E8 with the additional components of neuregulin, ultrapure water, antibiotics/antimycotics, and recombinant albumin (Andrew Stout et al., 2021).

In sum, understanding the environmental impacts of producing E8/E9 animal cell growth media requires identifying tracking, and consolidating the embedded resources utilized and waste outputs generated in the production of each media ingredient, assuming that all medium components are produced and purified individually and then mixed in the appropriate proportions during the media preparation. This is no small task considering that both E8 and B9 growth media are composed of more than 50 different input ingredients when DMEM/F12 is broken down into its constituent components. Thus, to understand the potential environmental impact of E8/B9 production, we include all these ingredients (or at least as many as could be included given data availability) in our comparative life cycle assessment (LCA).

Materials and methods

All of the individual components of the E8/B9 media were identified and categorized into eight broad categories (Figure 5.1) based upon their production method (Chen et al., 2011; L. Specht, 2019). Table 5.1 provides a breakdown of each growth media for composition. The LCA was conducted following the ISO 14040 and 14044 standards to estimate the potential environmental impact of each E8/B9 component (International Organization for Standardization, 2006a, 2006b). A combination of peer-reviewed literature, OpenLCA v.1.10 software, information from databases, stoichiometric calculations and engineering judgement was utilized to understand each E8/B9 component production process and to complete the LCA.

Table 5.1 Essential 8 and Beefy-9 growth medium composition

Component	**Concentration in Essential 8™ (**μg/mL)	**Concentration in Beefy-9 (**μg/mL)
DMEM/F12 basal media	-	-
2-Phospho-L-ascorbic acid trisodium salt	64	200
Insulin (human, recombinant)	19.4	20
Transferrin (human, recombinant)	10.7	20
Sodium selenite	0.014	0.02
Fibroblast growth factor (FGF-2)	0.1	0.04
Neuregulin (NRG1)	-	0.0001
Transforming growth factor (TGF-β3)	0.002	0.0001
UltraPure Water	-	58000
Antibiotic/Antimycotic	-	10000
Recombinant albumin	-	800
Additional $NaHCO_3$	543	-

Goal and Scope

The goal of this LCA was to estimate the environmental impact of current/near-term E8 and B9 growth medium production. It is hoped that the LCA results will provide clear environmental impact information

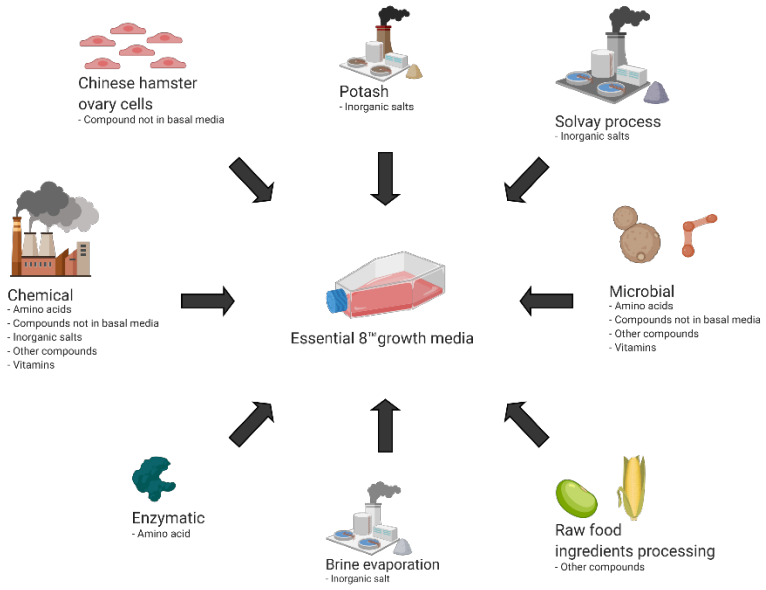
to producers utilizing large volumes of E8/B9 growth mediums or other mediums produced in a similar manner. The analysis was conducted as a cradle-to-production gate analysis. A system boundary was set at the cradle (raw material extraction) to the E8/B9 production facility gate. A limit of 0.1 kg reactant or precursor per kilogram of input was deemed the minimum limit to continue to track a component. For the sake of this study, precursor refers to a material/chemical used to produce an ingredient in E8/B9 growth medium (ex. starch hydrolysate is a precursor to glucose). The functional unit was defined as a liter of growth medium with the reported concentrations of each component (Andrew Stout et al., 2021; L. Specht, 2019). The functional unit was chosen to allow for comparison with other defined cell growth mediums.

Life cycle inventory (LCI)

Production process information was initially searched for in the ecoinvent (v.3.8) database. If available, then the material and energy input flows were tracked utilizing the ecoinvent (v.3.8) datasets (ecoinvent Association, 2021). If the initial production process information was not available in ecoinvent, then other literature sources and calculations were utilized to estimate material inputs and outputs (see following section and appendix A-H). Ecoinvent's global datasets were utilized throughout the life cycle inventory to limit the effect of geographic variation. The ecoinvent database can be examined with five different settings (undefined, allocation (cutoff by classification), allocation at the point of substitution, substitution (consequential, long term) and allocation (cut-off, EN15804)) which unlink or link datasets using several different methodologies. The database search was configured to "undefined" to maximize the LCI analysis transparency (ecoinvent Association, 2021). An undefined system model unlinks unit processes and allows for multiple outputs from each unit process. The flows and processes were then imported and configured in OpenLCA software which tracks inputs/outputs for a product system. The estimated material and energy flows should be considered non-exhaustive as some industrial production processes (e.g. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and lipoic acid production) were

excluded and other E8/B9 component production processes were only partially represented. It should also be noted that the reported E8/B9 component production processes are not to produce cell culture grade materials. Production of cell culture grade materials would likely require additional resources, and this is addressed in the scenario analysis section. The methods, calculations, limitations, and assumptions are detailed in the subsequent sections.

Figure 5.1 Broad production categories for E8/B9 components



Raw Food Ingredients

Corn was assumed to be the source for glucose due to being widely used for biorefining and food/beverage production in the United States (Capehart & Proper, 2021). Cottonseed oil production was utilized to estimate linoleic acid production due to the cottonseed oil fatty acid profile (Yang et al., 2019). Ecoinvent datasets were utilized to estimate the material flow for both glucose and linoleic acid. Appendix A provides details on the calculations and procedures utilized to determine the material flows of glucose and linoleic acid.

Microbial Fermentation Products

Components of E8/B9 which are or have potential to be produced via microbial fermentation were identified (Tables A1.0 and A2.0). The total mass of each component was determined from literature (Andrew Stout et al., 2021; Chen et al., 2011; L. Specht, 2019). The glucose mass requirement for each component was determined utilizing microbial yields (g product/g glucose) and microbial titers (g/L of media) from literature sources (see appendix B). Microbial yields with greater than 0.01 g product/g glucose were utilized if available in literature since the glucose concentration can vary depending on organism growth requirements, fermentation system, and operating parameters (Wu & Maravelias, 2018).

When a microbial yield was unavailable for a growth medium component, microbial titers (g/L) from the literature were utilized to estimate the required mass of glucose. The glucose concentration of the media was assumed to be 10 g/L for calculations which utilized titer to estimate the required glucose mass. A batch system without the capabilities to add nutrients/glucose was assumed. Given this assumption, a glucose concentration of 10 g/L was deemed acceptable (Millipore Sigma, n.d.).

The inputs/outputs other than glucose for microbially-produced compounds were estimated utilizing data from industrial lysine production as a proxy system. Varying yields between compounds indicated that a correction factor was necessary, i.e. more resources are utilized if more batches are required for the same mass of product. Each correction factor was calculated utilizing the reported lysine yield and the reported compound yields (Marinussen & Kool, 2010). When microbial titer was reported and utilized in the model, an assumed glucose concentration (10 g/L) was used to calculate the correction factor. Table A1 and A2 in appendix B provide correction factors and sources for yields and titers (See calculations A2 and A3 in appendix B).

Enzyme-derived Products

The embedded resources for the enzymatic production of E8/B9 components were estimated utilizing a similar approach as the microbial method previously described in microbial fermentation products section. L-aspartic acid was the only E8/B9 component identified to be produced enzymatically and the description of the assumed process can be found in appendix C.

Chemical Products

The ecoinvent database was utilized to estimate embedded energy and material flows for compounds produced via chemical synthesis (ecoinvent Association, 2021). If the ecoinvent datasets were not available, reported production methods for the compounds were analyzed and stoichiometric calculations were conducted to determine mass of E8/B9 component precursors (reactants). This process was repeated if the E8/B9 precursor was not available in the ecoinvent dataset. Figure 5.2 provides an example of this process with the components encased in the red outline having datasets available in ecoinvent and these datasets are utilized to account for the environmental impact of each component. Substitution was also utilized if the ecoinvent dataset was not available for an E8/B9 component (ex. ascorbic acid was substituted for ascorbic acid 2-phosphate). Compounds which were utilized in the production of more than one E8/B9 component were accounted for separately. The material and energy flows for these compounds were accounted for in OpenLCA and their material and energy flows were utilized as they appeared as inputs for multiple growth medium components. Table A5.3 in appendix D provides a list of each component and the components' precursors. If industrial production information was unavailable, embedded resources could not be quantified and/or a reasonable substitute could not be identified, then no data were entered for these components. Components without data were still entered into OpenLCA, but without any inputs or outputs. It should be noted that the described method for

estimating the inputs and outputs should be considered non-exhaustive due to some of these gaps in the data.

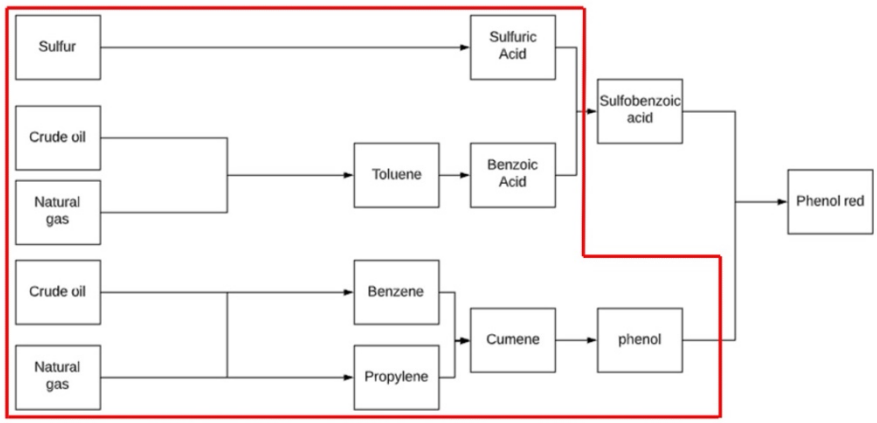


Figure 5.2 Phenol Red example for ecoinvent dataset utilization.

Compounds within the red outline have ecoinvent datasets which account for their material and energy flows. The material and energy flows are not accounted for components outside the red outline. Stoichiometric calculations (theoretical yield utilized) were conducted to estimate mass of each compound when information was not available in ecoinvent (Ex. A minimum of 0.519 kg of sulfobenzoic acid and 0.5311 kg of phenol is needed to produce 1 kg of phenol red).

Solvay and Potash

These categories of E8/B9 components utilize soda ash or potash as major components in their manufacture. For these components, both ecoinvent and available literature estimates were utilized in the same manner as previously described in the chemical category.

Brine evaporation

Sodium chloride is assumed to be produced from a mix of brine and mining operations. Sodium chloride in brine is utilized for soda ash production and is accounted for by utilizing the brine production dataset which does not include cleaning and drying steps. Sodium chloride utilized for other component

production processes or as an E8/B9 component are assumed to be produced from a mix of brine and mining operations. The reported embedded resources for non-soda ash related sodium chloride production include extraction, drying, and purification.

E8/B9 Components and precursors utilized in multiple production processes

Several E8/B9 component precursors are utilized in the production of multiple E8/B9 components. The material and energy flows necessary to produce these components were accounted for utilizing ecoinvent datasets (ecoinvent Association, 2021). Appendix G lists the components that are utilized in the production of multiple E8/B9 components.

Animal cell-produced product

TGF-b an be produced using animal cell culture (Beatson et al., 2011; Zou & Sun, 2004). One advantage of producing TGF- b ia animal cell culture rather than a more traditional fermentation organism like *Escherichia coli* is absence of endotoxin. One disadvantage is that the growth medium must be suitable for animal cell culture which has more complex nutrient requirements. To explore the environmental impact of utilizing animal cells for growth factor production, it was assumed that TGF-b was produced via animal cell culture. Chinese hamster ovary (CHO) cells are the most used animal cell line and are particularly important for glycoprotein overexpression (Beatson et al., 2011; Zou & Sun, 2004). CHO cells require a more complex growth medium as compared to more basic media inputs used for bacteria or yeast growth. DMEM/F12 was utilized as the basal medium for E8/B9 and was deemed to be an acceptable growth medium for CHO cells. The CHO cells were assumed to not require the other 7 components of E8/B9 (ascorbic acid 2-phosphate, additional $NaHCO_2$, sodium selenite, insulin Transferrin‡ and FGF-2) (L. Specht, 2019). The material and energy flows were estimated for TGF-b utilizing the data collected for the basal medium production and reported titers of TGF-b.

Components not included in assessment

The following components are not accounted for due to the authors' inability to find either production data or environmental impact data.

Lipoic Acid

Lipoic acid was first chemically synthesized in the 1950s (Colingsworth et al., 1952). At an industrial scale, lipoic acid is currently chemically synthesized in three stages (National Center for Biotechnology, n.d.). There has been interest expressed in utilizing biotechnology for lipoic acid production and the overproduction of lipoic acid has been reported in genetically modified *E. coli* (Sun et al., 2017). Despite the advances in biotechnology, lipoic acid is largely produced via chemical synthesis. Data related to the energy and material flows during the chemical synthesis of lipoic acid were not able to be obtained and thus are not included in the LCA.

HEPES

A-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, HEPES is a hydrogen ion buffer which is commonly utilized in cell culture (Good et al., 1966). The procedure for HEPES production was first described in 1966 (Good et al., 1966). Data related to industrial manufacture of HEPES was not able to be obtained, so the embedded resources associated with HEPES production were not included in the LCA.

Additional B9 components

The composition of B9 is similar to E8, but has additional components: neuregulin, antibiotics/antimycotic, ultrapure water and recombinant albumin. Additional analysis was conducted to evaluate the environmental impact of these supplemental components. antibiotic/antimycotic production typically utilizes 100 kg of solvent and 50 kg of water per kilogram of compound produced (Ho et al., 2010). An ecoinvent-provided equal mix of 15 different organic solvents (acetone, butanol, cumene, cyclohexanol, dichloromethane, ethyl benzene, ethyl glycol, isopropanol, methanol, methyl ethyl ketone, nitrobenzene, styrene, tetrachloroethylene, toluene and xylene) was utilized to estimate the impact of

generic organic solvent use. The neuregulin and recombinant albumin environmental impacts were estimated utilizing reported titers (5 mg/L and 17 g/L, respectively) and the method described in microbial-titer methods section (Mautino et al., 2004; Zhu et al., 2021).

Transportation resources

The ecoinvent database v.3.8 was utilized to provide an estimate of the transportation-related resources for each E8/B9 component and their precursors when available. When available transportation was accounted for utilizing ecoinvent datasets which estimate transportation requirements for each product. The ecoinvent datasets provide data on metric ton-km which can be converted to energy via the energy intensities of different modes of transport (MJ/metric ton-km). The energy intensities can vary depending on location and type of transport (Fraser et al., 1995; Gucwa & Schäfer, 2013). OpenLCA software was utilized to consolidate transportation requirements of all inputs and estimate the combined environmental impact of transportation. Appendix H has additional information related the accounting of resources used for transportation.

Life cycle impact assessment

The life cycle impact assessment (LCIA) was conducted utilizing the OpenLCA program v.1.10 and OpenLCA LCIA v2.1.2 methods software. The total direct and indirect energy used throughout the lifecycle of a product, known as, cumulative energy demand (CED) and the tool for reduction and assessment of chemicals and other environmental impacts (TRACI) 2.1 were the LCIA methods utilized in OpenLCA. CED was chosen as metric because its correlation with other environmental impacts including such as global warming, resource depletion, acidification, eutrophication, tropospheric ozone formation, ozone depletion, and human toxicity (Huijbregts et al., 2006). CED calculations also separate the estimated energy demand by energy source and classifies each source as renewable or non-renewable. TRACI is an often-cited LCIA tool which utilizes peer-reviewed characterization factors to provide metrics for ozone depletion, climate change, acidification, eutrophication, smog formation, human health

impacts, and ecotoxicity (J. Bare, 2011; J. C. Bare et al., 2003). The use of CED and TRACI provides environmental impact metrics which are reproducible and standardized so that the results are comparable with other product systems. Sensitivity and scenario analyses were also conducted to examine potential uncertainty in the LCIA results and additional environmental impacts associated with the production of high purity products.

