ABSTRACT

GOODWIN, CORBIN MARIE. Towards Enabling Scale-Up of Cultivated Meat: Techno-Economic Analysis and Circular Cell Culture System. (Under the direction of Dr. Rohan Shirwaiker).

The accelerating demand for resource-intensive, industrially produced meat should be supplemented by cultivated meat (CM), which offers critical efficiency gains in land, water, and fossil fuel use. Drastic scale-up is necessary for CM to meaningfully compete with conventional livestock agriculture. Despite this, the majority of edible lab-grown meat is being produced in R&D laboratories on the scale of spinner flasks and well plates. Large-scale modeling should be leveraged to uncover knowledge gaps and direct resource allocation. As such, techno-economic analyses (TEAs) offer insights into what industrial production of CM should look like to achieve price parity with conventional meat. By applying current bench-scale knowledge in combination with facility-scale variables and cost sensitivities, we can interrogate the state of progress in CM.

The specific aims of this thesis are:

1. Analyze current TEAs on CM for insights on the challenges to industrial-scale production and compare with trends in primary CM literature.
2. Devise an industrial-scale process design and TEA for a circular cell culture system to assess the feasibility of a novel, sustainable, and cost-effective replacement of conventional media.

In the first aim, a scoping review of primary CM literature was conducted as well as an analysis of the six available TEAs and related works. The essential assumption that frames each of the TEAs is the intent to utilize scaling practices, data, and facility design of related bioprocessing fields, such as large (≥ 20,000 L) stirred-tank bioreactors (STRs) and suspension-tolerant, continuously available cell lines. While these ideas are not entirely new, they are

markedly different from trends within almost all other primary CM literature which more closely resemble bench-scale tissue engineering. The TEAs demonstrate that, under the current technological paradigm, CM is unlikely to be competitive with traditional meat. They reinforce the importance of research on several topics, such as cell type, media formulation, and suspension culture. Feasibility may hinge on specific cost-saving areas, such as the use of complex, plant-based media components, food-grade aseptic conditions, and drastic scaling of related supply chains. The TEAs also point to other high-priority research directions that are scarce in primary literature, including serum-free differentiation, bioreactor design, and facility design.

The second aim presents a TEA of a novel circular cell culture system that entirely replaces conventionally defined, serum-dependent, cell culture media. Based on the bench-scale design in literature, nutrients are supplied by *Chlorococcum littorale* microalgae biomass, and spent media is recycled by a live microalgae culture. This TEA also draws from large-scale microalgae bioprocessing designs from the biofuel sector. As the first CM TEA to incorporate the in-house production of medium macronutrients, the model requires very large capital expenditures (industry-wide CAPEX, ranging from $5B to $76B), but achieves the lowest base-case estimate of cell culture medium cost (<$1/L) due to low operating expenditures (OPEX). It also reveals that microalgae biomass production and media recycling should be separate systems due to the imbalance in medium requirements and divergence of optimum processing conditions. The cost and environmental efficiency of the circular cell culture (CCC) facilities rely most crucially on the intensity of microalgae biomass demand by the CM animal cells.

In summary, this thesis comprehensively analyzes CM TEAs and identifies high priority research directions. Process design of a novel circular media system to reduce media costs, one key direction identified from the TEA review, and improve sustainability is also analyzed. The outcomes of this thesis can help advance research toward achieving CM scaleup to achieve price parity with conventional livestock agriculture.

Towards Enabling Scale-Up of Cultivated Meat: Techno-Economic Analysis and Circular Cell Culture System

by
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DEDICATION

To the unparalleled "Rain and Thunderstorm Sounds on Study Ambience with Crackling Fireplace" by New Bliss on YouTube.

BIOGRAPHY

Corbin Goodwin completed her B.S. in Bioengineering at Clemson University in May 2022. In the Fall of 2022, she began her M.S. in Industrial and Systems Engineering at North Carolina State University. Corbin joined the Biomanufacturing lab and started her thesis research in the Spring of 2023 under the guidance of Dr. Rohan A. Shirwaiker.

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1.1 Food and the Environment

Prudent management of the relationship between food and the environment has become critical to sustain the growing global population amidst mounting ecological pressure. Compared to 15 years ago, it is estimated that 70% more food will be needed by 2050, yet climate change and ecological decline are increasingly straining the global food supply already[1]. Intensified and more frequent heat waves, droughts, storms, floods, and crop diseases, diminishing arable land, and heat-induced human and crop productivity decline are leading to compounding risks of malnutrition and mortality[2]. Conversely, the food system itself is a driver of ecological degradation. It creates around a quarter of humanity's total greenhouse gas emissions and without intervention is expected to far overshoot emissions limits that keep the globe below 2°C over pre-industrial averages[1,3]. Drastic changes to the food system are an essential component of the plan to reach climate change targets laid out by the Intergovernmental Panel on Climate Change (IPCC). It is also responsible for a number of negative feedback loops, such as the simultaneous dependence on and destruction of invertebrate pollinators, 40% of which face extinction[4,5]. Further, without intervention, current agricultural practices will not meet the demands of coming decades. Land use, for example, is a severe issue; agriculture currently uses 50% of global habitable land, and will demand an impossible additional area two times the size of India by 2050[1,6,7]. To sustainably feed the world, food production must be strongly adapted to increase efficiency while simultaneously reducing energy, land, and water use, amongst other impacts.

Meat production is key to these interventions, as it is the single food group with the greatest effect on the environment. While meat tends to be an excellent source of complete protein and provides economic and food security in many regions, meat production especially disproportionately consumes global resources and is a main driver of deforestation, methane emissions, and loss of biodiversity[8,9]. 41% of the contiguous US is used for feeding and raising livestock, a majority of which goes to meat production and does not account for land use by imported livestock, and yet meat provides only 17% of consumed calories. Meanwhile, agricultural land for all other food makes up around just 4% of the contiguous US[10]. This severe inefficiency in land and energy use cannot be maintained alongside the planet's growing food and resource demands. Indeed, globally, livestock consume five times the amount of food that humans do[11]. This presents a clear opportunity: many more people can be fed with far less environmental impact if conventional meat consumption is partially replaced or reinvented. Thus, alongside plant-based food movements, there is a growing impetus on developing cultivated meat (CM) to supplement conventional industrial livestock farming. Also known as lab-grown meat, clean meat, and cell-based meat, CM is real animal tissue produced using biotechnology.

1.2 Environmental Impact of CM

CM has the potential to offer a significant reduction in consumption of most resources in comparison to conventional beef production[12–14]. Animals must use feed calories for many outputs, muscle biomass production (i.e., meat) being just one. On the other hand, the efficient caloric conversion of CM requires reduced feed inputs to produce the same amount of food. Recent life cycle analyses (LCAs) offer insights into the impacts of this tradeoff. Reduced feed requirements lead to a predicted 84-90% reduction in land use by CM in comparison to conventional beef. This reduces fertilizer use, and in combination with the elimination of manure, also results in less fine particulate matter and acidification potential. LCAs also predict

CM will require ~43-65% less blue water in comparison to beef. CM is estimated to have variable but overall similar environmental impact in comparison to chicken, sausage, and fish[12–14]. Though CM converts calories from crops more efficiently, this is done with higher energy use: ~38% higher than beef cattle and ~88% higher than chicken[13]. However, this still presents an opportunity similar to that of vertical farming: the electrification of conventional agriculture can further our independence from fossil fuels, given that the electricity supply is renewable. Indeed, recent LCAs predict that CM reduces global warming potential by 83-93% through the use of renewable energy[12,13] in comparison to beef. Though CM is projected to be less resource-efficient than plant-based protein[12], as income increases, so typically does demand for meat and dairy products. By 2050, the global demand for meat is expected to increase 88% compared to from 2010 data[1]. If produced on an industrial scale, CM could help fill this gap while remediating the enormous resource use issues of conventional livestock agriculture.

1.3 Current CM Research Trends

CM is real animal tissue, biopsied and expanded in culture. It is most commonly sourced from livestock animal species, such as cows, pigs, chickens, and fish, and grown to mimic the texture, taste, and nutritional profile of native muscle tissue[15]. CM combines disciplines such as tissue engineering, food science, and bioprocessing. A summary of primary CM literature is provided in **Table 1.1**.

Table 1.1: Summary of primary CM literature from scoping review. A total of 113 primary peer-reviewed articles, defined by an explicit focus on CM and the inclusion of cell-based lab work, are organized into six key overarching topics: scaffold, media, cell growth, cell type, bioreactor, and adipogenic and co-culture, with some articles included in more than one category. The common themes within each topic are also listed.

Topics	Papers	Common Themes & Current Work
Scaffold	56	• ~18 papers focused on fabrication • ~38 papers focused on biomaterials or other scaffolding aspects • Plant-based polysaccharides • Plant-based proteins • Functionalizing plant-based scaffolds • Decellularized plants • Tuning mechanical properties
Media	21	• Plant-based compounds, like protein isolates, to replace serum • Microalgae-based compounds, like antioxidants, to replace serum or basal media • Optimizing commercial serum-free media for relevant cell types • Circular media system, media recycling, and feeder cells • Bacteria for inexpensive recombinant protein production and optimizing production
Cell Growth	20	• Plant or microalgae-based supplements to support growth • Exploring growth characteristics, such as the effect of hypoxia, aligned scaffolds, serum-starvation • Exploring how growth supplements or cell maturation impact organoleptic properties
Cell Type	16	• Creation of immortal lines (2 papers on engineered iBSC, 1 paper on naturally immortalized BSCs, 1 paper on naturally immortalized chicken fibroblast) • Transdifferentiation of somatic cells • Smooth muscle cells for proliferation and ECM production • Insect muscle and fat cells • Cell engineering to eliminate need for specific growth factors or other characteristics
Bioreactor	11	• Optimizing spinner-flask conditions for MCs • Few instances of STR cultures • 1 instance of perfusion culture, also the only instance of suspension culture • 1 instance of disposable rocking bags
Adipogenesis & Co-culture	24	• ~20 papers on adipogenesis • ~4 papers on muscle and fat co-culture

1.4 Scale Up

Several major advancements are still required to produce CM on a scale that could realize these benefits[16]. The cost of CM at scale was first considered in 2014 with a simple analysis that introduced the "village scale" production model[17]. Using common animal cell growth characteristics, the cell mass that one $20m^3$ STR could produce per year was estimated and compared at an assumed value of $5/kg against the cost of a minimal volume of defined media. The results showed that the cost of the most optimistically priced medium alone eclipsed the value of the CM produced. Even without the inclusion of production expenses, this early publication lucidly demonstrates the distance to an economically viable CM industry.

One of the most significant challenges to large-scale CM production is developing methods to inexpensively and efficiently produce vast quantities of cells. To adjust to a food-scale economy, there is growing consensus that cells must be grown in bioreactors as large as or several times larger than the maximum volume of 20,000 L used for animal cell culture in the pharmaceutical industry[18,19]. As bench-scale research in CM progresses, techno-economic analysis (TEA) offers detailed analysis of the hypothetical application of such research in combination with facility-scale variables and costs inspired by related fields.

There are currently six TEA studies published on CM. Using cell growth characteristics such as doubling time, maximum density, and media consumption from well-characterized animal cells such as human embryonic stem cells (hESCs), Chinese hamster ovary cells (CHOs), or the C2C12 mouse myoblast cell line, the production capacity of the chosen bioreactor volume is estimated and then parallel production lines or duplicate facilities required to achieve the yearly production goal are enumerated. The facility design often includes seed trains, media sterilization and storage, oxygen supply, and minimal downstream processing. Raw material, utility, maintenance, and labor costs may be incorporated into annual operating expense (OPEX) and equipment and installation costs are incorporated in a capital expenditure (CAPEX) estimate. The OPEX is combined with a portion of the CAPEX, which is distributed to annual costs over an estimated lifetime of the facility, to form the annual operating cost, which is divided by the annual production of CM to estimate a minimum cost of goods sold (COGS). Thus, the feasibility of scaled production of CM is judged by the minimum price required to cover the cost of production.

1.5 Motivation and Research Objectives

Additional sustainable and accessible protein supply is urgently needed. CM, if able to scale to a sufficient production volume, could deliver the taste, texture, and familiarity of conventional meat while severely reducing the associated resource demand. CM is a nascent field in which the majority of scientific publications are either reviews, perspectives, or based on bench-scale experimentation. Within primary publications, as shown in **Table 1.1**, a majority of work is centered on scaffolding techniques, which are generally limited to small-scale production. Instead, it is the goal of this thesis to reframe CM as a scalable industry aimed at the widespread provision of affordable and nutritious protein. This will be achieved by assessing current CM research directions and gaps within the framework of the few published CM TEAs. Based on the most urgent insights from that analysis, namely the need for an inexpensive, animal-free cell culture medium, a cross-disciplinary TEA combining microalgae and animal cell culture is constructed to analyze the potential of a novel circular cell culture system.

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CHAPTER 2: CM TEA REVIEW

2.1 Background

Cultivated meat (CM) may offer a sustainable protein supply in the coming decades provided it can be produced on an industrial scale. Large-scale modeling, such as techno-economic analysis (TEA), can generate insight into the feasibility of CM scale-up and uncover knowledge gaps. TEAs apply detailed process design and profitability analysis to assess novel production systems, and as such, should be employed at the early stage of technological development to direct resource allocation and research efforts[1]. This analysis is invaluable to CM, for which several TEAs have been published, but have not yet been comprehensively reviewed. Thus, this review analyzes current CM TEAs for opportunities and challenges presented by the state-of-the-art models of scaled-up production design and compares and prioritizes these needs to research directions found within other primary CM literature.