Sensitivity analysis and scenario analysis

A one-at-a-time sensitivity analysis was conducted to examine how increases in concentration of each E8/B9 component affect the environmental impact of the functional unit (1 liter E8/B9). It was found that the basal medium, i.e. the core component for both E8 and B9 growth mediums, was responsible for >90% of the LCIA outputs, so an additional one-at-a-time analysis was conducted on the basal medium. The concentration of each basal medium component was individually increased by 25% and the LCIA calculations for cumulative energy demand and TRACI 2.1 were conducted and recorded using OpenLCA. The percentage of change from the original values was used as an indicator of sensitivity for each variable.

While LCA can be an important decision-making tool for stakeholders, there is significant level of uncertainty in the results (Igos et al., 2019). This uncertainty can arise due to a variety of reasons including, but not limited to generalizations, estimations, spatial considerations, model assumptions, and limited availability of information. To address the uncertainty in our assessment, we conducted a scenario analysis on the E8 growth medium to gain an additional understanding of the potential environmental impacts of producing a highly refined growth medium capable of animal cell culture at currently reported cell densities (cells/ml). We believe the results of the scenario analysis are highly transferable to B9 given its similar composition to E8. We examined three scenarios as described below.

Baseline scenario: This scenario accounts only for the data which we were able to obtain from our described methods. It does not account for any additional processing or resources which are associated with pharmaceutical grade ingredient production. This scenario should be considered a minimum due to the limited nature of the analysis which is described in the method section.

Partial purification scenario: This scenario increases the concentration of non-basal media components of E8 (ascorbic acid 2-phosphate, additional $NaHCO_3$, sodium selenite, insulin, transferrin and FGF-2) by 20-fold to account for additional processing associated with active pharmaceutical ingredient production (Wernet et al., 2010). The increase in concentration accounts for the additional energy and resources used for purification process.

High purification scenario: This scenario increases the concentration of all components by 20-fold to examine the impact of all E8 components being processed to the purity of active pharmaceutical products (Wernet et al., 2010). Again, the increase in concentration accounts for the additional energy and resources used for purification process.

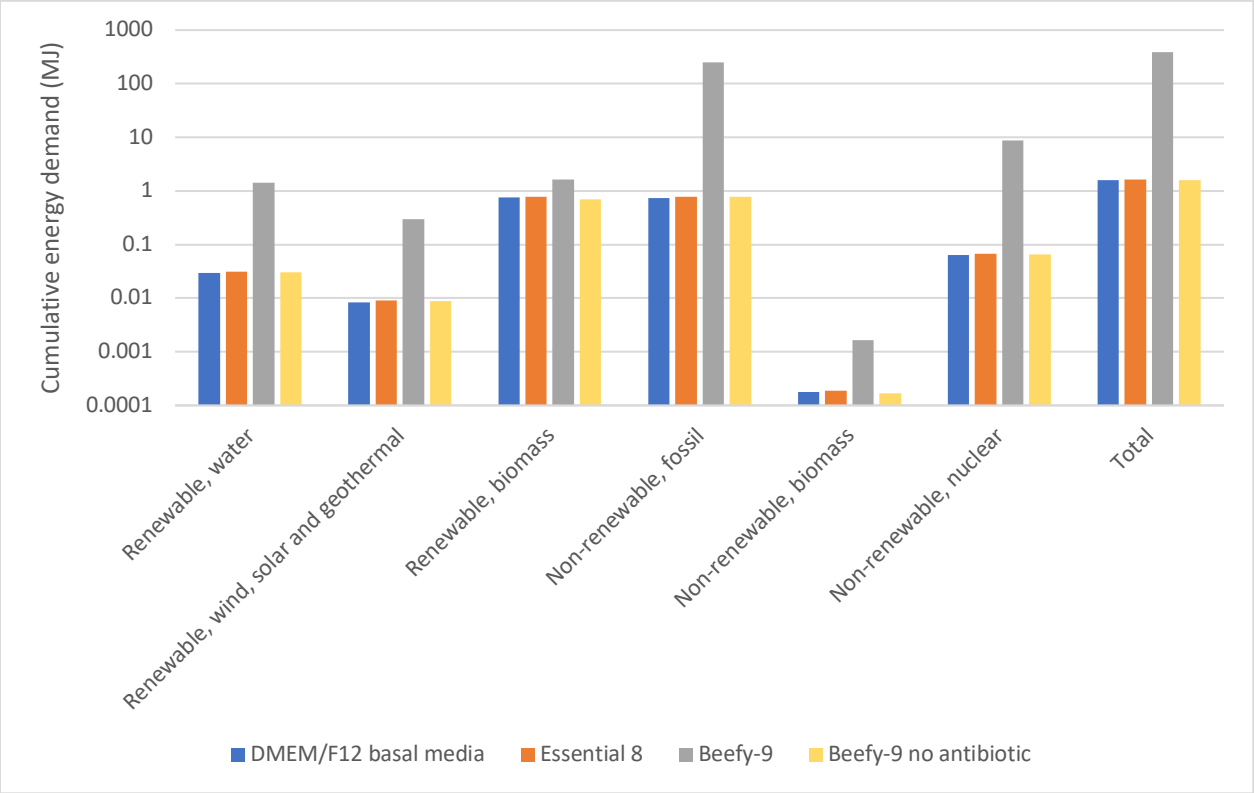
Results

The baseline results indicate a dramatic difference in E8 and B9, however this difference can be attributed to the inclusion of antibiotics in the B9 formulation. When an antibiotic free version of B9 (B9af) is considered the energy use and environmental impacts are analogous. We examined the LCIA results for CED and TRACI LCIA methods for the E8, B9 and B9af. OpenLCA attributed the majority of the environmental impacts (>90%) of both E8 and B9af to the DMEM/F12 basal medium. To further analyze the environmental impacts of DMEM/F12, a sensitivity analysis was conducted on each DMEM/F12 component. This analysis found that glucose was the most environmentally impactful component of the DMEM/F12 medium and this is largely due to its relatively high concentration (3.151g/L) in relation to the other DMEM/F12 growth medium components. Additionally, our scenario analysis of the E8 growth

medium indicated that if E8 components are purified or refined to pharmaceutical/fine chemical standards then the environmental impact of the growth medium will increase significantly.

For E8, the DMEM/F12 basal medium was the most environmentally impactful constituent when considered as a single ingredient/component of the media composition. Figure 5.3 provides a breakdown of the energy sources used for growth medium production and provides the total CED for a liter of each growth medium. The change in the total cumulative energy between the DMEM/F12 basal medium and E8 was ~4%. This change is even less significant when comparing DMEM/F12 growth medium and the B9af growth mediums (Figure 5.3). The replacement of a portion of DMEM/F12 with ultrapure water in B9af growth medium can be attributed with the lower CED of B9af. However, when the addition of antibiotics/antimycotic is accounted for in B9 the total cumulative energy increases nearly two orders of magnitude when compared to E8 or B9af (390 MJ/L vs. ~1.7 MJ/L, respectively). This increase can be attributed to the high volumes of organic solvent and water (100 kg of solvent/1 kg of antibiotic/antimycotic and 50 kg of water/1 kg of antibiotic/antimycotic) associated with antibiotic/antimycotic or high-purity small molecule production (Ho et al., 2010). These organic solvents originate from fossil fuels which accounts for the order of magnitude increase in fossil fuel CED.

Figure 5.3 Cumulative energy demand of each growth medium (MJ/L)



Note use of log scale on vertical axis

Table 5.2 TRACI impact category results for one liter of growth medium

a. Table 5.2, Part 1

	Smog (kg O_3 eq)	Acidification (kg SO_2 eq)	Respiratory effects (kg PM2.5 eq)	Non-carcinogenic* (CTUh)	Ecotoxicity (CTUe)
DMEM/F12 basal media	3.66E-03	5.30E-04	6.62E-05	-1.62E-08	1.50E+00
Essential 8	3.89E-03	5.60E-04	7.05E-05	-1.56E-08	1.61E+00
Beefy-9 antibiotic free	3.73E-03	5.20E-04	6.65E-05	-1.36E-08	1.50E+00
Beefy-9	4.06E-01	3.43E-02	4.65E-03	1.08E-06	6.15E+01

b. Table 5.2, Part 2

	Global warming (kg CO_2 eq)	Ozone depletion (kg CFC-11 eq)	Carcinogenics (CTU)	Eutrophication (kg N eq)	Fossil fuel depletion (MJ surplus)
DMEM/F12 basal media	6.20E-02	5.75E-09	7.09E-09	3.80E-04	7.10E-02
Essential 8	6.57E-02	6.00E-09	7.55E-09	3.90E-04	7.43E-02
Beefy-9 antibiotic free	6.40E-02	7.11E-09	7.07E-09	3.90E-04	7.70E-02
Beefy-9	8.03E+00	2.92E-05	3.65E-07	1.18E-02	3.33E+01

*Levels of non-carcinogenic ecotoxicity reported as near zero negative and positive values according to LCIA software.

PM2.5= particles less than 2.5 micrometers in diameter

CTUh= Comparative toxic unit for humans; CTUh per kg emitted = disease cases per kg emitted

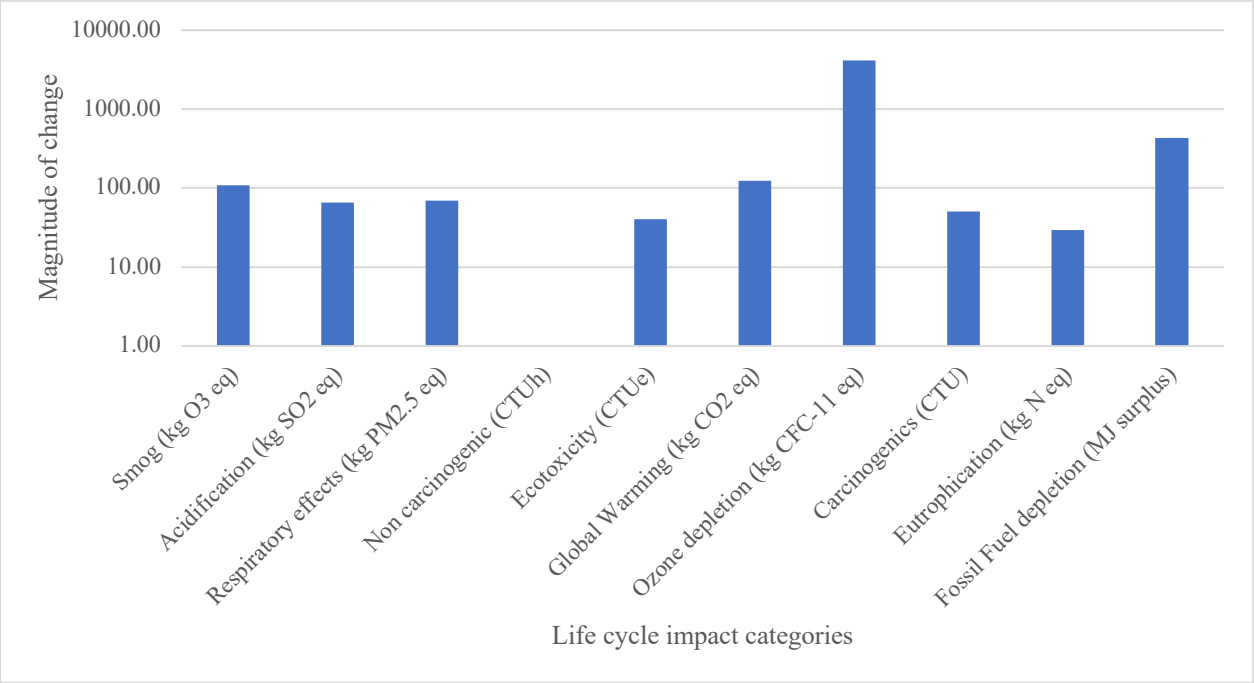
CTUe= Comparative toxic unit for aquatic ecotoxicity impacts; CTUe per kg emitted = PAF × m^3 × day per kg emitted

PAF = Potentially affected fraction of species

CTU= Comparative toxic unit

The results of the TRACI LCIA indicate minimal differences in E8, DMEM/F12 and B9af growth mediums (Table 5.2). When antibiotic containing growth mediums are included, the B9 TRACI LCIA results are orders of magnitudes higher than E8 and DMEM/F12 growth mediums across most impact categories. For example, the global warming potential (GWP) is ~122x higher for B9 than E8, and B9 would deplete ~448x more fossil fuel than E8. Figure 5.4 illustrates the magnitude of change between B9 and B9af. Thus, from an environmental perspective, the reduction and/or elimination of antibiotic/antimycotic growth medium components would be particularly advantageous. It is also important to note that this analysis does not account for the antibiotics being released into the environment during production. Additional analysis would be necessary if an antibiotic containing growth medium is used for industrial scale production of non-vital products.

Figure 5.4 Magnitude of change of each TRACI impact category for B9 relative to B9af



Note use of use of log scale on vertical axis

Magnitude of change calculated via (B9 result-B9af result)/B9af result

Levels of non-carcinogenic ecotoxicity are near zero according to LCIA software.

PM2.5= particles less than 2.5 micrometers in diameter

CTUh= Comparative toxic unit for humans; CTUh per kg emitted = disease cases per kg emitted

CTUe= Comparative toxic unit for aquatic ecotoxicity impacts; CTUe per kg emitted = PAF × m³ × day per kg emitted

PAF = Potentially affected fraction of species

CTU= Comparative toxic unit

Sensitivity Analysis

DMEM/F12 basal medium was found to be the most environmentally impactful component of both E8 and B9af in all impact categories. To further understand the genesis of these impacts, an additional sensitivity analysis was conducted to determine which of the DMEM/F12 basal medium components (>50) most influenced its environmental impact. The sensitivity analysis indicates that the glucose input is the most environmentally impactful DMEM/F12 component. This is due to the glucose concentration being orders of magnitude greater than most other inputs except sodium chloride and HEPES. A 25% increase in glucose concentration changed each TRACI 2.1 output by 6-20% and each cumulative energy demand output by 6-12% (Figure 5.5 and 5.6). A 25% increase sodium bicarbonate or sodium chloride concentration increased some TRACI outputs by ~3% and cumulative energy demand outputs by ~2%. Looking at broader categories of inputs, a 25% increase in all amino acids increases CED and TRACI 2.1 outputs by ~15%. It should also be noted that HEPES concentration is greater than glucose concentration, but the HEPES environmental impacts are not accounted for due to a lack of manufacturing data.

Figure 5.5 Percentage of change in each TRACI impact category from a 25% increase in DMEM/F12 glucose concentration

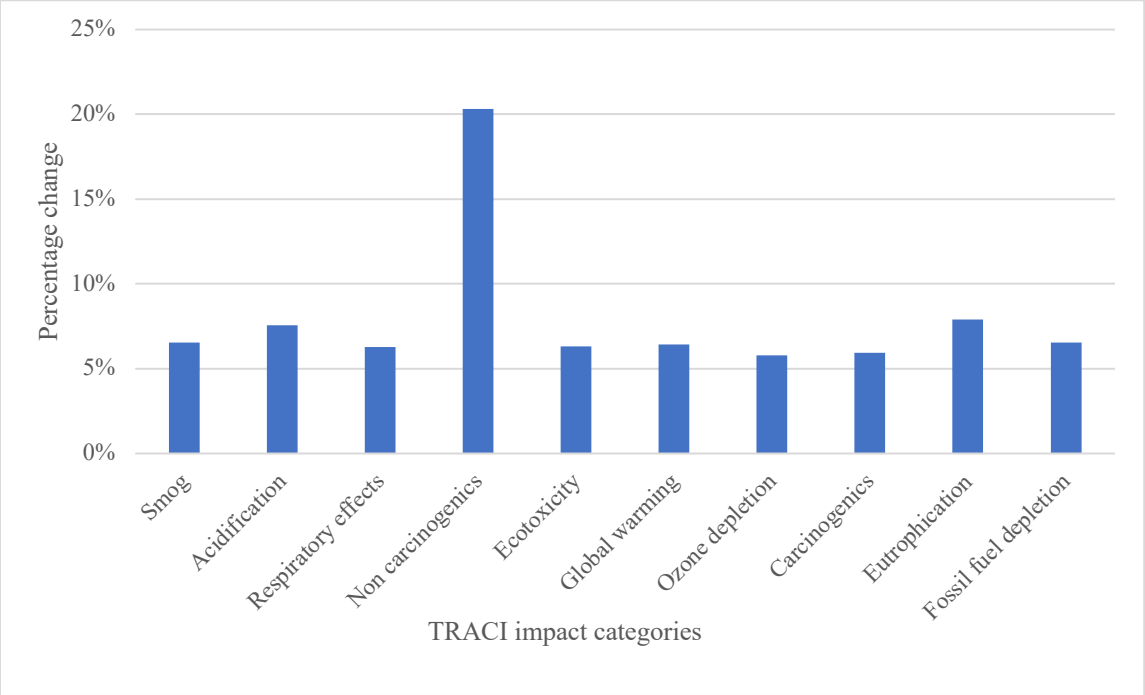
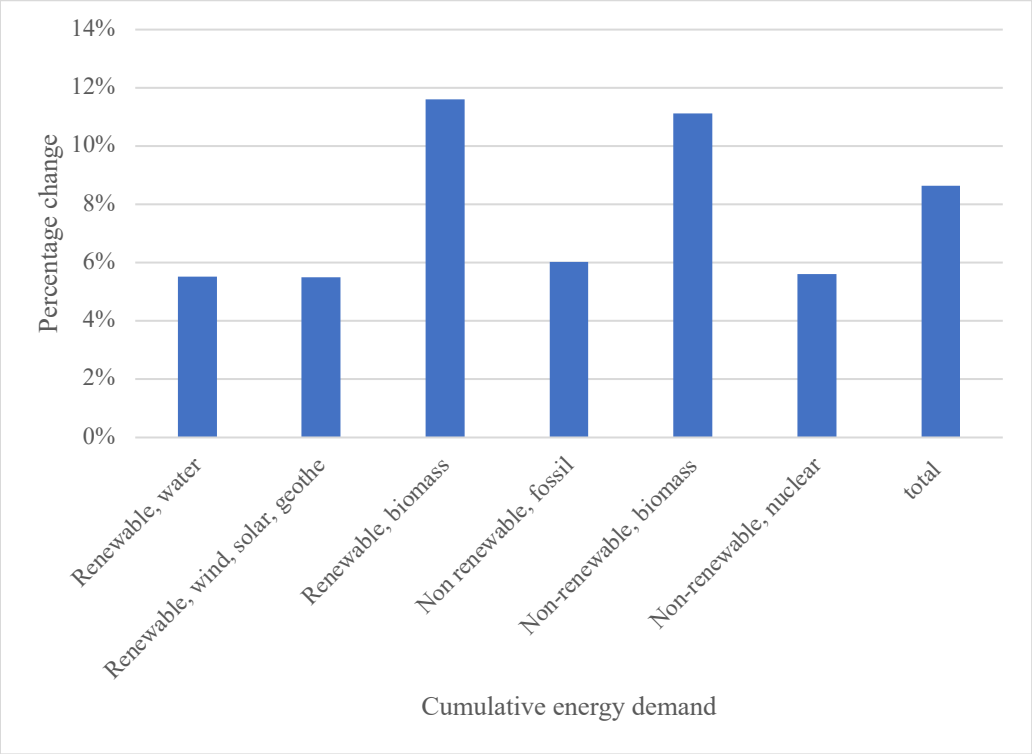


Figure 5.6 Percentage of change in each cumulative energy demand category from a 25% increase in DMEM/F12 glucose concentration



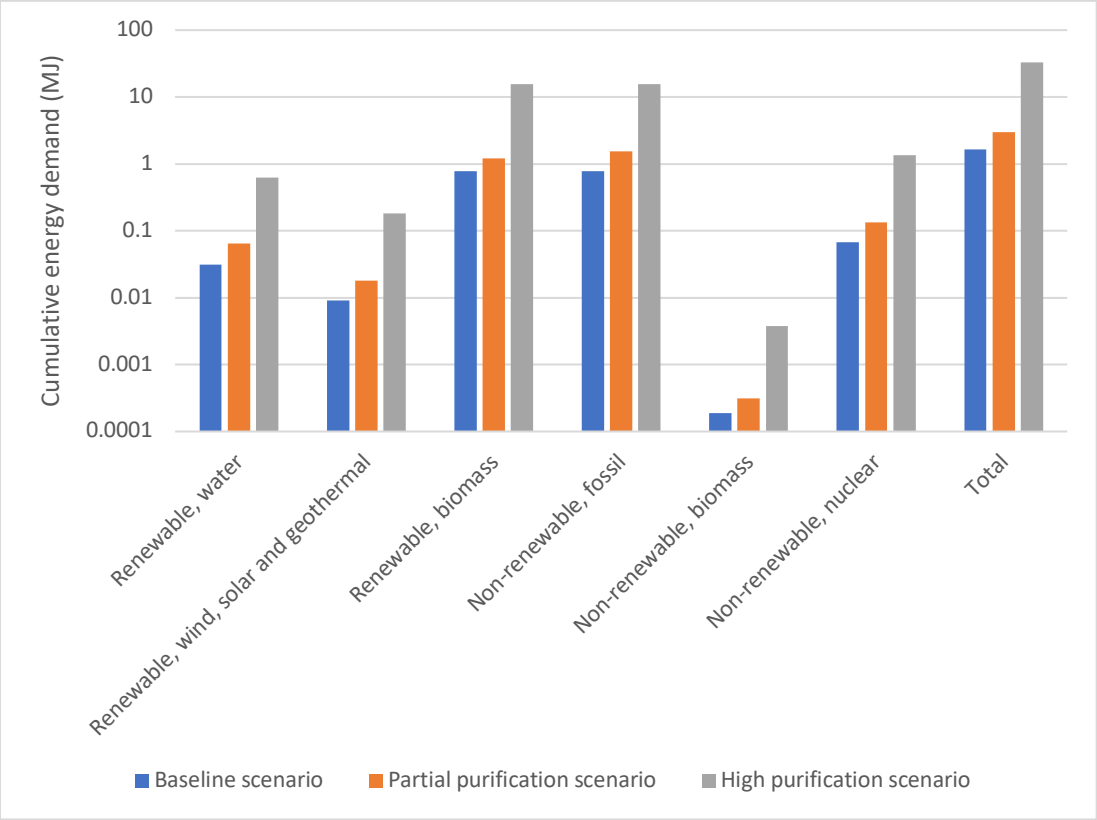
Scenario Analysis

A scenario analysis was conducted to further explore the potential environmental impacts of media production, including the additional impacts of producing high-purity or pharmaceutical grade E8 components (Wernet et al., 2010). The scenario analysis examined the most established growth medium, E8, and was utilized to examine how the environmental impact might change as the level of refinement increases for some or all E8 components. The first scenario is the baseline scenario and should be considered the minimum environmental impact of E8 production due to the limited nature of this study. The CED for the baseline scenario was 1.65 MJ/per liter of E8 with the majority of the energy being supplied by non-renewable fossil fuel and renewable biomass. This is greater than seven times the amount of energy used to light a 60-watt incandescent lightbulb for an hour.

Partial purification scenario increased the seven components other than DMEM/F12 growth medium by a factor of 20 to account for the additional impact associated with high purity/pharmaceutical compound production (Wernet et al., 2010). This increase in partial purification scenario nearly doubled the total cumulative energy demand compared to the first scenario. This highlights the potential impact that high purity substances can have on total cumulative energy demand for the production process.

The final scenario examines the potential environmental impacts if all E8 components (including DMEM/F12) are produced as high purity compounds with their potential resource use being increased by a factor of 20. The total cumulative energy demand for scenario 3 was 33 MJ/L of E8, which is more energy that is contained in a liter of gasoline (Engineering Toolbox, 2008). This indicates that the level of purification and refinement of each component will heavily influence the cumulative energy demand of E8 (Figure 7.0). Orders of magnitude differences in the TRACI 2.1 outputs can be observed within the scenarios as well.

Figure 5.7 Cumulative energy demand results for scenario analysis of Essential 8 growth medium (MJ)

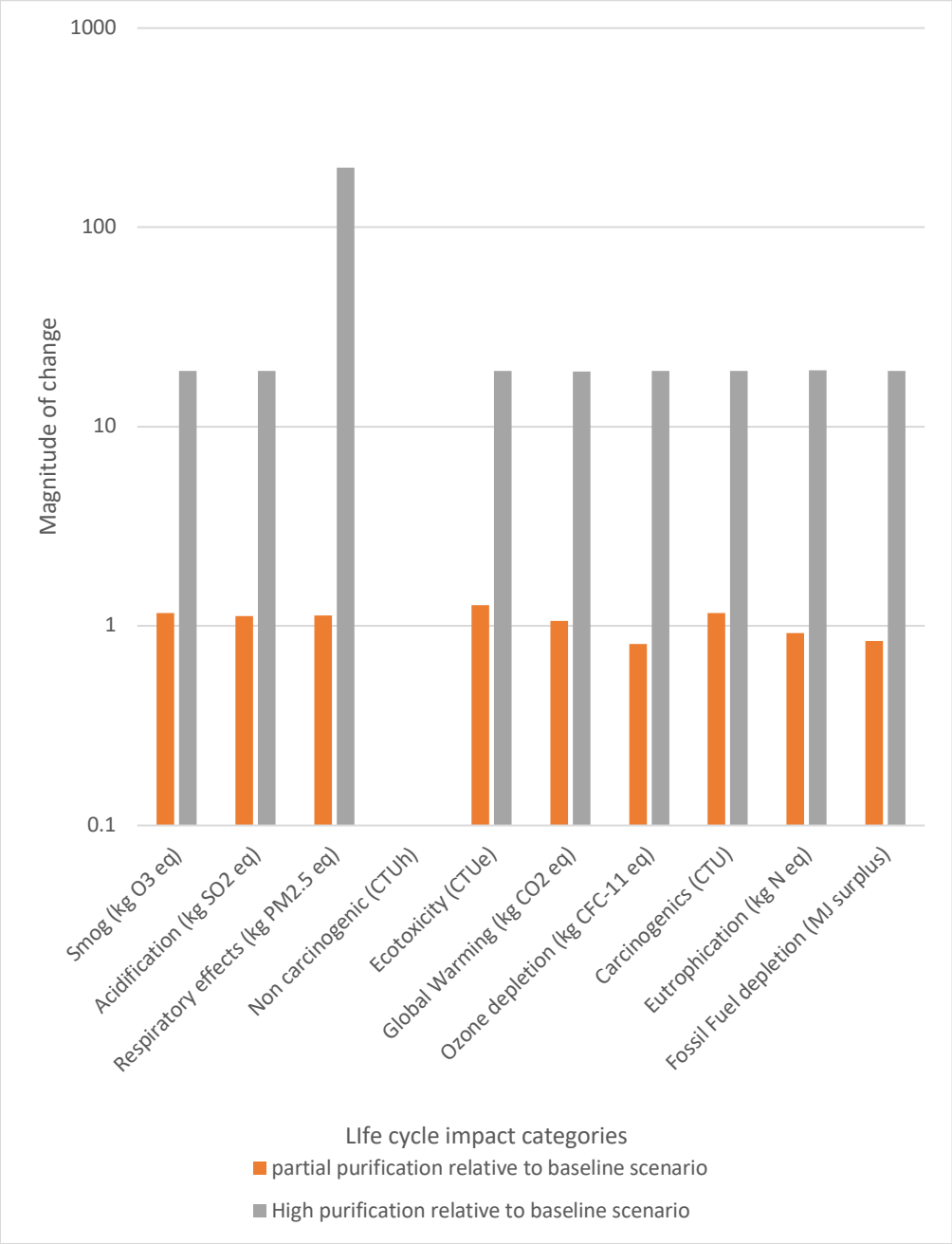


Note use of use of log scale on vertical axis

The TRACI 2.1 results for each scenario are similar to the order of magnitude changes observed in the cumulative energy demand. The GWP increases linearly from the baseline scenario to highly purified scenario, reflecting the 20x increase associated with complete reliance on high purity inputs for Scenario 3. The GWP doubles between the baseline scenarios and partial purification scenario which indicates that utilizing a few high purity E8 components can substantially increase the environmental impact of the E8 production (Figure 5.8). Also, it should be recognized that the baseline scenario is the minimum GWP of E8 production. The fossil fuel depletion impact category increased by 20x when comparing the baseline scenario and high purification scenario. The overall TRACI 2.1 results indicated

that a ~20x increase in resource use produced roughly linear results in each impact category with the exception of respiratory effects (Figure 5.8).

Figure 5.8 Magnitude of change between each TRACI impact category for partial/high purification scenarios relative to the baseline scenario



Note use of use of log scale on vertical axis

Magnitude of change calculated via ((Partial/High purification result-baseline result)/baseline result)

Levels of non-carcinogenic ecotoxicity are near zero according to LCIA software

PM2.5= particles less than 2.5 micrometers in diameter

CTUh= Comparative toxic unit for humans; CTUh per kg emitted = disease cases per kg emitted

CTUe= Comparative toxic unit for aquatic ecotoxicity impacts; CTUe per kg emitted = PAF × m^3 × day per kg emitted

PAF = Potentially affected fraction of species

CTU= Comparative toxic unit

Discussion

Understanding the minimum environmental impacts of animal/human cell growth mediums provides a starting point for developing a cradle-to-grave environmental footprint of animal or human stem cell based bioproducts. This LCA provides existing biomanufacturers and laboratories with data that can be utilized to help better understand their current environmental impacts. The data from this LCA can also be used to provide environmental impact metrics for increases in production efficiency (increased titer) or reduction in overall growth medium usage. The economic importance of increasing a production facility's titer, production scale, or reducing growth medium use is often cited (Humbird, 2021; Risner et al., 2020; Wu & Maravelias, 2018), however our LCA provides the metrics to quantify the potential positive environmental impacts of increases in titer or overall reduction in growth medium use.

Understanding the potential environmental impact of E8/B9 consumption can also help with the environmentally responsible scaling of nascent biotechnology industries, such as the animal cell-based meat or stem cell therapy industries. Researchers and companies currently use highly refined growth mediums for animal cell culture and understanding the environmental ramifications of their work may be important to environmentally and socially conscious investors or financiers. This consideration may be less important for manufacturers of therapeutics, but it should be an important consideration for those

wishing to change large-scale commodity systems like our food and agriculture systems. These emerging industries could use this initial study to understand the near-term environmental implications of developing large scale systems before maximizing metrics like achievable cell concentration (cells/ml). Advances in technology which reduce growth medium use and/or increase the achievable cell concentration will play an important economic and environmental role for biobased products particularly those which seek to be alternatives to commodity-type products, such as animal cell-based meat. This convergence of economic and environmental interest is one of the strengths of the developing bioeconomy, however this work also highlights the potential environmental challenges of utilizing large volumes of growth medium for bioproduct production.

The reported environmental impacts of the baseline scenario should be considered the minimum environmental impacts due to some key limitations in the analysis. First, the overall system boundary was partially truncated since we were unable to fully account for all E8/B9 components such as HEPES and lipoic acid. Incomplete LCI information in ecoinvent and the literature (See Methods section) also contributed to the limited assessment of the environmental impact of some components in the growth medium. It should be noted that the HEPES buffering agent whose concentration was greater than glucose (3.575 vs. 3.151g/L) was not accounted for and may substantially contribute to the environmental impact given the concentration and its chemical synthesis. The environmental impacts of other compounds, such as phenol red were only partially accounted for as well. In addition, the assumption of utilizing microbial fermentation to produce some compounds may not be true in all cases. This highlights why the reported environmental impacts of this LCA should be considered as a minimum.

E8 is an established animal cell growth medium which has been used for animal cell research since at least 2011 and does not include antibiotics within its formulation (Chen et al., 2011). B9 which includes antibiotics/antimycotics and other additional components (neuregulin, ultrapure water, and recombinant albumin) as yet to be commercially established (Andrew Stout et al., 2021). If antibiotic use

is required for B9 to be a viable growth medium, then it's environmental impact is likely to be two orders of magnitude greater than E8. If the B9 growth medium can be utilized without the addition of antibiotics, then our findings indicate that these growth mediums have highly similar environmental impacts. This indicates that from an environmental standpoint that antibiotic use should be limited/eliminated from any large-scale cell proliferation system.

When E8 and B9af were compared it was found that DMEM/F12 basal medium was the major contributor to the environmental impacts of both growth mediums. A sensitivity analysis was conducted which analyzed the environmental impact of the components of DMEM/F12 basal medium. The sensitivity analysis found that the glucose component was the most impactful component and approximately half of glucose's global warming potential can be attributed to its transport (26%) and production from starch slurry (24%). However, due to the quantity of DMEM/F12 basal medium components (>50) there was not a component that could be attributed to the majority (>50%) of the environmental impacts. Approximately half of glucose's global warming potential can be attributed to it's transport (26%) and production from starch slurry (24%). Our results should be transferable to growth mediums which utilize DMEM/F12 as a basal medium. However, additional analysis would be needed for growth mediums whose basal medium composition are different than DMEM/F12's composition.