2.2 Key Results and Characteristics of CM TEAs

A scoping review of primary CM literature was performed (additional details can be found in Appendix A), to determine the primary TEAs. **Figure 1.1** presents a schematic overview of production methods amongst the CM TEAs and design discrepancies due to scientific unknowns. The designs and foremost results of these TEAs are further described in **Table 2.2**. In this section, the main features of the CM TEAs are summarized and contextualized, which offer a number of cohesive conclusions, as well as uncertainties and opportunities for growth.

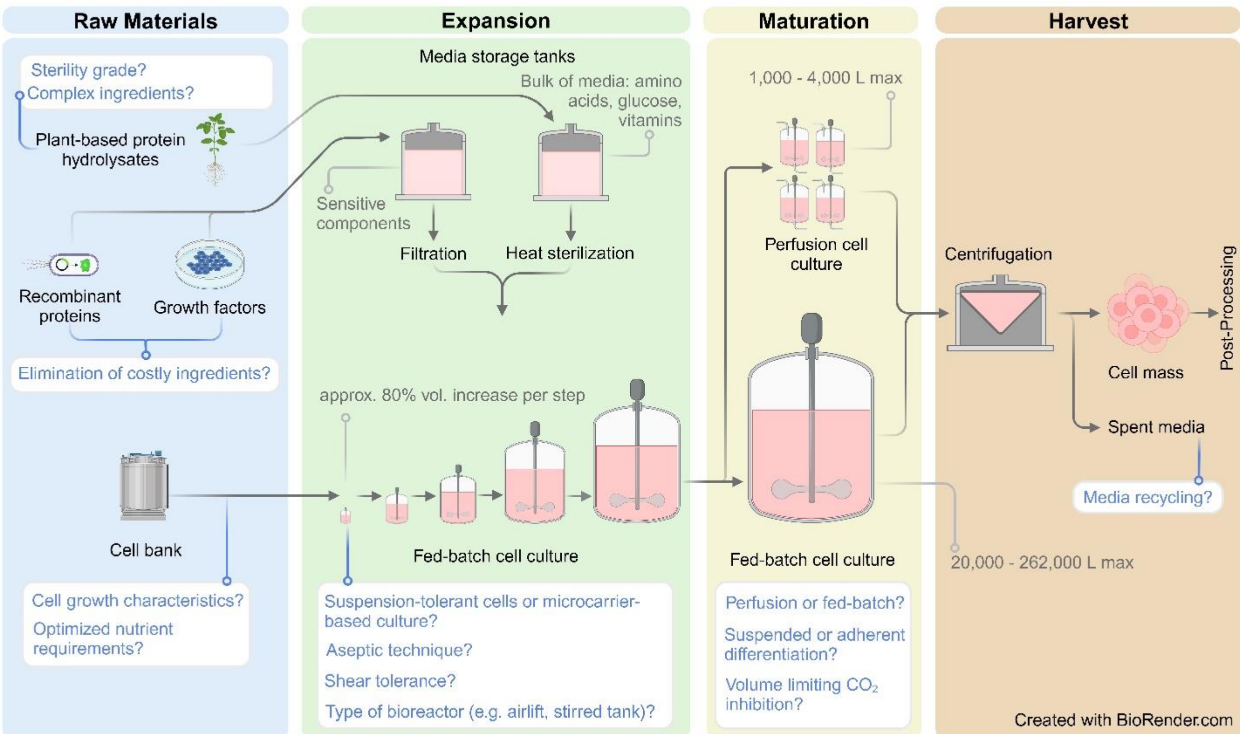

Figure 2.1: Schematic overview of scaled cultivated meat (CM) production informed by techno-economic analyses (TEAs). Key decision questions or discrepancies at various stages of production amongst the TEA models found in literature are highlighted in the white boxes.

Table 2.2: Summary of cultivated meat (CM) techno-economic analysis (TEA) studies. *Media cost in terms of $/L were not provided.

Better-case Scenarios	Better-case Cost of Production ($/kg product)	Better-case Facility Metrics
- Media cost of $0.24/L (Specht[28] Scenario 8) via low basal media cost and zero cost for recombinant proteins & growth factors - Reduced glucose consumption by order of magnitude - Density max volume fraction of 1 - Reduced doubling time (24h to 8h) & cell maturation time (240h to 24h)	2	50 STRs, $37M CAPEX
- Most amino acids sourced from soy hydrolysate	Fed-batch: 22 Perfusion: 35	Fed-batch: 24 STRs, $328M CAPEX Perfusion: 96 STRs, $663M CAPEX
- Reduced growth factor cost (1000-fold) - Reduced recombinant protein cost (100-fold) and need (5-fold) - Social investment allowing payback time of 30 years (from 4 years) - Reduced production time (25%) - Larger cell diameter	6.49	$320M CAPEX
- Larger cell diameter - Media turnover driven by lactate accumulation while supplementing glucose - Reformulated the medium composition (yeast extract, soy hydrolysate, & decrease amino acid concentrations) - Added growth factor IDGF-2	7.87	Unspecified
- +/- 30% OPEX & CAPEX - 10% deduction in operating time - Facility location in India or China	39.17	Unspecified
- Neglected depreciation - 42,000 L STR: N/A, cannot reach target - 211,000 L STR: media reduced to $0.45/L - 262,000 L ALR: media reduced to $0.75/L	<10	Unspecified

Table 2.2 [continued]