This LCA also does not evaluate the complex potential health and environmental implications of antibiotics entering our water or terrestrial systems (J. Wang et al., 2020). However, our scenario analysis explores how increased levels of purification which are required for pharmaceutical or fine chemical production can increase the embedded resources and energy within a growth medium. The results of the E8 scenario analysis highlight how the level of refinement can influence the environmental impacts of a growth medium like E8. Additional energy production and use is responsible for 65-85% of the additional environmental impacts associated with fine chemical production (Wernet et al., 2010). This indicates that employing energy efficient means of refinement will be important for the sustainable

production of growth medium components, and especially critical for applications which may require high volumes of growth medium. All components may not require the same level of refinement as fine chemicals but utilizing less refined components has the potential to increase the risk of contamination, batch variation, or failure.

An additional challenge related to cell culture purification is endotoxin removal. Endotoxin is a heat and pH stable lipopolysaccharide with a molar mass ranging from 2.5-70 kDa (Magalhães et al., 2007). These characteristics can make endotoxin removal challenging and different purification strategies must be deployed based on the substance's properties (EMD Millipore, 2012). The heat stability of endotoxin can make traditional steam sterilization protocols (121°C for 45 min or 132°C for 4 min) ineffective since inactivation requires temperatures 250 °C for 30 min or 180 °C for 3 h (Centers for Disease Control and Prevention, 2016; Magalhães et al., 2007). Other methods of removal which utilize filtration or charged/hydrophobic membrane interactions are highly dependent upon the characteristics of the proteins/compounds which are being purified (EMD Millipore, 2012). A variety of these methods have been utilized to separate and remove endotoxins from laboratory grade components including LPS affinity resins, two-phase extractions, ultrafiltration, hydrophobic interaction chromatography, ion exchange chromatography, and membrane adsorbers (Magalhães et al., 2007). Of course, these additional processing steps would likely increase production costs as well as the environmental impact of the E8/B9 production. Due to these purification challenges, it is likely worth prioritizing the development of an efficient and environmentally friendly method of endotoxin removal to reduce the environmental impact of E8/B9 production.

Conclusion

The bioeconomy has been touted as one of the solutions to the United Nations SDGs, however critical environmental, economic and social assessment is needed to understand the sustainability of biobased products. An environmental assessment of animal or human stem cell growth mediums (E8 and

B9) is necessary to understand the environmental impact of a multitude of potential products ranging from therapeutic stem cell therapy to animal cell-based meat products. Further, individually specified sustainability assessments of biobased products are necessary due to the multitude of different factors (e.g. type of production organism, doubling time, product yield, and titer, among other factors) which can affect resource use.

This LCA provides a foundation for determining the minimum near-term environmental impacts of growth mediums utilized for animal cell proliferation. It examined the environmental impacts of an established stem cell growth medium (E8) and compared it with an emerging growth medium, B9. It was found that antibiotic/antimycotic use was highly environmentally impactful, however if an antibiotic free version of B9 (B9af) was utilized then the E8 and B9af environmental impacts were similar. The similarity in environmental impacts of E8 and B9af can be attributed to the fact both mediums utilize DMEM/F12 as the basal medium. Scenarios were utilized to explore how increased levels of refinement and purification of growth medium components can potentially increase the environmental impact of a growth medium. The environmental impacts in this LCA should be viewed as the minimum impacts of E8, B9, B9af and DMEM/F12 production due to the truncated nature of this assessment. This LCA should provide a starting point for researchers working at the convergence of emerging animal cell-based biotechnology and sustainability.

The quantified environmental impacts of these human and animal cell growth mediums will be an essential resource for assessing environmental impacts of new potential bioproducts. Further, this information can be used to understand how the reduction in growth medium use or an increase titer can have a positive environmental impact. This work acts as a foundation for future LCAs or other environmental assessments which examine products produced from animal or human stem cells. Future work could involve a more regional assessment of each growth medium component, detailed assessment of individual growth medium components, impact assessment of missing E8/B9 components (Ex.

HEPES), the development of environmentally friendly bioprocesses and/or sustainability assessments of near-term human or animal cell products.

Appendices

Appendix A- Raw ingredients

Glucose

The material flow for glucose production was utilizing ecoinvent 3.8 datasets. The maize was assumed to be dried during initial harvest and flow the can be described in calculation A1. The conversion factors were all taken from the ecoinvent 3.8 datasets. Transportation was assumed between each step and is accounted for utilizing ecoinvents datasets.

Calculation A5.1. Example of ecoinvent mass determination: Maize mass needed for glucose production

Maize → Starch → Glucose

1 kg of glucose x 0.9 kg starch/1 kg glucose = 0.9 kg of starch

0.9 kg of starch x 1.261 kg of dry corn/1 kg of starch = 1.1349 kg of dry corn

Calculation notes:

1 kg of glucose requires 0.9 kg of starch to produce (ecoinvent Association, 2021)

1 kg of starch requires 1.261 kg of maize (ecoinvent Association, 2021)

Linoleic acid

Cottonseed oil production was utilized to estimate the linoleic acid production. The separation of linoleic acid from fatty acids was not accounted for and would likely increase the input/outputs of this E8/B9 component. The ecoinvent datasets were utilized to estimate the material/energy flow from cottonseed

production to cottonseed oil refining. Transportation was assumed between each production step. Refined cottonseed oil was assumed to have a similar triglyceride content (96% w/w) (Matthäus, 2010). Cottonseed oil's fatty acid profile has been reported consist of the 52% linoleic acid. This indicates that a minimum of ~2 kg of refined cottonseed oil would be required to produce 1 kg of linoleic acid.

Appendix B- Microbial yield

Table A5.1 Reported microbial yields and calculated correction factor for some E8/B9 components

Compound	Yield ($g/g_{glucose}$)	Correction factor	Source
i-Inositol [1]	0.820	0.788	(Y. Li et al., 2021)
L-Alanine	0.520	1.24	(Drauz et al., 2007)
L-Arginine-HCl [2]	0.431	1.50	(Man et al., 2016)
L-Asparagine-H_2O [2]	0.836	0.773	(Drauz et al., 2007)
L-Cysteine-HCl-H_20 [2]	0.060	10.8	(H. Liu et al., 2018)
L-Cystine [2]	0.060	10.8	(H. Liu et al., 2018)
L-Glutamic acid	0.630	1.03	(Wen & Bao, 2019)
L-Histidine-HCl-H_2O [1,2]	0.080	8.08	(Schwentner et al., 2019)
L-Isoleucine	0.300	2.15	(Drauz et al., 2007)
L-Leucine	0.150	4.31	(Drauz et al., 2007)

L-Lysine-HCl [2]	0.646	1.00	(Félix et al., 2019)
L-Methionine[1]	0.130	4.97	(Zhou et al., 2019)
L-Phenylalanine	0.180	3.59	(Drauz et al., 2007)
L-Proline	0.360	1.79	(Wendisch et al., 2016)
L-Serine	0.430	1.50	(Zhang et al., 2018)
L-Threonine	0.400	1.62	(Drauz et al., 2007)
L-Tryosine	0.100	6.46	(Lütke-Eversloh et al., 2007)
L-Tryptophan	0.200	3.23	(Drauz et al., 2007)
L-Valine	0.280	2.31	(Drauz et al., 2007)
Pyridoxal-HCl [2]	0.325	1.99	(Y. Wang et al., 2021)
Pyridoxine-HCl [2]	0.325	1.99	(Y. Wang et al., 2021)
Sodium Pyruvate [2]	0.678	0.953	(Y. Li et al., 2001; Miyata & Yonehara, 1999)
Thymidine	0.019	34.0	(Lee et al., 2010)

[1]Coverted from mol/mol $_{glucose}$ to g/g $_{glucose}$

[2]See information below for additional clarification

Calculation A5.2 Correction factor calculation example for compounds with reported yields

L-Leucine correction factor = $0.646^{*} / 0.150^{**} = 4.31$

[*]L-Lysine yield (g/g $_{glucose}$) constant in all calculations

[**]L-Leucine (g/g $_{glucose}$)

Table A5.2 Reported microbial titer for some E8/B9 components

Compound	Titer (g/L)	Correction factor	Source
D-Calcium pantothenate	86.0	0.0751	(Acevedo-Rocha et al., 2019; Hohmann et al., 2017)
FGF-2	2.00	3.23	(Sauer et al., 2019)
Hypoxanthine	1.23	5.24	(M. Liu et al., 2020)
Insulin	4.00	1.62	(Baeshen et al., 2014)
Riboflavin	16.4	0.394	(S. Liu et al., 2020)
Transferrin	2.33	2.77	(Finnis et al., 2010)
Vitamin B12	0.18	35.7	(K. Li et al., 2013)

Calculation A5.3 Correction factor calculation example for compounds with reported titer

Insulin correction factor = 4.00 $g_{insulin}$ / 10 $g_{glucose}$ = 0.4 $g_{insulin}$ / $g_{glucose}$ → 0.646*/0.4 = 1.62

*L-Lysine yield ($g/g_{glucose}$) constant in all calculations

L-Arginine-HCl

Microbial L-arginine production is utilized as a substitute for L-arginine-HCl production. Additional resources with processing L-arginine into L-arginine-HCl-H_2O are not accounted for.

L-Asparagine-H_2O

L-Asparagine can be synthesized utilizing L-aspartic acid which is esterified followed by treatment with ammonia (Drauz et al., 2007). To estimate the embedded resources in asparagine production, the yield (.836 g/g glucose) for aspartic acid production was utilized. The resources utilized for esterification, ammonia treatment and additional steps for the conversion of L-asparagine to L-asparagine H_20 are not accounted for.

L-Cysteine-Cl-H_2O

Microbial L-cysteine production is utilized as a substitute for L-cysteine-HCl-H_2O production. Additional resources with processing L-cysteine into L-cysteine-HCl-H_2O are not accounted for.

L-Cystine

It is the oxidized dimer formed from a pair of cysteine molecules. The reported yield for the microbial production of cysteine is 0.06 g/g glucose and this value is utilized for cystine production (H. Liu et al., 2018).

L-Histidine-HCl-H_2O

Microbial L-histidine production is utilized as a substitute for L-histidine-HCl-H_2O production. Additional resources with processing L-histidine into L-histidine-HCl-H_2O are not accounted for.

Sodium Pyruvate

Microbial pyruvate production is utilized as a substitute for sodium pyruvate production. Additional resources with processing pyruvate into sodium pyruvate are not accounted for.

Pyridoxal-HCl and Pyridoxine-HCl

Pyridoxal HCl and pyridoxine HCl are forms of B_6 and have been produced microbially via recombinant *Sinorhizobium meliloti* (Acevedo-Rocha et al., 2019; Y. Wang et al., 2021). A titer of 1.3 g/L of B_6 has been report and this titer was used to estimate yield based upon a minimal media containing 4 g of glucose/L (King, 2015; Y. Wang et al., 2021). The estimated yield was utilized to estimate the embedded resources for both pyridoxal HCl and pyridoxine HCl production and additional processing was not accounted for.

Appendix C- Enzymatic

L-Aspartic acid

L-aspartic acid has been described as being produced enzymatically with yields of up to 0.95 (g/g fumaric acid) being reached while utilizing fumaric acid as a feedstock (Appleton & Rosentrater, 2021; Yukawa et al., 2010). Fumaric acid can be produced utilizing glucose as feedstock with a yield of 0.88 g/g glucose (Martin-Dominguez et al., 2018). To estimate embedded resources, the required mass of glucose was determined (~1.20 g glucose per g of L-aspartic acid produced). The embedded resources associated with the mass of glucose was then attributed to L-aspartic acid production.

Appendix D- Chemical

The following section provides the methodology utilized to estimate the environmental impact of E8/B9 components. Table A5.3 provides the E8/B9 precursor components for each chemical classified E8/B9 component and additional details related to the life cycle inventory methodology can be found in this subsequent section.

Table A5.3 Chemically manufactured E8/B9 components' life cycle inventory accounting methods and precursors components

E8/B9 component	Life cycle inventory method/s*	Precursor components used to produce E8/B9 component
Ascorbic acid 2-phosphate	sub	*Glucose*
Biotin	stoi	*cysteine, glucose,* others-not accounted for
Sodium selenite	stoi	Selenium dioxide, *sodium hydroxide*, selenium, *hydrochloric acid, soda ash,* sulfur dioxide, *sulfur*
Cupric sulfate	eco	Copper oxide, *sulfuric acid,* copper cathode
Ferric nitrate	stoi	Ferric nitrate, iron, nitric acid, iron ore concentrate
Ferrous sulfate	eco	N/A (only ferrous sulfate accounted for due to being a by-product)
Folic acid	N/A	Only water use estimated, others-not accounted for

Zinc sulfate	eco	*Sulfuric acid*, zinc oxide
Phenol Red	stoi, sub	O-sulfobenzoic acid, phenol, benzoic acid, *sulfuric acid*, cumene, *oxygen*, toluene, benzene, *propylene*
Putrescene 2HCl	stoi	Putrescine, *HCl, acrylonitrile*, hydrogen cyanide
Glycine	eco	*Ammonia*, chloroacetic acid, *sodium hydroxide*, acetic acid, *chlorine*, carbon monoxide, *methanol*
Choline chloride	stoi	Ethylene oxide*, HCl,* trimethylamine, ethylene, *oxygen, ammonia, methanol*
Niacinamide	stoi	3-cyanopyridine, 3-methylpyridine, *ammonia, oxygen*, acrolein, *propylene*
Thiamine hydrochloride	stoi, sub	Thiamine chloride, *HCl, Acrylonitrile*

sub- Substitution of acceptable component/s and ecoinvent database was utilized to estimate embedded resources.

stoi- Stoichiometry was utilized to determine required mass of components and ecoinvent database was utilized when datasets were available.

eco- Only ecoinvent datasets were utilized to quantify embedded resources

Compounds which are italicized are utilized in the production of multiple E8/B9 components and their embedded resources were account as well (see Table A5).

*Additional explanation of life cycle inventory accounting methods detailed below

AA2P (ascorbic acid 2-phosphate)

An ecoinvent dataset for ascorbic acid 2-phosphate was not reported and ascorbic acid was deemed as an acceptable substitute. The required mass of glucose, the major component utilized in ascorbic acid production was determined via E8/B9 concentration of ascorbic acid 2-phosphate and ecoinvent database. The energy and material flows were determined from the ascorbic acid ecoinvent dataset and from the material/energy associated with glucose produced from corn (see raw ingredients: glucose section).

Biotin

Biotin can be synthesized from multiple precursors utilizing multi-step reactions (Bonrath et al., 2009; Casutt et al., 2011; de Clercq, 1997; Tang et al., 2020). Multiple reaction schemes have been reported to be used for the conversion of cysteine to biotin and we assume cysteine as the starting reactant (de Clercq, 1997). We assumed a yield of 50% due to the multiple reaction steps with potential for loss and differences in molecular weight. The yield could be potentially lower due to inefficiencies in production. Adequate data were not available to estimate inputs/outputs during the multiple step conversion process. Cysteine can be produced microbially utilizing glucose as a feedstock and the embedded resources were determined utilizing the method described in the microbial methods section and appendix B (Martin-Dominguez et al., 2018). The embedded resources associated with mass of glucose was then attributed to biotin. The reported embedded resource estimates for biotin production should be considered a minimum due to unavailability of input/output data related to energy and material flows during the production.

Sodium selenite

The following production route was chosen for sodium selenite, however sodium selenite can be prepared utilizing other methods (Brauer, 1963). This production route was chosen to minimize the additional compounds required for sodium selenite production. After stoichiometric calculations were complete, ecoinvent datasets associated with selenium production (selenium, soda ash, sulfur dioxide and hydrogen

chloride) were utilized to quantify the some of the embedded resources in sodium selenite production. Sodium hydroxide, soda ash, sulfur dioxide and hydrogen chloride are utilized in production of this component and multiple E8/B9 components (See appendix G for complete list). Ecoinvent datasets were not available for sodium selenite and selenium dioxide production.

Equation A5.1 Selenium dioxide production

$Se + air + heat \rightarrow SeO_2 + products\ of\ combustion$

Assumption: No Se is lost during conversion of Se to SeO_2. Theoretical yield is utilized. Energy usage is not calculated for stoichiometric equations.

Equation A5.2 Sodium selenite production

$SeO_2 + 2\ NaOH \rightarrow Na_2SeO_3 + H_2O$

Assumption: Theoretical yield is utilized. Energy usage is not calculated for stoichiometric equations.