Author & Date	Summary	Assumptions	Production Goal (kg/yr)	Media Type & Cost Estimate	Baseline Media Cost ($/L)	Bioreactor	Animal Model	Cell Type	Max Cell Density (cells/mL)	Cell Doubling Time (hr)	Baseline Cost of Production ($/kg product)	Media Cost as % of Cost of Production	Baseline Facility Metrics	Baseline CAPEX linearly scaled to 1% of beef market
Risner et al.[21] (2020)	A preliminary TEA	- Food-grade - Total cell volume per bioreactor set equal to bioreactor volume	121,000,000	- Essential 8 - Vendor prices from Specht[26]	377 (Specht[26] Scenario 1)	20,000 L STR, fed-batch	Beef	Bovine myoblast/MSC, growth variables based on human embryonic stem cells (hESCs)	Scenario 1: 1×10^7 Scenario 2, S3: 9.5×10^7 Scenario 4: 2×10^8	Scenario 1: 24 Scenario 2, S3: 16 Scenario 4: 8	400,000	>99%	5205 STRs, $4B CAPEX	$4B
Humbird[19] (2021)	TEA & in-depth analysis if raw material supply chain is also scaled	- Pharma-grade - Scaled raw materials industries - Metabolic engineering to reduce density inhibition - CO_2 inhibition at large volumes	6,800,000 in a 100,000,000 market	- Defined, serum-free - Regression & bulk prices to estimate component costs at scaled production	Fed-batch: $22/kg of CM Perfusion: $21/kg of CM*	20,000 L STR, fed-batch *or* 2,000 L STR, perfusion	Mammalian	General mammal, growth variables based on CHO cells	8.6×10^7	24	Fed-batch: 37 Perfusion: 51	60%	Fed-batch: 24 STRs, $328M CAPEX Perfusion: 96 STRs, $663M CAPEX	Fed-batch: $4.84B Perfusion: $11.8B
Vergeer et al.[24] (2021)	Current & projected (to 2030) TEA using data from 7 CM companies & 7 suppliers	- Incorporates proprietary data from CM companies - Food-grade	10,000,000	- Defined, serum-free - Vendor prices from Specht[26] & supplier quotes	High: 537.3 Mid: 126.2 Low: 16.6	10,000 L STR & 2,000 L, perfusion with scaffolds, semi-continuous	Unspecified	Industry data averages	5×10^7	30	High: 22,421 Mid: 1,708 Low: 150	>99%	$450M CAPEX	$5.45B
Ashizawa et al.[22] (2022)	Examines how insect cells can reduce TEA costs, adapting the Risner et al. model	- Total cell volume per bioreactor set equal to bioreactor volume - Food-grade	121,000,000	- Yeastolate-Primatone (YPR) medium *or* Scheider's Drosophila medium - Vendor & bulk pricing	YPR: 28.88 Drosophila: 13.65	20,000 L STR, fed-batch	Insect	Lepidopteran (Sf-9 and Hi-Five) *or* Drosophila melanogaster (S2) insect cell lines	Lepid: 2×10^7 S2: 3.01×10^7	Lepid: 22.72 S2: 38.5	Lepid: 4193 S2: 6426	>99%	Lepid: $3.67B CAPEX S2: $12.3B CAPEX	Lepid: $3.67B S2: $12.3B
Garrison et al.[23] (2022)	Based on Risner et al.[21] & Specht[26], additional fixed, operational, & labor costs	- FGF-2 and TGF-β alternatives - Improved media efficiency - Food-grade	560,000	- Essential 8 - Vendor prices from Specht[26]	3.74 (Specht[26] Scenario 5)	20,000 L STR, expansion & 30,000 L STR, differentiation	Unspecified	Unspecified	Unspecified	Unspecified	63.69	27.90%	16 bioreactors, $60M CAPEX	$12.96B
Negulescu et al.[18] (2023)	TEA using large-scale STR or ALR bioreactors	- CO_2 does not inhibit cell density in large production volumes - Food-grade	100,000,000	- Modified Beefy-R - Humbird[19] price correlations for amino acids, vendor prices	1.4	42,000 L STR, 211,000 L STR, *or* 262,000 ALR; all fed-batch	Beef	General mammal, medium consumption experimentally determined by C2C12 cells in B8	3.3×10^7	23	42,000 L STR: 25 211,000 L STR: 20 262,000 L ALR: 16	42,000 L STR: 41.3% 211,000 L STR: 71.5% 262,000 L ALR: 83.3%	- 42,000 L STR (25 facilities): $10.95B CAPEX - 211,000 L STR (5 facilities): $5.86B CAPEX - 262,000 L ALR (4 facilities): $1.58B CAPEX	- 42,000 L STR: $13.25B - 211,111 L STR: $7.09B - 262,000 L ALR: $1.91B

2.2.1 Cost of Production Baseline

Assuming that scaled suspension cultures are possible, each TEA includes baseline cost of production results based on real-world data that attempts to reflect the scaled output of the current state of CM technology. Although the TEAs model a number of different protein products including bovine, insect, or general animal cells in slurry or paste format, this review will use the beef industry as a benchmark for CM advancements and goals; LCAs and other analyses repeatedly demonstrate CM typically lowers environmental impact in comparison to ruminant animals, primarily cows[2–4]. In 2023, the average wholesale price of choice-grade beef was $10/kg[5]. This is close to the cost of production goal ($9/kg) set by Negulescu et al.[6] by which CM may reliably compete with conventional beef. While others[7] suggest higher price points to initially market CM amongst premium goods, achieving price parity with conventional meat is necessary for longer-term widespread adoption, without which the potential environmental impact of CM is severely limited. Further, scaled CM will most likely resemble ground beef, which is less expensive than average beef products.[8]

In the first published CM TEA, Risner et al.[9] found an economically unviable baseline that required a vast number (5,205) of 20,000 L stirred tank bioreactors (STRs), and an extremely high cost of production of $400,000/kg. Ashizawa et al.[10] reported a two orders of magnitude reduction in cost of production using common fruit fly or lepidopteran insect cells with the aforementioned model[9]. The cost difference between the two is largely due to the insect cell's more efficient use of glucose and lower incubation temperature, however, the cost is still impractically high. Garrison et al.[11] presented a model with additional fixed and operational costs, including a seed train and labor, but selected an ambitious baseline media cost, two orders of magnitude lower than Risner et al.[9], resulting in a cost of production of $63.69/kg. Later, Negulescu et al.[6] presented a comprehensive revision of the earlier model[9] assuming very large bioreactors (42,000 L-262,000 L), a more cost-efficient media formulation, and bulk media pricing (adopted from Humbird[7]). For a facility using 262,000 L airlift bioreactors (ALRs), they found a cost of goods (COGS) of $16/kg, nearing but still well over the average wholesale price of beef. Humbird's[7] detailed analysis of the CM industry and TEA under the assumption that surrounding supply chain industries would scale alongside CM also resulted in a cost of production on the same order of magnitude as Negulescu et al.[6]. Vergeer et al.[12] took a unique approach, using proprietary cell growth and media requirement data from seven CM companies and seven related suppliers. Their cost of production is mid-range among peer TEAs, estimated from $22,421/kg to $150/kg.

Discrepancies between the various TEAs have clearly led to a wide range of CM cost estimates at scale. They are unified, however, by the result that with current technology (with the exception of significant cell line and suspension culture assumptions), CM would not reach a price competitive with conventional meat. If the industry is restricted to bioreactors at or below 20,000 L, CM approaches cost parity only under Humbird's[7] assumption of a scaled economy.

2.2.2 Capital Costs

All aforementioned TEAs share results that suggest CM will require massive capital investment. To replace just 1% of the US beef market, around 121 million kg/yr, Risner at al.[9] estimated a capital expenditure (CAPEX) of $4 billion. Notably, Negulescu et al.[6] reduced this cost by >50% using bioreactors approximately 2-13x larger in volume, but still assumed an impracticably large CAPEX ($1.58 billion), which is less easily broken into smaller investments. Both of these are underestimations, however, as they only include the cost of production bioreactors while neglecting components such as seed trains, media preparation, and downstream processing. The CM production goals of the other TEAs have been roughly scaled to 121 million kg/yr for comparison (**Table 1.2**). Humbird's[7] robust analysis of facility needs for 6.8 million kg/yr (CAPEX of $328 million for a fed-batch model) resulted in a scaled CAPEX of $4.84 billion. Similarly, the Vergeer et al.[12] scaled CAPEX was $5.45 billion. The smaller facility designed by Garrison et al.[11] presented a scaled CAPEX of more than double these estimates. To put these figures in perspective, the USDA reported a total CAPEX of $49.4 billion for all US farming in 2023[13] – a $5 billion CM facility could require ~10% of that to produce just 1% of the supply of one type of meat.