Cuperic Sulfate

Ecoinvent datasets were utilized to estimate material and energy flows associated with cuperic sulfate production (copper sulfate, copper oxide and copper cathode). Sulfuric acid is utilized in production of this component and multiple E8/B9 components (See appendix G) for related ecoinvent datasets. Copper cathode production data set includes embedded resources from copper mining.

Ferric Nitrate

Ferric nitrate can be prepared by adding nitric acid to iron pellets, powder or scrap iron (National Center for Biotechnology, 2021). After stoichiometric calculations were complete, ecoinvent datasets (Iron mining and beneficiation, iron pellet and nitric acid) were utilized to quantify the some of the embedded

resources and outputs in ferric nitrate production. Ecoinvent data set was not available for ferric nitrate production.

Equation A5.3 Ferric nitrate production

$Fe + 4\ HNO_3 \rightarrow Fe(NO_3)_3 + NO + 2\ H_2O$

Assumption: Theoretical yield is utilized.

Ferrous sulfate

Ferrous sulfate is generally produced as a by-product of steel manufacture (Wildermuth et al., 2000). The utilized ecoinvent dataset (iron sulfate) accounts for this and only the embedded resources from additional refining is accounted for in the dataset and our model.

Folic acid

Chemical synthesis of folic acid largely occurs in an inexpensive, one-pot process (Mair et al., 2019). The economic viability of the chemical synthesis of folic acid largely prevents other production methods such as microbial fermentation or refinement from raw materials. Adequate data was not available to estimate embedded energy, but wastewater production was estimated in be 250-300 kg of wastewater for each kg of folic acid produced(Bryne, 2015).

Zinc Sulfate

Ecoinvent data sets were utilized to estimate embedded resources and outputs in zinc sulfate production (zinc sulfate and zinc oxide). Sulfuric acid is utilized in production of this component and multiple E8/B9 components (See appendix G). The embedded resources for zinc scrap are not included due to being a by-product of other production process and iron scrap being utilized as a stand in the dataset.

Phenol red

Phenol red (phenolsulfophthalein) can be obtain by the condensation of o-sulfobenzoic acid anhydride with phenol (Gessner & Mayer, 2011). Sulfobenzoic acid (mostly m-sulfobenzoic acid) derived from benzoic acid and sulfuric acid is used as a substitute for pure o-sulfobenzoic acid (Reese, 1932). Stochiometric calculations were conducted to determine the mass of compound needed when information was unavailable in ecoinvent datasets. Ecoinvent datasets were utilized to estimate embedded resources and outputs in phenol red production (phenol, benzoic acid, toluene, and benzene). Sulfuric acid, propylene, oxygen, in ground natural gas and crude oil are utilized in production of this component and multiple E8/B9 components (See appendix G). Ecoinvent datasets were unavailable for phenol red and sulfobenzoic acid.

Putrescine-2HCl

Putrescine and HCl required mass and embedded resources were determined utilizing stochiometric calculations and ecoinvent datasets. Putrescine can be produced utilizing acrylonitrile and hydrogen cyanide in the presence of tertiary amine and with subsequent hydrogenation (Broadwith, 2011). Ecoinvent datasets were utilized to estimate the material flows in Putrescine-2HCl (Acrylonitrile, HCl, hydrogen cyanide). Both acrylonitrile and HCl are utilized in production of this component and multiple E8/B9 components (See appendix G). Ecoinvent datasets were unavailable for putrescine and putrescine-2HCl.

Glycine

Ecoinvent data sets were utilized to estimate energy and material flows during glycine production (glycine, chloroacetic acid, acetic acid, and carbon monoxide). Ammonia, chlorine (liquid), sodium hydroxide and methanol are utilized in the production of this component as well as multiple E8/B9 components (See section appendix G).

Choline Chloride

Choline chloride is produced via reaction between hydrochloric acid, trimethylamine and ethylene oxide (equation A5) (Johnson Matthey Davy Technologies, 2014). Ecoinvent datasets were utilized to estimate embedded resources (ethylene oxide and trimethylamine). Hydrochloric acid, ethylene oxygen, ammonia, methanol, in ground natural gas, and crude oil are utilized in the production of this component as well as multiple E8/B9 components (See appendix G). An ecoinvent dataset was unavailable for choline chloride.

Equation A5.4 Choline chloride production

$$HCl + N(CH_3)_3 + C_2H_4O \rightarrow C_5H_{14}ClNO$$

Assumption: Theoretical yield is utilized.

Niacinamide

Niacinamide can be produced utilizing microbial synthesis with precursor compounds being produced chemically (Shimizu et al., 2000; Z. Wang et al., 2017). Niacinamide can be produced via microbial conversion of 3-cyanopyridine to niacinamide with a 94.5% yield (Z. Wang et al., 2017). 3-cyanopyridine can be produced via the ammoxidation of 3-methylpryidine (equation A6) (Shimizu et al., 2000). Ecoinvent datasets were utilized to estimate embedded resources for niacinamide (3-methylprydine and acrolein). Ammonia, oxygen and propylene are utilized for the production of this component as well as multiple E8/B9 components (See appendix G). Embedded resources for niacinamide and 3-cyanopyridine production are not accounted.

Equation A5.5 3-cyanopydrine production

$H_3CC_5H_4N + NH_3 + 1.5\ O_2 \rightarrow NCC_5H_4N + 3\ H_2O$

Assumptions: Theoretical yield is utilized. Stoichiometric calculation does not account for high density cell growth in bioreactor.

Thiamine hydrochloride

Thiamine hydrochloride can be manufactured by combining thiamine chloride with one molar equivalent of hydrochloric acid (ChEBI, 2021). Thiamine production process was then utilized as substitute for thiamine chloride production process and acrylonitrile was utilized as the starting material for thiamine production (Létinois et al., 2020). Acrylonitrile and hydrochloric acid production was then utilized to estimate embedded resources in thiamine hydrochloride production. The embedded resources utilized during thiamine and the intermediates production processes are not included in the assessment.

Appendix E- Solvay

Sodium bicarbonate

Sodium bicarbonate production can be integrated into a soda ash production utilizing the Solvay process (European Commission, 2007b). To produce one ton of sodium bicarbonate approximately 0.7 tonnes of raw soda ash and 550 kg of CO_2 (approximately 53% of the CO2 is released to atmosphere) are ulitilzed (European Commission, 2007b). Equation A7 illustrates the theoretical mass balance to produce sodium bicarbonate. The CO_2 utilized for soda ash is considered to be a product of combustion and embedded resources for the CO_2 is not accounted.

Equation A5.6 Theoretical sodium bicarbonate production

$Na_2CO_3 + CO_2 + H_2O \rightarrow 2NaHCO_3$

Calcium chloride

Calcium chloride is assumed to be produced from the Solvay process and soda ash. The calcium chloride is assumed to dried and the embedded resources for liquor production and drying processes are accounted for (European Commission, 2007a). The drying process can produce a product that is 75-82% flake or 100% prills (European Commission, 2007a). The 100% prills production was assumed to be the product utilized for E8/B9 production. Table A3 provides estimates for embedded energy and water in use for calcium chloride production.

Table A3. Energy and water inputs for calcium chloride prills (100% w/w) production from soda process

Thermal energy for liquor production (GJ/ton)	7-9
Electrical energy for liquor production (GJ/ton)	0.3-0.5
Cooling water for liquor production (m^3/ton)	40
Thermal energy for prills production (GJ/ton)	7-9
Electrical energy for prills production (GJ/ton)	0.6-0.8
Cooling water for prills production (m^3/ton)	n/a

*Table developed from European Union reference document (European Commission, 2007a)

Sodium Phosphates

Sodium phosphates (monobasic and dibasic) are estimated to require approximately the same embedded resources and are not delineated between in embedded resource summation. Sodium phosphate production requires the use of soda ash or sodium hydroxide for production and are categorized as a product of the Solvay process due to soda ash being a major reactant. Ecoinvent datasets (sodium phosphate, purified phosphoric acid, phosphoric acid (fertilizer), quicklime, and beneficiated phosphate rock) were utilized to quantify the embedded resources in the sodium phosphate production process. Soda ash, sulfuric acid and crushed limestone are utilized for this component as well as multiple E8/B9 components.

Appendix F- Potash

Potassium Chloride

Potassium chloride is assumed to be produced from a mining operation and the ecoinvent dataset starts at the extraction at mine and ends with production of 1 kg of potassium chloride.

Magnesium Chloride

Magnesium chloride can be produced from a variety of sources including salt lakes, underground brines, residual brines from the potash industry (European Commission, 2007a). The embedded resources were estimated utilizing industry supplied data as an estimation for embedded energy and water (Table A4) (Compass minerals, 2016). Energy and water usage calculated using reported water and energy intensities of products (Compass minerals, 2016).

Table A4. Embedded water and energy in magnesium chloride production

Energy (MJ/kg)	4.9×10^{-1}
Water (m^3/kg)	4.7×10^{-3}

Magnesium Sulfate

Magnesium sulfate is assumed to be produced from a mining operation and the reported embedded resources and outputs in the ecoinvent dataset starts at the extraction at mine and ends with production of 1 kg of magnesium sulfate.

Appendix G- List of components used in production of multiple E8/B9 components

Table A5. Materials used to produce more than one E8/B9 ingredient

Material used multiple times for E8/B9 ingredient production	**E8/B9 ingredients**
Sodium bicarbonate	E8/B9 media ingredient*
Sodium hydroxide	Sodium selenite, Glycine
Soda ash	Sodium selenite, Glycine, Sodium bicarbonate, Sodium phosphates
Limestone	Sodium selenite, Glycine, Sodium bicarbonate, Sodium phosphates
Brine (NaCl)	Sodium selenite, Glycine, Sodium bicarbonate, Sodium phosphates
HCl	Putrescine-2HCl, Choline chloride, Thiamine hydrochloride
Chlorine (liquid)	Putrescine-2HCl, Choline chloride, Thiamine hydrochloride, Glycine
Chlorine (gas)	Putrescine-2HCl, Choline chloride, Thiamine hydrochloride, Glycine
NaCl (brine excluded)	E8/B9 media component, Putrescine-2HCl, Choline chloride, Thiamine hydrochloride, Glycine

Sulfuric acid	Copper sulfate, Zinc sulfate, Phenol red, Sodium phosphates
Sulfur	Sodium selenite Copper sulfate, Zinc sulfate, Phenol red, Sodium phosphates
Acrylonitrile	Putrescine-2HCl, Thiamine hydrochloride
Ammonia	Glycine, Choline chloride, Niacinamide, Putrescine-2HCl, Thiamine hydrochloride
Propylene	Phenol red, Niacinamide, Putrescine-2HCl, Thiamine hydrochloride
Methanol	Glycine, Choline chloride
Oxygen (liquid)	Phenol red, Choline chloride, Niacinamide,
High pressure natural gas	Ferric nitrate, Glycine, Choline chloride, Niacinamide, Putrescine-2HCl, Thiamine hydrochloride
In ground natural gas	Phenol red, Choline chloride, Niacinamide, Putrescine-2HCl, Thiamine hydrochloride
Crude oil	Phenol red, Choline chloride, Niacinamide, Putrescine-2HCl, Thiamine hydrochloride

Table excludes E8/B9 components whose embedded resources were quantified using raw/microbial/enzymatic methods described in the material and methods section.

*Used in DMEM/F12 basal media and as an addition to E8 basal media (one of the 7 components besides DMEM/F12 basal media)

Appendix H- Transportation

Ecoinvent datasets were utilized to account for the resources used for transportation across each components supply chain. These data sets were then entered into OpenLCA and the LCIA methods software package was utilized to determine the environmental impacts of transportation across the supply chain. Additional information related to the transportation of E8/B9 can be found in the following appendix subsections.

Raw ingredients

Glucose

Each market was utilized for each stage of production (dry maize to glucose). The total embedded energy related to corn/glucose transport was determined then the embedded energy used per kg of glucose was determined.

Linoleic acid

Refined vegetable oil market as a substitute in the ecoinvent dataset. The corn transport was not accounted for on based on the assumption that wet milling will be utilized and is onsite.

Microbial- Yield and titer

Glycine which is an amino acid produce in bulk and the market is reported in ecoinvent will be utilized as a substitute for the transportation. The total required glucose will also be accounted for utilizing transportation per kilogram of glucose.

Enzymatic, Aspartic acid

Glucose and it's embedded resources utilized for transportation was utilized to estimate the embedded energy related to aspartic acid transportation.

Chemical, Biotin

The embedded transportation energy from cysteine and glucose transport was utilized to estimate embedded transportation energy.

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Chapter 6. Environmental examination of near-term animal cell-based meat: Is kill-free meat sustainable?

Journal article formatted for and awaiting submittal: Nature Foods

Environmental examination of near-term animal cell-based meat: Is kill-free meat sustainable?

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Abstract

Interest in animal cell-based meat (ACBM) or cultured meat as a viable environmentally conscious replacement for livestock production has been increasing, however a life cycle assessment for the current production methods of ACBM has not been conducted. Currently, ACBM products are being produced at a small scale and at an economic loss, however ACBM companies are intending to industrialize and scale-up production. This study assesses the potential environmental impact of near term ACBM production. Updated findings from recent technoeconomic assessments (TEAs) of ACBM and a life cycle assessment of Essential 8™ were utilized to perform a life cycle assessment of near-term ACBM production. A scenario analysis was conducted utilizing the metabolic requirements examined in the TEAs of ACBM and a purification factor from the Essential 8™ life cycle assessment was utilized to account for growth medium component processing. The results indicate that the environmental impact of near-term ACBM production is likely to be orders of magnitude higher than median beef production if a highly refined growth medium is utilized for ACBM production.

Introduction

Livestock production is an integral component of the global food system, providing staple proteins (milk, eggs, and meat) consumed worldwide, contributing to crop productivity via utilization of manure as fertilizer, and providing critical nutrition and income to underprivileged households in low to middle income countries (Gilbert et al., 2018; Robinson et al., 2011). Global meat production has increased from 70.57 million tonnes in 1961 to 337.18 million tonnes in 2020, though the consumption of different meat sources is highly regionalized (FOA, 2022; Ritchie et al., 2019). Looking forward, the overall demand for meat is expected to double by 2050 (Food and Agriculture Organization of the United Nation (FAO), 2019), and this trend has raised concerns about the environmental impact of scaling up meat production to meet these expected demands.

In 2020, beef and buffalo meat accounted for ~22% of global meat production, and poultry and pork accounted for ~39% and ~32% of worldwide meat production, respectively (FOA, 2022; Ritchie et al., 2019). When the top three livestock production systems are examined from an environmental perspective, beef is the most impactful per kilogram, though this value varies significantly by production system (Poore & Nemecek, 2018). The environmental impact of beef production includes greenhouse gas emissions (GHG) from enteric fermentation and manure, nutrient loading in the nitrogen and phosphorus cycles, reduction in biodiversity from overgrazing, and land-use change (Gilbert et al., 2018; Steinfeld et al., 2006).

Multiple life cycle assessments (LCAs) have examined different beef production systems and the global warming potential or GWP (kg of carbon dioxide equivalent, CO_2-eq) was the most highly utilized environmental metric for these assessments (de Vries et al., 2015). This impact is then normalized by the functional unit of the beef product (e.g. live weight, carcass weight and boneless meat), which varies across studies. For example, skeletal muscle is only one product produced from a slaughter facility (Desjardins et al., 2012). Approximately 78.3% mass of the animal is utilized as primal cuts of meat

(37.8%), rendering products (32.8%), raw hide, (4.9%) and offal (3.2%) in the United States and Canada (Desjardins et al., 2012). A 2015 review of beef LCAs reported a range of 7.6 kg (live weight) to 29.7 kg (carcass weight) of CO_2e per kg of beef (de Vries et al., 2015).

The reported values in the literature vary significantly due to differences in functional unit, as mentioned above, but also by the production system (Ex. origin of calf, organic vs. non-organic, and type of diet), and geographic location (de Vries et al., 2015). A study that examined the environmental impact of multiple foods at the retail level indicated GHG emissions ranged from 9.6 to 432 kg of CO_2e for each kilogram of fat and bone-free meat and edible offal (FBFMO) produced (Poore & Nemecek, 2018). The reported GHG emissions from meat produced from a beef herd (cattle raised with primary purpose of meat production) ranged from 35-432 kg of CO_2e per kg of FBFMO. After statistical analysis, the mean and median for the beef herd was 99.5 and 60.4 kg of CO_2e per kg of FBFMO. The greenhouse gas emissions from FBFMO produced from dairy herds ranged from 9.6 to 73.9 kg of CO_2e per kg of FBFMO. The mean and median of the greenhouse gases produced from FBFMO production from dairy herds was 33.4 and 34.1 kg of CO_2e per kg of FBFMO, respectively (Poore & Nemecek, 2018). The relative closeness of the mean and median indicate fewer outliers for dairy herd produced FBFMO. Due to the potential environmental impacts of increased beef production and animal welfare concerns, beef production has been identified as a large-scale food production system that could be modified, significantly curtailed, or even eliminated (McMichael et al., 2007; Pierrehumbert & Eshel, 2015).

Alternative Protein Products and Animal Cell-Based Meat (ACBM)

Several methods or system alternatives have been proposed to reduce the environmental impact of human-consumed proteins including alternative protein production, regenerative agriculture, and bovine methane reduction (“clean cow”) efforts (Cusworth et al., 2022; Min et al., 2022; Molfetta et al., 2022). During the last five to ten years, alternative proteins or meat alternatives have gained popularity with a multitude of stakeholders. These stakeholders have coalesced around this concept to augment or

replace conventional beef production (Tziva et al., 2020). The interest of these stakeholders is multifaceted and includes concerns for animal welfare, environmental concerns and/or monetary motivations. The multifaceted nature of these stakeholders can be illustrated by non-profit groups like The Good Food Institute which exhibits interests in a mix of social activism, scientific inquiry, and monetary investment.

Alternative proteins can be broadly categorized into three distinct categories: plant-based proteins, fermentation-based proteins, and animal cell-based meat (ACBM) (Asgar et al., 2010; Tziva et al., 2020). Plant-based and fermentation-based proteins are currently commercially available, and these products have been for several decades (Ex. Tofurky and Quorn®, respectively) (Tziva et al., 2020). The core concept of ACBM production is that animal cells such as pluripotent stem cells can be proliferated in industrial scale bioreactors (>1,000 L), differentiated into a variety of cell types (e.g. adipocytes, myotubes, fibroblasts), and then processed for human consumption in place of conventionally produced meat (Humbird, 2021; Risner et al., 2020). At the time of this writing, no ACBM products are produced at a large enough scale to be considered commercially available. The authors acknowledge the small-scale production of ACBM products in Singapore, however these products utilize animal serums such as fetal bovine serum and are not widely available (Hasiotis, 2022). Additional challenges related to organoleptic quality of these novel products are also evident (Fraeye et al., 2020).

Despite the highly limited availability of ACBM products, investment in ACBM companies has continued to increase with a total investment of over $2 billion at the time of writing (Turi, 2021). This investor excitement is likely linked to analyst's reports which are bullish on meat alternatives with some reports predicting a 60-70% displacement of ground beef by 2030-2040 (Suhlmann et al., 2019; Tubb & Seba, 2019). More recent reports seem to be more modest with their predictions of replacing a half of a percent of conventional meat products with ACBM products by 2030 (Brennan et al., 2021). With 12.6

billion kg of beef produced in the United States in 2021 (Maples, 2021), even this more conservative estimate of predicted displacement would have a massive impact on the food system.

Existing Technoeconomic Assessments of ACBM

Given the reported potential impact of ACBM production, researchers at the University of California, Davis (UC Davis) published a preliminary, peer-reviewed techno-economic assessment (TEA) of ACBM that examined the core capital and operating expenditures required to produce ACBM at scale (Risner et al., 2020). Given the uncertainty of auxiliary processes (i.e. scaffolding, product forming or shaping, etc.) the TEA focused on the core cell proliferation and differentiation processes in production scale bioreactors. The production scale bioreactors represented the system capital costs and the variable operating expenditures included ingredients, raw materials, some utilities, and labor cost. The Risner et al. TEA included Essential 8^{TM} (E8) as the animal cell growth medium for their model. E8 is a defined growth medium designed for stem cell research and had been previously suggested as a growth medium which could be scaled and slightly modified for industrial production of ACBM (Chen et al., 2011; E. A. Specht et al., 2018; L. Specht, 2019). The authors believe that use of E8 or similar refined growth medium will be necessary given *in vitro* animal cells sensitivity to media impurities in comparison to yeast or bacterial cells.

Given the uncertainty inherent to modeling an emerging technology, the Risner et al. TEA included an assessment of four potential scenarios for the production of 122 million kg of ACBM (wet cells) or alternatively, 36.6 million kg of dry cells and 25.62 million kg of protein. Scenarios 1 and 4 represented "bookend" scenarios where Scenario 1 represented the initial state of ACBM production mirroring the economics of early proof of concept demonstrations and Scenario 4 represented achieving the physical and biological limits of the bioreactor (thus, not an operationally realistic scenario for actual ACBM production). Scenarios two and three represented "midpoint" scenarios where a few particularly critical cost hurdles were overcome.

Shortly after the Risner et al. TEA was published, a more complete TEA commissioned by Open Philanthropy was peer reviewed and published in *Biotechnology and Bioengineering* (Humbird, 2021). This TEA examined a complete production system and examined what equipment would be necessary at a scale of 100 million kg of ACBM produced per year. The Humbird TEA examined a more simplified growth medium with commodity level pricing and refinement for the carbon source. The Humbird TEA also utilized chemical engineering scaling equations to estimate costs at scale.

The Endotoxin Challenge

These TEAs highlighted many of the technical challenges related to ACBM production, but growth medium refinement was identified as one of the most important consideration for near-term analysis. One aspect of this refinement is the endotoxin reduction/removal for each growth medium component. Endotoxins, also known as lipopolysaccharides (LPS) are a critical component of the outer membrane of Gram-negative bacteria. Endotoxins contain a hydrophilic polysaccharide fraction, which is covalently bonded to a hydrophobic lipid known as lipid A (Magalhães et al., 2007). Gram negative bacteria are ubiquitous to the environment and are commonly found in tap water (Vaz-Moreira et al., 2017). In cell culture the presence of endotoxin can have a wide variety of effects. For example, at an endotoxin concentration as low as 1 ng/ml it reduced pregnancy success rates by 3 to 4-fold during *in vitro* fertilization of human IVF embryos (Dawson, 1998; Fishel et al., 1988; Snyman & van der Merwe, 1986). Gram negative bacteria shed small amounts of endotoxin into the environment when they proliferate and shed large amounts when they are inactivated (Corning, 2020).

Animal cell culture is traditionally done with growth medium components which have been refined to remove/reduce endotoxin (Corning, 2020). The method of endotoxin reduction or elimination is highly dependent upon the properties of the substance being purified (EMD Millipore, 2012). There are a multitude of methods employed for the separation of endotoxin from growth medium components and

these include use of LPS affinity resins, two-phase extractions, ultrafiltration, hydrophobic interaction chromatography, ion exchange chromatography, and membrane adsorbers (Magalhães et al., 2007). In turn, the use of these refinement methods contribute significantly to the economic and environmental costs associated with pharmaceutical products since they are both energy and resource intensive (Wernet et al., 2010).

The Limitations of existing ACBM LCAs

Previously conducted LCAs of ACBM have significant limitations, namely , the high levels of uncertainty in their results and the lack of accounting for endotoxin removal. Despite study authors clearly reporting high levels of uncertainty in their LCAs, the results are often cited as clear evidence for the sustainability of ACBM production. The potential environmental impact of producing ACBM has been evaluated to be less than conventionally produced beef in previously conducted LCAs of ACBM (Mattick et al., 2015; Tuomisto et al., 2014; Tuomisto & Teixeira de Mattos, 2011). An often-cited LCA of ACBM production claims 1.9-2.2 CO_2eq GHG emissions are emitted and 26–33 MJ energy will be utilized per kg of ACBM produced. This assessment utilizes a cyanobacteria hydrolysate as feedstock for the animal cells (Tuomisto & Teixeira de Mattos, 2011). The cyanobacteria would be grown in an open pond made of concrete, harvested, sterilized, hydrolyzed and used as an animal cell growth medium. To these authors' knowledge, this is not a technology or feedstock that is currently used for animal cell proliferation, nor is it one that is currently near feasibility given the current technical challenges of ACBM production. An amendment to the original study was later published that acknowledged technical challenges that the original study didn't address (Tuomisto et al., 2014). While the published amendment also examined different scenarios with different feedstocks and bioreactor combinations, the authors acknowledged the high levels of uncertainty inherent to these untested approaches (Tuomisto et al., 2014).

An additional ACBM LCA which provided an increased level of detail was published in 2015 (Mattick et al., 2015). However, a close examination of the assumptions reveal some significant shortcomings of this study as well (Zimberoff, 2022). The process assessed in the study assumes the use of soy protein hydrolysate as an amino acid source, neglects to apply specific consumption rates to estimate the utilization of basal media and amino acids, and proposes the use of corn starch microcarriers for cell proliferation (Mattick et al., 2015). These assumptions are not accurate representations of current ACBM production.

In sum, the existing LCA literature on ACBM does not provide reliable estimates of the environmental impact of current or near-term ACBM production. This study seeks to address this gap in knowledge and provide a meaningful understanding of the environmental consequences of ACBM production. The assessment is based on a detailed model of ACBM production that is entirely based on peer-reviewed TEAs of ACBM systems as well as an existing LCA of the most representative ACBM media currently in use (Humbird, 2021; Risner et al., 2020). Given the existing level of investment, technological forecasting, and public funding associated with ACBM enterprises, this type of detailed environmental assessment of near-term ACBM production is critically needed (Zimberoff, 2022).

Methods

This LCA was conducted utilizing the ISO 14040 and 14044 standards (International Organization for Standardization, 2006b, 2006a). The work builds on existing process models developed in peer-reviewed TEAs of ACBM (Humbird, 2021; Risner et al., 2020) as well as an existing LCA of an animal cell growth medium (Risner et al., 2023). The ISO process requires the following steps for a complete LCA: identifying the goal and scope, conducting a life cycle inventory (LCI), calculating the life cycle impact assessment (LCIA) and ongoing interpretation of all components throughout the process.

Goal and scope

An updated environmental assessment is needed given the high levels of uncertainty of previously conducted ACBM LCAs. We aim to identify environmental challenges that should be addressed before seeking to industrialize a new meat production technology with assumed environmental benefits. In accordance to the ISO 14040 and 14044 standards, we have identified a functional unit which can be utilized to compare similar products. We have chosen the functional unit of a single kilogram of ACBM (wet basis) to allow for comparison with a similar conventionally produced ground beef product and ACBM products produced utilizing different growth mediums.

Lifecycle inventory assessment

The development of a process model is an important element in identifying the inputs and outputs of a system. The Risner et al. and Humbird TEAs are the most complete studies that contribute to our understanding of the ACBM production process at this time. This study leverages the best components of both TEA models to create the ACBM process model for our LCA. Both the Risner et al. and the Humbird TEAs highlight the importance of the growth medium in influencing the economic viability of future ACBM products. A study examining the environmental impact of animal cell growth media has been utilized to further enhance the quality of our environmental assessment of near-term ACBM (Risner et al., 2023).

Risner et al. TEA

The Risner et al. TEA estimated the required volume of growth medium based on cellular glucose consumption rates and did not examine cellular amino acid consumption rates at the time. However, animal cells must have an amino acid source. The theoretical limit of the mass balance of the amino acids provided and protein produced is 1:1. In reality, it is lower since amino acids are also used as an energy source as well as for nucleic acid production. Table 6.1 provides a breakdown of animal cell composition (Humbird, 2021).

Table 6.1 Animal cell composition

Moisture content	70%
Dry matter	30%
Protein (dry basis)	70%
Lipid (dry basis)	15%
Carbohydrate (dry basis)	10%
Nucleic acid (dry basis)	5%

These are key assumptions for the new model which explores utilizing both the minimum glucose and amino acid requirements to generate minimum viability scenarios for our production system. We have taken the approach of utilizing a fed-batch system that supplies the cells with the nutrients in E8 as necessary. This approach allows for a concentrated feed to be added to bioreactor and prevents cells from experiencing issues related to osmotic pressures from increased nutrient concentrations. Risner et al. scenario 1 utilizes a glucose requirement would require 1,148 liters of E8 to produce a kilogram of ACBM. When E8 provision is scaled to match the amino acid requirements for cell cultivation, then it would require ~292 liters of E8 to produce a kilogram of ACBM. Applying this amino acid requirement assumption to the previous UC Davis model shows that the Scenario 4 minimal requirement of E8 is actually not technically feasible, and Scenarios 2 and 3 may or may not be feasible depending on the protein content of the original inoculum. However, this limitation is accounted for in the updated model presented in this paper.

Humbird Technoeconomic assessment

To determine the cellular metabolic requirements, a "wild type" cellular metabolism and an "enhanced" cellular metabolism were examined. The wild type metabolism was deemed too inefficient for economic production due to lactate and ammonia production which inhibit cell growth. We only examined the enhanced cellular metabolism due to the economic concerns of the Humbird TEA. Equations 6.1 and 6.2 were utilized in the Humbird TEA to determine the mass of glucose, oxygen and amino acids needed for cellular proliferation. Dry cell matter (DCM) was determined, and the mass of each compound needed to produce a kg of ACBM (wet basis) was calculated.

Equation 6.1 Wild type cellular metabolism from Humbird TEA

0.333 Glc + 0.342 0_2 + 0.007 Arg + 0.004 Cys + 0.055 Gln + 0.003 His + 0.007 Ile + 0.010 Lys + 0.002 Met+ 0.005 Phe + 0.009 Thr + 0.002 Trp + 0.005 Tyr + 0.010 Val + 0.013 Ala + 0.006 Asn + 0.008 Asp + 0.011 Gly + 0.011 Leu + O.007 Pro + O.010 Ser —> DCM + 0.005 Glu + 0.070 NH_3 + 0.474 Lac + 0.435 $C0_2$ + 0.495 H_20.

Equation 6.2 Enhanced cellular metabolism from Humbird TEA

0.147 Glc + 0.378 0_2 + 0.007 Arg + 0.004 Cys + 0.022 Gln + 0.003 His + 0.007 Ile + 0.010 Lys + 0.002 Met+ 0.005 Phe + 0.009T hr + 0.002 Trp + 0.005 Tyr + 0.010 Val + 0.013 Ala + 0.006 Asn + 0.008 Asp + 0.011 Gly + 0.011 Leu + O.007 Pro + O.010 Ser —> DCM + 0.005 Glu + 0.004 NH_3 + 0.041 Lac + 0.455 $C0_2$ + 0.613 H_20.

Glutamine is not an E8 component, so a literature source was used to determine microbial yield (0.368 g/g glucose) and the microbial method described in the E8 LCA was applied to determine the environmental impact of glutamine inclusion in the growth medium (Lv et al., 2021). It is likely not included in E8 due to stability issues; however, it plays an important role in cellular metabolism (Lu et al.,

2019). Masses of minor protein ingredients such as insulin, transferrin, fibroblast growth factor (FGF) and transforming growth factor (TGF) were also accounted for on a functional unit basis.

The Humbird TEA also accounted for the power usage per batch. We examined the energy usage based upon batches per year (54,000 batches per year at 1852 kg/batch). Table 6.2 provides energy usage and unit conversions. This was then examined on a functional unit basis of 1 kg of ACBM.

Table 6.2 Humbird TEA energy estimates

	kwh per batch	BTU/year	MJ/year
Vacuum pressure swing adsorption oxygen gas generator power	2,139	394,106,472,000	415,805,974
Compressor power	156	28,742,688,000	30,325,260
Agitator	47	8,659,656,000	9,136,457
Chiller	257	47,351,736,000	49,958,923
Dewatering	22	4,053,456,000	4,276,639
Facility*	11,511	2,120,878,728,000	2,237,654,311
Natural gas (reported in MMBTU)	N/A	540,000,000,000	569,732,400
Total	14,132	3,143,792,736,000	3,316,889,964

*Includes clean rooms facilities

In sum, it was determined that the Humbird TEA had more complete accounting of energy use and capital expenditures than the Risner et al. TEA, but Humbird TEA assumptions about the growth medium needed to be updated to include additional necessary vitamins and minerals for animal cell growth.

Combined production system

Utilizing the best information from the Risner et al. TEA, E8 LCA, and the Humbird TEA, a new production system was modeled to understand the near-term impact of ACBM production. The capital expenditures described in the Humbird TEA were complete, however these were not considered for this assessment. The energy requirements from the production facility modeled from Humbird were used to estimate potential energy requirements. Figure 6.1 is a process flow diagram of a fed-batch ACBM production system with associated energy requirements.