2.2.3 Bioreactors

The TEAs do not offer a consensus on the volume or process model of bioreactors for CM production. Each TEA incorporates STRs at a minimum volume of 20,000 L. In the simplest models, a seed train of fed-batch STRs drive the expansion phase of production, and the final bioreactor (STR or ALR) also houses the differentiation phase[6,7,9,10]. Specht[14] presented a process model of a fed-batch STR seed train with a final 20,000 L expansion bioreactor that feeds semi-continuously into 4,000 L perfusion reactors. Vergeer et al.[12] adopted this model[14] but reduced the volume of the final expansion bioreactor and the perfusion reactors by half, the rationale for which was not presented. Humbird[7] directly compared a fed-batch 20,000 L STR process to an alternative facility using 2,000 L perfusion reactors for expansion and differentiation. As a third approach, Garrison et al.[11] adopted Specht's[14] semi-continuous design but followed the 20,000 L expansion STRs with 30,000 L "harvest" bioreactors, which presumably are STRs with some kind of scaffolding capacity (e.g., microcarriers). Notably, models that use perfusion bioreactors have limited volume because the largest high-density perfusion filters run up to 1,000 L per day. Humbird[7] found that the high cost of these filters and bioreactor volume limitation more than offset the benefit from increased cell density, resulting in a 38% larger cost of production.

2.2.4 Medium

Though each TEA uses a defined medium in its baseline scenarios, there are some stark differences in formulation. Of those modeling mammalian cell cultivation, two[9,11] used the well-known Essential 8 medium and one[7] used a simplified version. Negulescu et al.[6] used Beefy-R medium, an optimized descendant of Essential 8 with cost reductions such as the replacement of albumin, a recombinant protein, with rapeseed hydrolysate[6,15]. Vergeer et al.[12] used media formulations informed by industry sources, revealing striking contrasts in approach. They presented high, medium, and low media price estimations, differing by two orders of magnitude. These price variations were primarily attributed to the surveyed CM companies' differences in the use of albumin, which is notably absent in the Essential 8 formula and replaced by rapeseed in the Beefy-R formula. Further, the weight of recombinant proteins needed per kg of CM was an order of magnitude higher in Vergeer et al.'s[12] formulation than, for example, Humbird's[7] simplified Essential 8. This resulted in a contribution to the cost per kg of CM of $99[12] and <$2.50[7], respectively. In fact, recombinant proteins and growth factors contributed over 99% of the cost of Vergeer et al.'s[12] media formulations, while Humbird's[7] analysis showed their costs were negligible at scale. These discrepancies alone make comparison amongst the CM TEAs difficult. The necessity of growth factors and recombinant proteins may, as Vergeer et al.'s[12] high and low media cost estimations show, make the difference between an economically viable and nonviable product.

Additionally, the cost of CM media is difficult to predict and varies significantly among the TEAs. Specht[14] and the TEAs informed by it[9,11,12] used prices for individual basal medium components like amino acids and growth factors, assuming in-house mixing. However, current vendor prices for individual components at smaller scales still result in a very high price point: Specht[14] and Risner et al's[9] baseline price estimate is $377/L. An alternative assumption is that a scaled CM industry will necessitate the expansion of the markets of some ingredients, substantially reducing the media cost. Humbird[7] made this point central to their analysis by scaling CM and surrounding industry markets to accommodate 100 million kg of CM annually, which is "roughly equivalent" to the size of the plant-based meat industry. This relies on the assumption that raw material industries would choose to scale to accommodate CM, and could do so on a reasonable timescale, and at appropriate purity. The annual production of half of the amino acids in Humbird's[7] defined media formulation would need to at least double, increasing in hundreds of metric tons, to meet current demand plus that of a 100 million kg of CM.

Additionally, a number of essential amino acids already produced at appropriate scales, such as L-lysine, are primarily feed-grade and therefore likely require the creation of new supply chains to achieve the purity required for CM. Although there is no consensus on aseptic production requirements for CM, as will be discussed in more detail later, it is expected that raw materials should be at least food-grade[16]. These supply challenges are a reality for all CM production, whether the TEAs assume a scaled market or not. It is additionally notable that although Negulescu et al. [6] adopted Humbird's[7] media price correlation model, their media formulation assumptions favorably shifted the market and lowered amino acid costs, resulting in significantly lower media cost contributions than predicted by Humbird.

Overall, media was almost always the most expensive component of CM production in each TEA. The severity of this impact on cost of production is dictated by media pricing and formulation methods combined with infrastructure cost considerations and other assumptions: in Humbird's[7] baseline model, due to bulk media pricing and comprehensive CAPEX analysis, media accounted for 60% of the cost of production while CAPEX and labor costs represented 32% and 3%, respectively. In Negulescu et al.[6], which used Humbird's[7] media pricing approach but different production scenarios and assumptions (e.g., less expensive media formulation, different types and sizes of bioreactors, exclusion of clean room setting), media represented 41-83% of the cost of production; corresponding CAPEX ranged from 56-14% while labor costs were consistently <1%. Garrison et al.[11] found media to be 31% of the cost of production due to inexpensive media assumptions and a higher cost of labor (28%), while CAPEX accounted for 30%. Meanwhile, the media cost represented >99% of the cost of production in the other models[9,10,12] which may have underestimated CAPEX.

2.2.4 Better Case Scenarios

Each TEA includes better or best-case scenarios that achieved competitive cost of production by implementing cost reduction measures, some additionally informed by sensitivity analysis. Unsurprisingly, media is the central focus in the better-case scenario cost reduction measures. Broadly, the models with a baseline COP far outside of a competitive range[9,10,12] performed more thorough sensitivity analyses and applied a variety of cost reduction measures. Meanwhile, Humbird[7] and Negulescu et al.[6], with more reasonable baseline results, made fewer changes in their better-case scenarios. Garrison's[11] baseline media cost assumption was already somewhat of a better case scenario that positioned their cost similarly to Humbird[7] and Negulescu et al.[6].

A variety of insightful media-reducing changes were also considered. Vergeer et al.[12] posited that growth factors such as FGF2 and TGF-ß may be able to be produced recombinantly and thus applied a 1000-fold cost reduction. They also reduced recombinant protein price by a factor of 100, citing industry sources. Humbird[7] and Ashizawa et al.[10] incorporated soy hydrolysate to replace more costly protein sources, such as animal derived or recombinant proteins. This was the only better case scenario Humbird[7] explored, and led to a $15 in the fed-batch scenario. Some better-case scenarios may be too lofty: Risner et al.[9] reached a competitive cost of production only through unlikely assumptions, including a cell density volume fraction of 1 (suspension culture viscosity limits are met at a volume fraction ~0.25[16,17]), and by setting recombinant protein and growth factor costs to zero, which account for over 99% of media costs in their baseline scenario. Ashizawa[10] and Vergeer et al.[12] increased cell size as a cost reduction measure, but did not change corresponding cell density and media consumption rates, resulting in an overestimation of the impact of a larger cell volume. Without considerable advancements in cell media technology, there is consensus through these TEAs that cost-effective production of CM may be untenable.

2.3 Analysis and Research Recommendations based on the TEA Insights

By applying current bench-scale knowledge in combination with facility-scale variables and cost sensitivities, TEAs allow us to interrogate the state of progress in CM science. There are many key lessons from the TEA studies that should inform future research. Some of these concepts are already being explored in primary literature and will be analyzed here, as summarized in **Figure 2.2**. Other areas are not yet present and therefore raise opportunities for growth.

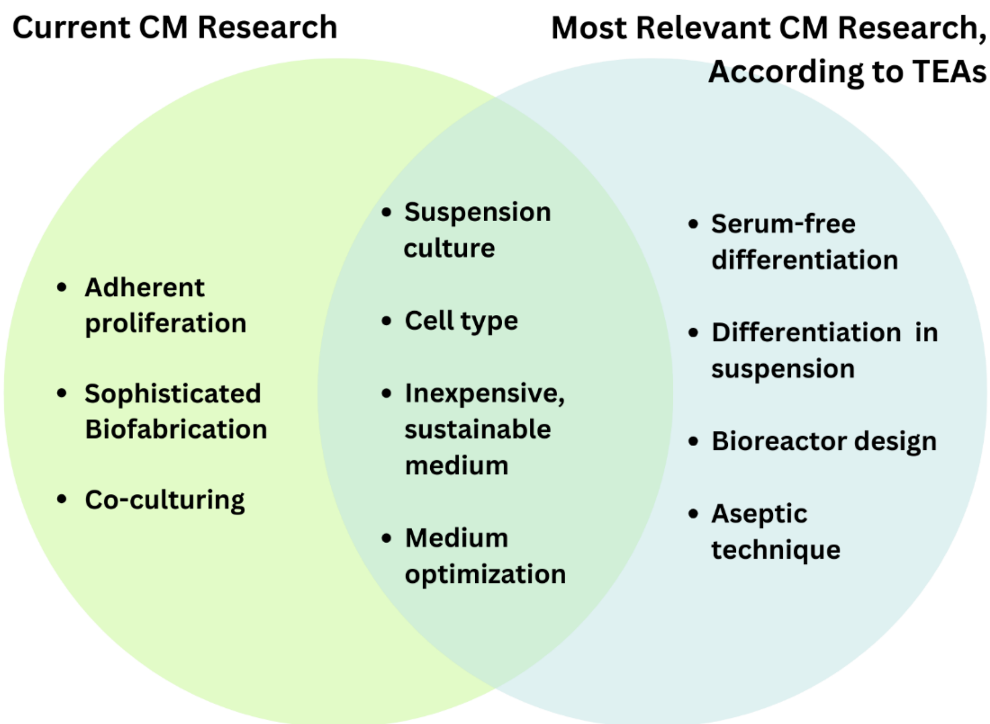

Figure 2.2: Informed by the CM TEAs, the relevance of current research directions found in primary literature, including missing research.

2.3.1 Current CM Research Relevant to Scaled Production

Suspension Culture

One critical characteristic shared by the TEAs is the use of ≥20,000L bioreactors. At this size, STRs are designed for bulk production and have only been used with suspension cell lines for the purpose of harvesting therapeutic proteins to date. Though this simplicity is ideal, it is not yet clear that maturation of relevant CM cell types, which are adhesion-dependent, can be achieved in suspension[16]. In the cell therapy field, STRs are paired with microcarriers to enable the suspension culture of adherent human mesenchymal stem cells (hMSCs), but at a much smaller scale (35-50 L)[18]. The scale-up production of CM may therefore depend on the success of large suspension culture either with suspension-tolerant cell lines or considerable improvement in microcarrier technology. A small body of research in CM exists to examine this question.

~20 CM studies involve microcarriers, of which a few use static cultures, a majority use rocking, agitated, or stirred conditions in plates or spinner-flasks, and a few culture in true STRs. Primary CM literature has demonstrated the feasibility of expanding relevant cell types on microcarriers in spinner flasks[19] and the increasing cost-efficiency of STRs[20,21], confirming that cell types like bovine muscle satellite cells perform similarly in microcarrier-based suspension culture as hMSCs. This then underscores the need for further research to understand the limiting impact of shear stress on dynamic microcarrier culture. To this end, using CFD modeling, Zhang et al.[22] predicted a stir speed of 15 rpm would be sufficient to suspend microcarriers in a 20,000 L STR. However, due to the high shear stress introduced by gas sparging in STRs, this translates to a speed of 120 rpm in a 250 mL spinner flask, at least double what is typically used in spinner-flask studies. They speculated that this high shear may mean that large STRs are not compatible with CM production on microcarriers but are likely compatible with suspended cells. Alternatively, ALRs may facilitate large microcarrier culture; in addition to the more uniform shear stress characteristics, a CFD study on a novel 300,000 L ALR included many spargers to maintain oxygen uptake and a small bubble size, theoretically protecting microcarriers from bubble slip and rupture damage[23]. Microcarriers ultimately limit the maximum achievable density in suspension cell culture, as the scaffolding bead volume contributes to the maximum volume fraction[16,17]. However, if adherent culture is necessary, microcarriers may be the most viable scaffolding technique.

Only 2 CM studies have attempted non-microcarrier-based suspension culture[24,25]. In one, primary turkey muscle satellite cells were suspended at 110 rpm in a spinner flask for 3 months[24]. While the suspended cells maintained better plasticity, they differed more significantly from the muscle cell phenotype than adherent cells, calling for further research to understand this phenomenon. The other study established a naturally immortal chicken fibroblast line adapted to serum-free and suspension culture and cultured in 2 L STRs in both fed-batch and perfusion conditions at 320 and 325 rpm[25]. This demonstration is an excellent proof of concept of some of the most essential culture characteristics for CM scale-up, however with the heavy caveat that chicken fibroblast cells are more robust than bovine myoblasts.