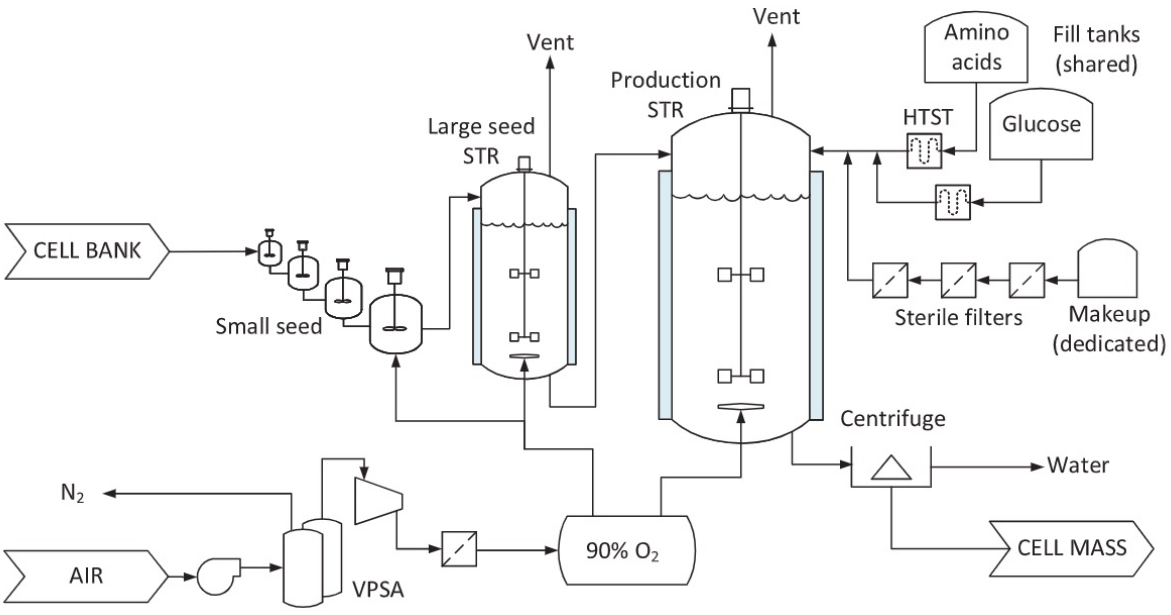


Figure 6.1 Fed-batch ACBM production system utilized in this LCA of ACBM

This image was taken from Scale-up economics for cultured meat (Humbird, 2021)

The use of glucose consumption rate and required amino acid content was taken from the Risner et al. TEA. Also, the idea of utilizing a highly refined growth medium can be attributed to the Risner et al. TEA. The Humbird growth medium requirements were also calculated and utilized for scenario development. The growth medium requirements were entered into OpenLCA which contained the datasets for the E8 growth medium components. Figure 6.2 provides a map of how the main literature sources were utilized to provide an updated LCA of ACBM.

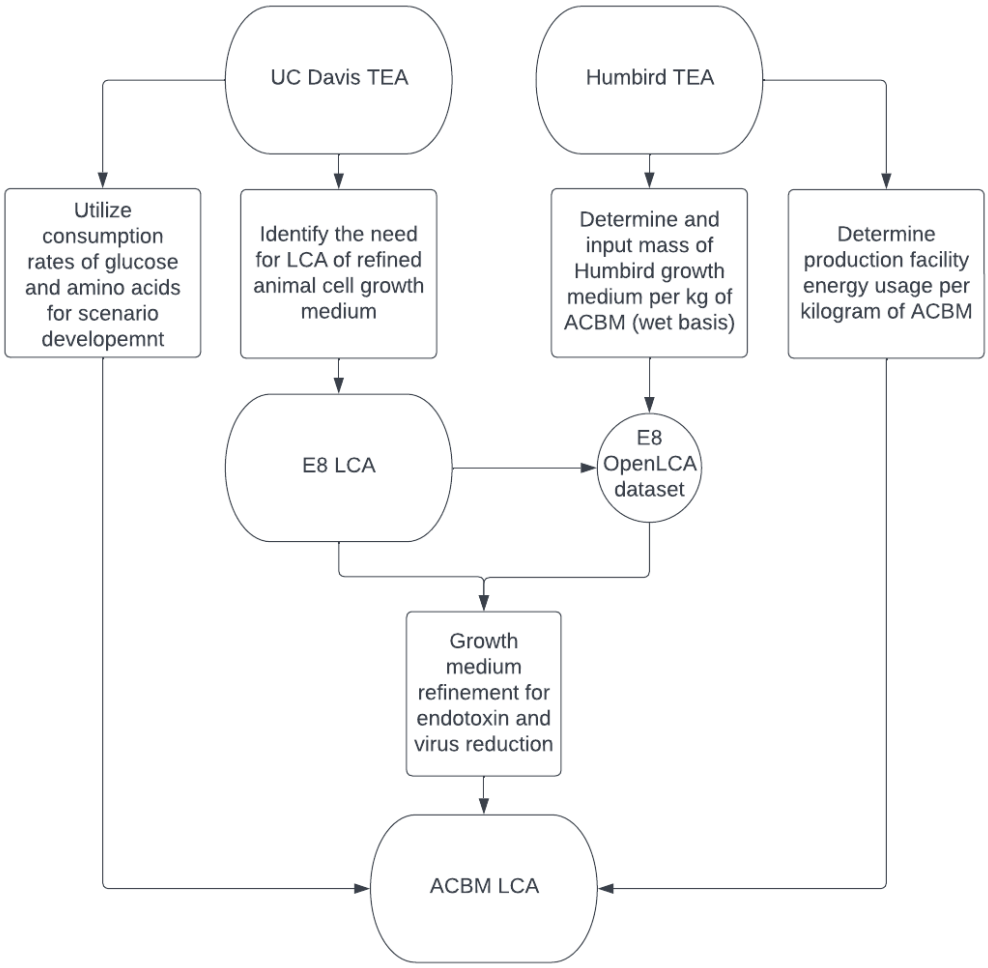


Figure 6.2 ACBM LCA main literature source map

Lifecycle Impact assessment (LCIA)

After all the inputs were identified and consolidated, a life cycle impact assessment was completed utilizing data and methods from the E8 LCA, OpenLCA v.1.10 software and OpenLCA LCIA v2.1.2 methods software. The tool for reduction and assessment of chemicals and other environmental impacts (TRACI) 2.1 was the LCIA methods utilized in the OpenLCA LCIA software, and these results were combined with the facility power data to determine the potential environmental impact of the production of 1 kg ACBM (wet basis).

Scenario analysis

All scenarios utilize a fed-batch system as described in the Humbird (2021) TEA. Energy estimates from the Humbird TEA are utilized in all scenarios. Growth medium components were assumed to be delivered to the animal cells as needed and the build-up of growth inhibiting metabolites such as lactate or ammonia are not accounted for unless specifically stated in the scenario. The growth medium substrates are also assumed to be supplied in a manner to achieve the highest possible specific growth rate in the production bioreactor. The three minimum/base scenarios were defined utilizing data from the Risner et al. and Humbird TEAs then a purification factor was applied based on the results from a LCA which examined the environmental impact of fine chemical and pharmaceutical production (Wernet et al., 2010). Each of the three base scenarios were examined independently and then with the purification factor applied for a total of six scenarios in the assessment (see descriptions below):

- *Risner et al. glucose consumption rate (GCR) scenario:* Reported estimates of the cellular glucose consumption rate were utilized to estimate the required growth medium volume in the Risner et al. TEA. This is same nutrient requirement as Scenario 1 from the Risner et al. TEA, however it is being delivered in a fed-batch manner as described by the Humbird system. The entire volume of growth medium is not assumed to be replaced, but the required nutrients are added as needed. This scenario utilizes E8 for its growth medium and it is estimated to require the equivalent of 1,148 L of E8 to produce one kilogram of ACBM wet basis.
- *Risner et al. amino acid requirement (AAR) scenario:* This scenario utilizes E8 as its growth medium and provides the minimum amount of amino acids needed to achieve the minimum amount of cellular protein mass for one kilogram of ACBM to be produced. This scenario indicates that 291.5 liters of E8 would contain the necessary amount of amino acids to produce a kilogram of ACBM wet basis with 21% (w/w) protein content.

- *Humbird growth medium scenario (HGM):* This scenario utilizes the Humbird TEA enhanced metabolism equation (equations 6.2) to estimate the total required growth medium nutrients. The wild-type metabolism was not utilized for scenario development due to it being deemed economically unfavorable. This scenario utilizes 0.35 kg of glucose, 0.16 kg of oxygen, 0.26 kg of amino acids, and minor protein ingredients (209.52 mg of insulin, 115.56 mg of transferrin, 1.08 mg of FGF and 0.02 mg of TGF) to produce one kg of ACBM wet basis.

- *Purification factor (PF):* Fine chemical or pharmaceutical production is more energy and resource intensive than bulk chemical production (Wernet et al., 2010). To account for this, the authors have utilized an LCA which compared fine chemical production to bulk chemical production. It was reported the cumulative energy demand (MJ) was 20x greater than bulk chemical production and the global warming potential (GWP) was 25x greater than bulk chemical production (Wernet et al., 2010). In animal cell culture, growth mediums are highly refined to prevent contamination from endotoxin and other contaminates. Given the resource intensity of fine chemical production, a purification factor of 20x is utilized to account for the resources associated with high levels of refinement.

The scenarios were developed to examine a range of potential environmental impacts utilizing the information available to the authors. As more complete information is ascertained about ACBM production, additional scenarios could be developed to provide a more complete understanding of the true environmental impact of the ACBM products.

Results

The LCIA was conducted on both the base scenarios and scenarios with purified growth medium components. TRACI 2.1 results are shown in Table 6.3 The GWP for all scenarios was greater than the minimum reported GWP for retail beef (9.6 kg of CO_2e per kg of FBFMO) (Poore & Nemecek, 2018). The GWP of all purified scenarios ranged from 246 to 1,508 kg of CO_2e per kilogram of ACBM which is 4 to 25 times greater than the median GWP of retail beef (~60 kg CO_2e per kg of FFBMO). Without purification of the growth medium components, the GWP of the GCR scenario is approximately 25% greater than reported median of GWP of retail beef (Poore & Nemecek, 2018).

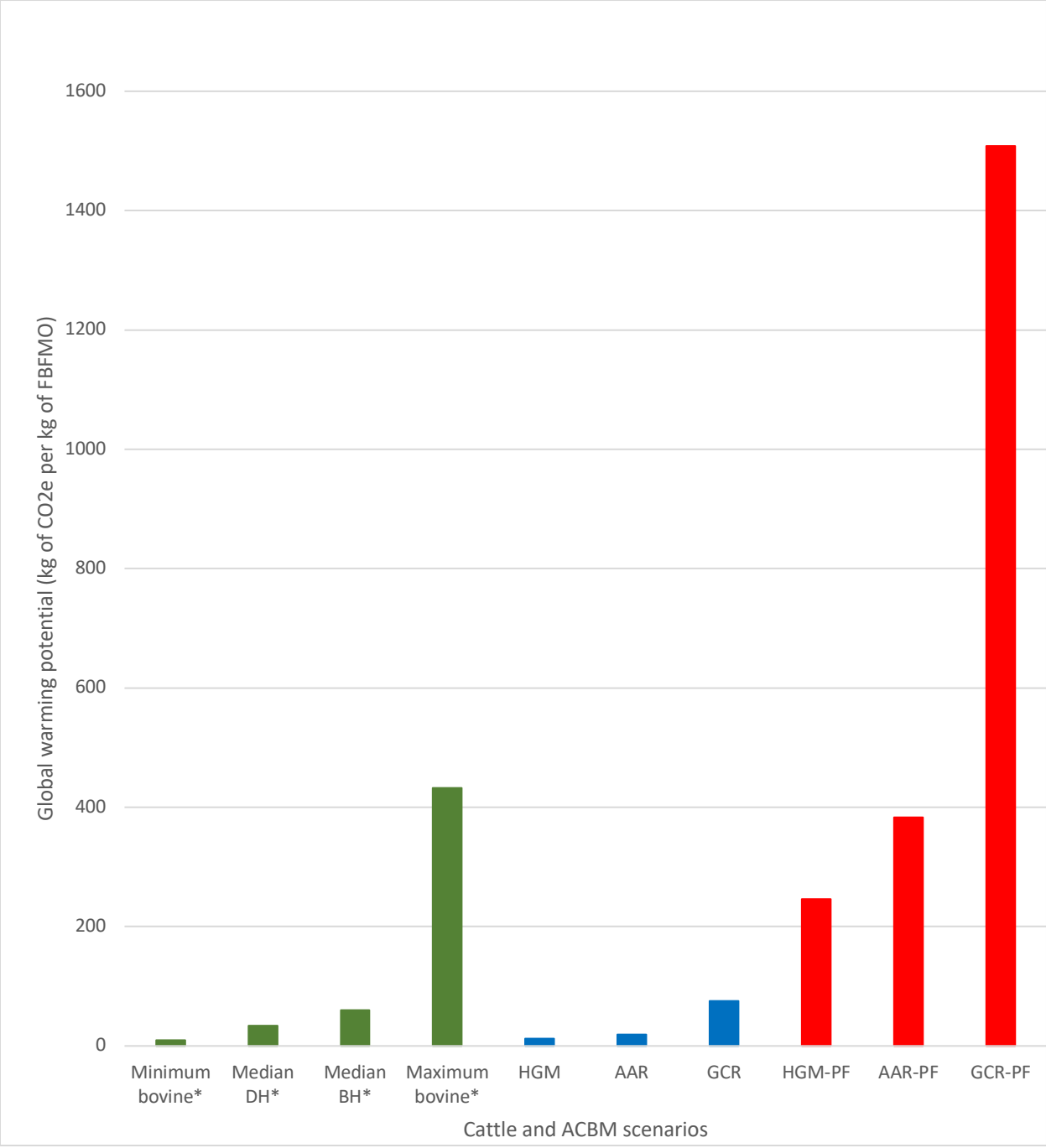
Table 6.3 TRACI 2.1 LCIA results for each unprocessed and purified growth medium scenarios

	GCR	GCR-PF	AAR	AAR-PF	HGM	HGM-PF
Smog (kg O3 eq)	4.5	89.4	1.1	22.7	0.69	13.8
Acidification (kg SO2 eq)	0.6	12.9	0.2	3.3	0.10	1.9
Respiratory effects (kg PM2.5 eq)	0.1	1.6	0.0	0.4	0.01	0.3
Non carcinogenic (CTUh)	0.0	0.0	0.0	0.0	0.00	0.0
Ecotoxicity (CTUe)	1,848.9	36,977.9	469.6	9391.7	229.92	4,598.4
Global Warming (kg CO2 eq)	75.4	1,508.3	19.2	383.1	12.31	246.1
Ozone depletion (kg CFC-11 eq)	0.0	0.0	0.0	0.0	0.00	0.0
Carcinogenics (CTU)	0.0	0.0	0.0	0.0	0.00	0.0
Eutrophication (kg N eq)	0.5	9.0	0.1	2.3	0.07	1.4
Fossil Fuel depletion (MJ surplus)*	85.3	1,706.4	21.7	433.4	14.89	297.8

*Energy usage by ACBM production facility not accounted for in the table

It should be noted that the system boundary of this LCA stops at the ACBM production facility gate and does not include product losses, cold storage, transportation, and other environmental impacts associated with the retail sale of beef. Inclusion of these post-production processes would increase the GWP of ACBM products. Figure 6.3 illustrates the difference in the GWP of retail beef and cradle to upstream ACBM production gate.

Figure 6.3 Comparison of GWP of the ACBM production scenarios and reported retail beef values (fat and bone free meat and edible offal).