Suspension culture is promising and possibly the only viable route to scaled-up production, but will require the development of robust, perhaps anchorage-independent, immortalized cell lines. Overall, parameters such as cell seeding density, substrate concentration, continuous versus batch or fed flow, agitation rate, and sparging rate are being thoughtfully examined in small-scale studies, and more efforts should be made to predict their interactions in large systems. Until then, it is ultimately unknown how relevant animal cells may behave in bioreactors with thousands of liters of volume[23].

Cell Type

The optimization of cell type is a critical prevailing area of focus in CM. Ideal cell lines do not exist or are not publicly known. Because of this, most CM studies still use primary animal muscle cells. Current TEAs are significantly limited by using generalized media formulations and growth kinetics estimated from adjacent cell lines, such as C2C12, CHO, and hMSC. Cell characteristics and cultivation methods are typically significantly variable amongst cell lines[26–28]. Ongoing research characterizing more CM-relevant cell lines[24,29–35] is essential to improve modeling accuracy.

The most relevant cell lines in literature that can withstand suspension culture are insect cells, also used in Ashizawa et al.[10], and the stem cell line of chicken fibroblasts naturally immortalized by Pasitka et al.[25,32,36] (studies led by the founder of Believer Meats). Just Eat and Upside Foods also use naturally immortalized chicken fibroblast lines in their FDA-cleared products[37,38]. An important advancement, Pasitka et al.'s[25] cell line claimed high densities while maintaining a stable phenotype and high growth rates. However, this cell line is not publicly available, nor is it myogenic. There is a great need to continue the development of accessible myogenic cell lines of the most impactful species, especially bovine[2–4]. The creation of an adherent bovine cell line was endeavored, but through genetic engineering, which is likely to face significant regulatory hurdles and additional challenges associated with consumer acceptance[39]. Further, for reasons not yet established, the immortalized bovine satellite cells (iBSCs) performed worse than primary cells in the Beefy-R medium developed by the same lab[15,39]. Efforts such as those by Pasitka et al.[25] require extensive funding and years to complete, but are essential for the viability of CM research and commercial potential.

Inexpensive, Sustainable Cell Medium

The widespread adoption of CM is especially contingent on the drastic cost reduction and optimization of sustainable, serum-free medium. The TEAs are far from consensus on medium formulation, leading to remarkably different cost outcomes, yet no medium modeled in literature has reached the <$1/L goal[6]. Additionally, the energy, land, and agricultural requirements for producing this cell feed are among the highest contributors to CM's overall environmental impact[2–4]. No current media formulation free of animal-derived sources is simultaneously inexpensive, sustainable, and effective. Kolkmann et al.[40] demonstrated the insufficiency of current commercial serum-free media to meet the needs of CM and developed a formula for bovine satellite cells that supported up to 97% of the cell growth of serum-containing media, however, their complex formulation offers no cost reduction. Stout et al.'s[15,28] more simplified medium, Beefy-R, is estimated to cost ~$1.39/L[6] with Humbird's[7] media cost regression analysis, but still underperformed in comparison to a typical 20% FBS medium; they reported an unexpected doubling time of 55 hours for iBSCs in Beefy-R[41], significantly slower than the iBSC's original doubling time of 15 hours in serum-containing medium[39].

Media costs can be reduced through innovations for producing ingredients at a reduced cost, using less expensive alternative ingredients, or by reducing the quantity of media needed by improving cell metabolism and other functional characteristics or optimizing media formulations. All these advancements are leveraged within the CM TEAs, but in primary literature, most efforts have been to reduce serum dependency or replace or reduce the cost of growth factors or recombinant proteins. Serum-dependency can be reduced via plant or algae-based compounds. Complex ingredients from fermented okara and microalgae *Auxenochlorella pyrenoidosa* extract have been shown to improve cell growth with 5% FBS in comparison to 10% FBS alone[42,43]. Non-complex ingredients such as microalgae-based antioxidant C-phycocyanin (C-PC) and plant or microalgae-based antioxidant and flavonoid Naringenin have also contributed to serum-free cell growth[44,45]. Eliminating the need for conventional growth factors and recombinant proteins is also likely necessary to avoid supply chain issues, for example, 100 million kg/yr of CM would require 4-12x the current global recombinant insulin supply[16]. Pioneering the cost reduction of recombinant proteins, *Escherichia coli* strains were screened with different animal genes and encouragingly produced growth factor orthologs as or more effective in supporting NIH-3T3 fibroblast proliferation than commercially available growth factors. For example, Atlantic salmon FGF2 ortholog provided the same level of proliferation as commercial human FGF2 with a 10-fold reduction in the amount needed[46].

The TEAs also point to a unique observation: replacements for serum, growth factors, and recombinant proteins alone are likely not enough to bring CM to market. Humbird[7] and others demonstrate that ingredients in basal media, especially amino acids, significantly contribute to the cost of CM. Cost-reduction through economies of scale for these ingredients is not a secure assumption: CM products of the near future are expected to be hybrids of plant-based and CM biomass, thus reducing scaling pressure on raw material industries such as precision fermented amino acids. Further, the nutrients in DMEM are sourced directly or indirectly from grain-based agriculture, which is fertilizer dependent, vulnerable to climate change, and competes with other forms of food production[47]. Research therefore must also focus on replacing basal media components or perhaps conventionally defined media altogether to increase sustainability and reduce cost. Accordingly, in their better-case TEA scenario, Humbird[7] replaces most individually produced amino acids with soy hydrolysate, a complex ingredient. Though there is some support that soy hydrolysate may be a viable alternative[15,16,42], this choice remains speculative until there is more comprehensive research to validate the new formulation's efficacy. Another rare example of research along these lines is a series of studies that used live microalgae and feeder cell lines in tandem with mammalian cell culture to recycle cell medium and entirely replace DMEM and FBS[47–50]. Herein, they demonstrated that extract from the microalgae *Chlorella vulgaris,* a complex ingredient that can be grown in areas unsuitable for conventional agriculture, could replace DMEM while improving the proliferation and viability of bovine myoblasts[47]. There are many promising but disparate ideas in literature for improving cell media. These must be systematically optimized in combination for relevant cell types in relevant bioreactors, outputting data that can inform accurate facility-scale production models.

As such, first principles research on media design and development is warranted to holistically address the challenges of CM.

2.3.2 Current CM Research Less Relevant to Scaled Production

All CM TEAs accept the premise held by current biomanufacturing industries that large-scale suspension culture is the most efficient method of expanding a culture of animal cells. This is notably different from trends within primary CM literature. Almost half of all primary CM publications to date focus on scaffolding, assuming adherent growth and static culture, with the exception of a subset of studies on microcarriers. Examples from primary CM literature include constructing 3D scaffolds from decellularized mycelium[32], cultivating bovine stem cells on fibroblast-derived ECM[51], and myogenic and adipogenic cells printed in GelMA and co-cultured in a microchanneled block[52]. As has been pointed out elsewhere, many CM studies draw directly from tissue engineering principles, where complex culturing processes and extremely high-value products can be afforded[18,23]. Compared to agricultural products, the tissue engineering and pharmaceutical industries, in which complex, high-value products are sold in small quantities to consumers with little choice, scale by very different rules[23]. CM studies that emphasize adherent proliferation are mostly irrelevant for suspension culture and for the mission to scale CM production. Microcarriers were phased out of pharmaceutical practices to reduce cost and achieve higher cell density when cell engineering allowed the creation of suspension-tolerant cells from formerly adherent cell lines[53]. Indeed, some TEAs found the optimization of cell density to be a sensitive parameter in cutting media costs[6,10].