*FBFMO: Fat and bone free meat and edible offal

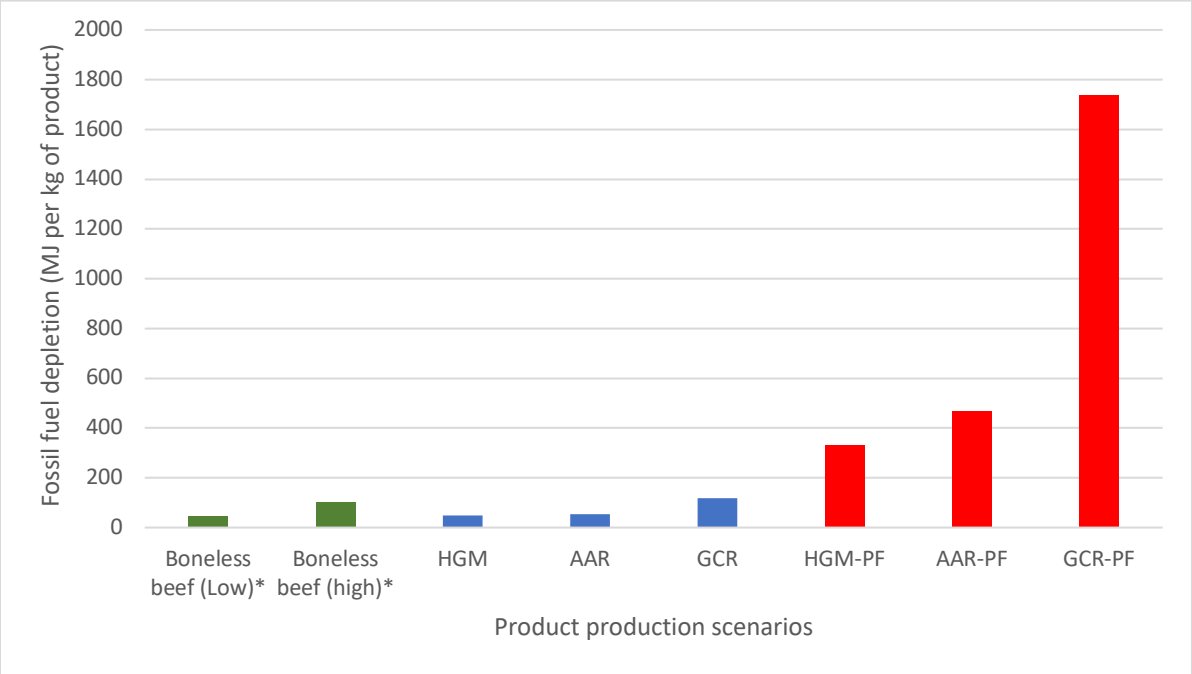
DH= Dairy herd

BH= Beef herd

Reported retail beef from Reducing food's environmental impacts through producers and consumers (Poore & Nemecek, 2018)

The fossil fuel depletion metrics were greater for all the ACBM production scenarios as compared to the low boneless beef metric (see figure 6.4). For unpurified scenarios, the higher level of energy use is largely associated with upstream processing facilities producing input products required for ACBM production. The HGM scenario was approximately ~1 MJ per kilogram greater than the lower estimate for boneless beef (Maysami & Berg, 2021). The AAR-PF and AGM-PF scenarios with growth mediums refined for animal cell culture required approximately an order of magnitude more energy than the reported low for boneless beef. The high cumulative energy demand for boneless beef was approximately double the fossil fuel depletion of the AAR and HGM scenarios. The fossil fuel depletion for scenarios with purified growth medium components were approximately 3 to 17 times greater than the reported high for boneless beef.

Figure 6.4 Fossil fuel depletion of each ACBM production scenario in comparison with boneless beef



*These are energy intensities which may include non-fossil fuel energy (Maysami & Berg, 2021)

Our system boundary for ACBM production does not include post-harvest handling, storage and transport which all require energy in some form. These additional energy inputs may increase the energy intensity/fossil fuel depletion of ACBM products indicating the reported results may be viewed as minimums.

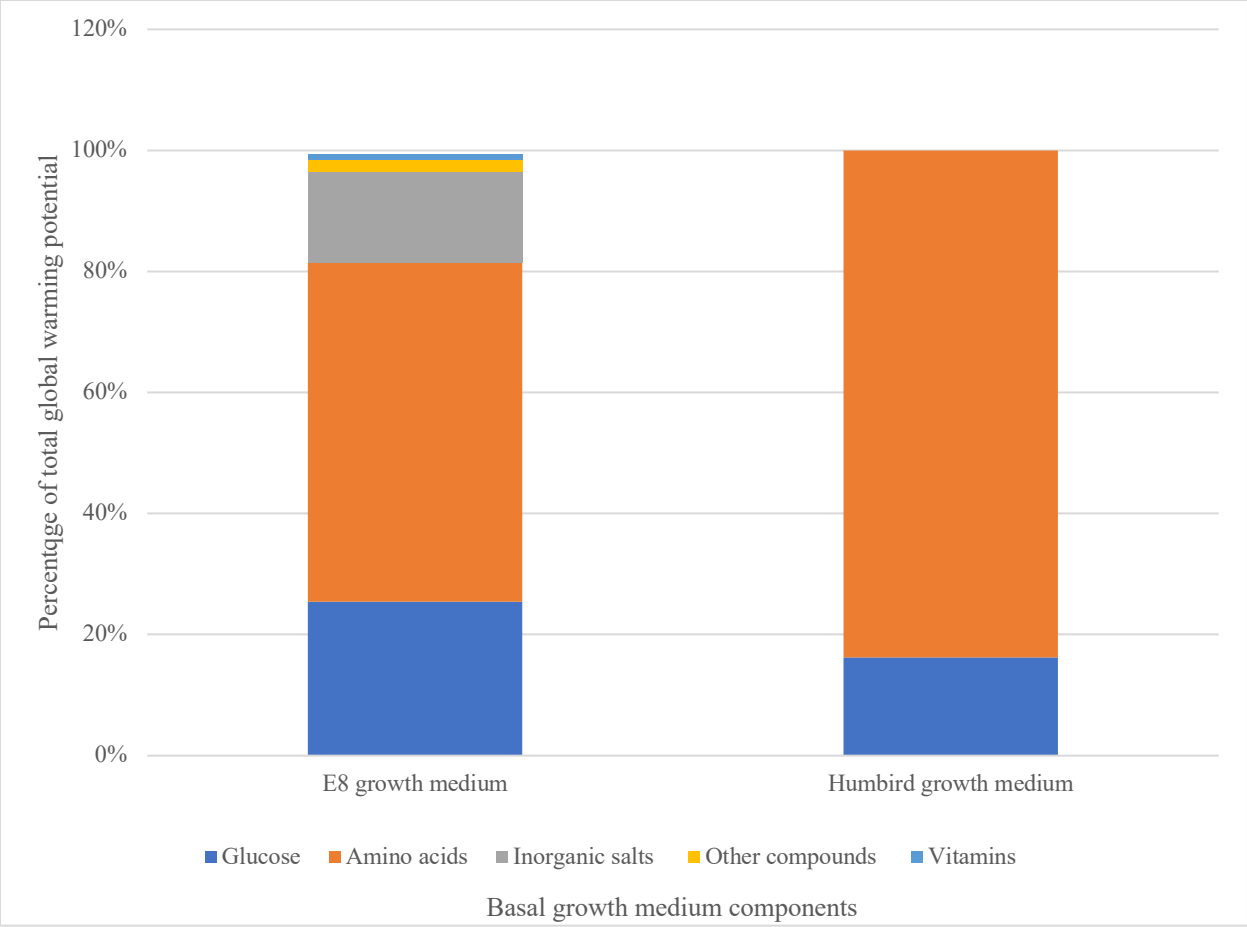
Discussion

Our results indicate that ACBM is likely to be more resource intensive than most meat production systems according to this analysis. In this evaluation, our primary focus has been on the resource intensity of the growth mediums. We have largely focused on the quantity of growth medium components (e.g. glucose, amino acids, vitamins, growth factors, salts, and minerals) and attempted to account for

purification requirement of those components for animal cell culture. We also acknowledge that our analysis may be viewed as minimum environmental impacts due to several factors including incomplete datasets, the exclusion of energy and materials required to scale the ACBM industry and exclusion of the energy and materials needed to scale industries which would support ACBM production.

We examined the growth mediums utilized in both UC Davis and Humbird TEAs and selected the UC Davis TEA as a more reasonable assumption given its more complete composition. Figure 6.5 compares the global warming potential of the different categories of basal growth medium components within each growth medium and illustrates differences in the basal mediums, such as the inclusion of vitamins, inorganic salts, and other components in the E8 growth medium.

Figure 6.5 Growth medium component contribution to global warming potential of each basal growth medium



Given the stringent medium component purity requirements for animal cell culture, the high purification scenarios with E8 as the growth medium are likely to represent the more accurate environmental impact of ACBM production. It should also be noted that these results should be considered a minimum since the E8 LCA is admittedly non-exhaustive (Risner et al., 2023). The E8 LCA does not account for 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and lipoic acid

production and there is only partial accounting of the embedded resources and energy for other E8 components (Risner et al., 2023). Scenarios AAR and AAR-PF assume a 100% conversion of amino acids to protein. This assumption is probably a poor assumption given the amino acids supply the nitrogen atom and amino group in the synthesis of nucleotide bases and nitrogen-containing sugars (Hu, 2020). The amino acid carbon skeleton is also utilized in the formation of groups like the functional methyl group (Hu, 2020). This indicates that AAR-PF may be an unlikely minimum as well.

Animal cell culture is inherently different than culturing bacteria or yeast cells due to their enhanced sensitivity to environmental factors, chemical and microbial contamination. This can be illustrated by the industrial shift to single use bioreactors for monoclonal antibody production to reduce costs associated with contamination (Jacquemart et al., 2016). Animal cell growth mediums have historically utilized fetal bovine serum (FBS) which contains a variety of hormones and growth factors (Jochems et al., 2002). Serum is blood with the cells, platelets and clotting factors removed. Processing of FBS to be utilized for animal cell culture is an 18-step process that is resource intensive due to the level of refinement required for animal cell culture. Thus, the authors believe that commercial production of an ACBM product utilizing FBS or any other animal product to be highly unlikely given this high level of refinement.

The requirement of endotoxin removal would also contribute to the environmental impact of ACBM products which makes our LCIA results for the minimum scenarios to be highly unlikely minimums. Utilization of commodity grade growth medium components such as glucose for animal cell growth is unlikely unless the components undergo an endotoxin separation process. The effect of endotoxin can vary greatly depending on cell type and source; however 25 ng/ml of endotoxin was shown to cause cell apoptosis when coupled with non-lethal heat shock (Corning, 2020). The necessity to remove endotoxin also indicates the use of a plant hydrolysate as an amino acid source will be challenging since endotoxin is amphiphilic with its hydrophilic polysaccharide fraction and hydrophobic

lipid fraction. The amino acids in the plant hydrolysate will interact differently depending upon their functional properties (e.g., hydrophobicity, charge). The multitude of interactions will potentially make separation difficult without additional separation steps which will further increase the environmental impact of the ACBM growth medium and subsequently ACBM products. Endotoxin does have an overall negative charge which may be beneficial for separation, however these extra processing steps will increase the environmental impact of ACBM products. For these reasons, the authors believe that scenarios which account for purification to be closer to a true minimum rather than the minimum baseline scenarios. An additional strategy for potential ACBM producers would be to develop cell lines which are endotoxin tolerate which may be help reduce the potential environmental impact of ACBM products.

We did not consider the environmental impact of scaling up ACBM production facilities. In 2021, the total cell culture bioprocessing capacity was 17.4 million liters with mammalian cell culture capacity being 11.75 million liters (Langer & Rader, 2021). The Humbird TEA states that each fed-batch production facility would require a total bioreactor volume of 649 m^3 and that it would require ~14.7 identical facilities to produce 100,000,000 kg of ACM annually, or an additional 9,540,300 liters of mammalian cell culture capacity. If this capital expansion was included in our LCA, we would need to expand our system boundary to include all the resources used in the mining of the materials and construction of these facilities. We also have not included the environmental impacts associated with scaling up multiple production facilities to produce the required mass of growth media components necessary for ACBM production at scale (Humbird, 2020, 2021). For these reasons, we believe that additional work is necessary to provide this expanded view of the environmental impact of producing ACBM at scale.

Conclusion

Critical assessment of the environmental impact of emerging technologies is a relatively new concept, but it is highly important when changes to societal-level production systems are being proposed

(Bergerson et al., 2020). Agricultural and food production systems are core to feeding a growing global population and the development of technology which enhances food production is important for societal progress. Evaluation of these potentially disruptive technologies from a systems-level perspective is essential for those seeking to transform our food system. Ideally, systems-level evaluations of proposed novel food technologies will allow policymakers to make informed decisions on the allocation of government capital. Proponents of ACBM have hailed it as an environmental solution that addresses many of the environmental impacts associated with traditional meat production. Upon examination of this highly engineered system, ACBM production appears to be resource intensive when examined from the cradle to production gate perspective for the scenarios and assumptions utilized in our analyses.

The existing LCAs of ACBM are insufficient for assessing the environmental impact of this emerging food technology. The main issue with these studies is that their technology models do not accurately reflect the current/near term practices which will be utilized to produce these products. Our environmental assessment is grounded in the most detailed process systems available that represent what is actually being done in this emerging food technology sector. Our model generally contradicts these previous studies by suggesting that the environmental impact of cultured meat is likely to be higher than conventional beef systems, as opposed to more environmentally friendly. This is an important conclusion given that investment dollars have specifically been allocated to this sector with the thesis that this product will be more environmentally friendly than beef.

Given this assessment, investing in scaling this technology before solving key issues like developing an environmentally friendly method for endotoxin removal or adapting cell lines which are endotoxin resilient would be counter to the environmental goals which this sector has espoused. Perhaps a focus on advancing these precompetitive scientific advances might lead to a better outcome for all. For example, solving the endotoxin challenge would also substantially benefit the biomedical and biopharmaceutical industries and their consumers by substantially reducing the cost of production.

Another example would be the development of a technological innovation which allow for the use of an inexpensive animal cell growth medium produced from an agricultural by-product. In short, our environmental assessment highlights the need for critical, detailed environmental assessments of emerging technologies to guide governmental agencies and the private sector *in advance of* allocating substantial research funding towards initiatives that assume transformational environmental benefits in the absence of rigorous analysis.

In sum, understanding the minimum environmental impact of near term ACBM is highly important for governments and businesses seeking to allocate capital that can generate both economic and environmental benefits (Zimberoff, 2022). We acknowledge that our findings would likely be the minimum environmental impact due to the preliminary nature of our LCA. This LCA aims to be as transparent as possible to allow the interested parties to understand our logic and why we have developed these conclusions. We also hope that our LCA will provide evidence of the need for additional critical environmental examination of new food and agriculture technologies.

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Chapter 7. Conclusion

The goal of this dissertation was to provide a critical analysis of the sustainability of near-term ACBM production. As a sum of the total parts of our analysis, there are strong indications that the sustainable production of ACBM has many challenges to overcome. The economic challenges appear to be substantial with growth medium costs being a key driving factor. Animal cell growth mediums generally contain more components than bacterial or fungal growth mediums due to the complex metabolism of multi-cellular animals. The need for additional costly growth medium components, such as growth factors or amino acids in lieu of a more economic nitrogen source like ammonia provides an economic hurdle which is different than bacterial or fungal products.

Seemingly straight forward problems related to the economic production of ACBM are deceptively complex. One example of such a problem is the lowering of growth medium cost. This is actually a multi-faceted problem which may involve the reduction/elimination of costs associated with growth factors, development of an economic method to purify growth medium components to required purity levels for large scale animal cell culture and scaling of multiple industries to provide growth medium components for the scaled production of ACBM. These are all examples which don't necessarily have easy or apparent solutions. It should also be noted that these are problems/issues which are not unique to ACBM production since these are issues the biopharmaceutical industry have not addressed.

Many of the economic issues with ACBM are linked to challenges related to the environmental impact of ACBM. One example is endotoxin removal from growth medium components which is both resource intensive and costly. One key takeaway that should be considered when examining ACBM from an environmental perspective is understanding where the system boundary is drawn. Tracing growth medium components to their "cradle" can be difficult, as illustrated in Chapter 5, but is necessary to truly understand the embedded resources within a growth medium. We have also assumed a scaled bioeconomy in our analysis, but this is not a current reality. What this indicates is additional research is necessary to truly understand the environmental impact of near-term ACBM products. Obtaining real-

world environmental data about the production of every component and component precursor would likely be difficult given the quantity of components in an animal growth medium and lack of access to industrial data. Individual producers of components and component precursors are unlikely to be willing to share their data and may not even have data on the level of detail necessary for an unassailable LCA to be conducted. In any LCA the assumptions should be carefully considered by the reader. This appears to be especially true for LCAs conducted for ACBM products which are not widely available seven years after the inception of the first ACBM company (Memphis meats, now known as Upside foods).

The ACBM industry is still in a nascent, uncertain state indicating that additional environmental and economic analyses are necessary as the industry matures or is scaled back. This dissertation aims to provide a more detailed evaluation of the sustainability of ACBM than what was previously available to the public, however it is far from comprehensive in its evaluation. While this evaluation is highly focused on the economic and environmental aspects of ACBM production, it neglects to delve into the third core tenant of sustainability which is the social aspect. The social aspect could potentially examine the impact of ACBM on traditional agriculture for those bullish on the emerging technology. For those bearish on the technology, it could be an examination of how or why so much capital was allocated to companies with unproven technological claims. Given the embryonic nature of the ACBM industry additional research in all aspects of sustainability related to ACBM are essential to extend knowledge and improve decision-making in this emerging industry.