Sophisticated bioprocessing techniques, including co-culturing, are also unlikely to play a role in the scale-up of CM. Though the goal of producing a CM steak with anisotropic fibrous structure, blood vessels, and integrated fat and muscle is exciting, it is perhaps too lofty to prioritize over the much more pressing and unexplored issues related to creating a scalable and affordable CM product. The numerous studies focused on granular details of complex scaffolding and culturing techniques have tended to neglect the overarching assumption of scaled animal cell production today and the challenges unique to competitive CM products.

2.3.3 Missing CM Research Relevant to Scaled Production

Differentiation in Serum-Free and Suspension Cultures

Finally, the CM TEAs depend on several assumptions that have not yet been researched in the context of CM. These represent new research opportunities to advance CM scale-up. Conventionally, after muscle cells are proliferated in growth media with a high amount of serum (10-20% FBS), differentiation is induced by serum-starvation with a differentiation media typically containing 2% serum. While research on new serum-free proliferation media formulations continues to advance, it is unknown how differentiation can be achieved without the ability to induce serum-starvation[54]. Additionally, it is unclear how differentiation is meant to take place in suspension culture. The current TEA studies have little to offer on this idea: Risner et al.[9], and by extension, Ashizawa et al.[10], assume an "unknown scaffolding process," presumably microcarriers, within the production bioreactors, which is not included in cost calculations. Similarly, Negulescu et al.[6] only mention a "differentiation step assumed to occur in the production bioreactor at the end of the batch". Other TEA studies assume the only distinction between proliferation and differentiation stages is a change in media formulation. This is also understudied in bench-scale literature; a majority of CM studies on scaffolding and biomaterials, which make up a large portion of the primary CM literature, involve studying differentiation and maturation on bioactive or anisotropic surfaces. Some of this work is relevant to microcarrier technology, but few microcarrier studies also evaluate differentiation potential. Given the uncertainty around the benefit of microcarriers versus a suspended cell culture, there should be additional work toward differentiating relevant cell types in suspension. The exclusion of scaffolding may lead to decreased cell maturity, but the tradeoff between this and expense saved is unknown. There is a significant opportunity to examine how suspended, serum-free proliferation stages can easily transition to a suspended, serum-free differentiation stage.

Bioreactor Design

There is a need for intense scrutinization of bioreactor design. The CFD study by Li et al.[23] is perhaps the only existing study which interrogates and reformulates a bioreactor design for CM-specific needs (other than the TEAs which examine the impact of bioreactor volume). Based on assumptions (e.g., a lower oxygen uptake rate in larger ALRs), they suggest that at volumes >20,000 L, ALRs are preferable to STRs because of their uniform shear stress and the elimination of an impeller. Indeed, Negulescu et al.[6] predicted that the ALR is preferable because it is cheaper to purchase at scale and more energy-efficient during operation. Yet, no relevant CM cell types have been grown in an ALR. Li et al.[23] also demonstrated that optimal bioreactor design changes depending on cell tolerance and whether microcarriers or suspended cell cultures are used.

Bioreactor volume limitation may be dictated by another important distinction between the CM TEAs: the assumption of CO_2 limitation. At bioreactor volumes >20,000 L, it is largely unknown whether the accumulation of CO_2 would limit animal cell growth[16]. Negulescu et al.[6] assumed it would not, enabling their bioreactor scale-up to 262,000 L with increasing cost efficiency. Humbird[7], however, concluded that the optimal bioreactor volume is 50,000 L, estimating that any higher would begin to experience CO_2 limitation.

Another understudied topic is the use of perfusion versus fed-batch bioreactors. Several benchtop studies have suggested that perfusion cultures are more efficient for both suspended cells and microcarrier cultures because of the higher cell density they enable[20,21,25]. Perfusion bioreactors which incorporate relatively immobile scaffolding or particles (e.g., cell aggregates, microcarriers), such as hollow fiber or packed bed bioreactors, are used within tissue engineering to achieve high density culture but their use in scaled-up applications is currently unrealized[18,23,55]. Perfusion culture techniques combined with agitated bioreactors such as STRs may have more scale-up potential. At the 2 L STR scale, Pasitka et al.[25] experienced growth-limiting waste accumulation in fed-batch mode while supplementing glucose and monitoring for lactate and ammonia accumulation, as suggested by the better-case scenario by Ashizawa et al.[10]. They concluded that a perfusion system is preferred, reporting a density of 1.08×10^8 cells/mL[25]. This is an improvement from the assumptions made in the CM TEAs, each of which assumes a maximum cell density on the order of 10^7, except for one best-case scenario[9]. However, this work is still reliant on several assumptions that need validation, such as cell size. Additionally, this conclusion opposes Humbird's[7] analysis which found that after incorporating bioreactor costs at scale, fed-batch systems were more cost effective than a complete perfusion system. If true, researchers must continue to develop ways to increase cell density without the use of perfusion bioreactors. It would additionally be useful in future TEAs to directly compare the hybrid system against an entirely fed-batch system.

Aseptic Production

One extremely impactful assumption without consensus or data within primary CM literature is the precaution necessary to achieve aseptic conditions. Though the final CM product may only need to be food-grade, animal cells are slow growing and defenseless against pathogens during production and may require more protection. This impacts infrastructure requirements. In agreement with pharma-grade production, including the large-scale STR cultivation of animal cells, Humbird[7] selected an ISO 8 clean room for the cell culture areas and an ISO 6 for the laboratory areas. Clean rooms are expensive, from both CAPEX and OPEX perspectives, and restrict the maximum volume of bioreactors that can be installed. If needed, this assumption would eliminate the possibility of bioreactors on the scale posited by Negulescu et al.[6] (211,000 L and 262,000 L) as they would be prohibitively expensive to house in an ISO 8 clean room. In fact, Humbird[16] postulates current industrial animal cell culture STRs have been restricted to 20,000 L in part because of clean room considerations. Additionally, some within these industries have recently transitioned to single-use bioreactors, in part, to decrease contamination risk. Kurt et al.[56] fitted an early process model[57] with single-use bioreactors for this reason, but found that it significantly increased CAPEX. Conversely, all CM TEAs except Humbird's[7] assume food-grade production facilities, and thus minimize CAPEX. As CM production facilities are projected to cost several billion dollars to build, achieving aseptic production with appropriate food-grade instead of pharma-grade facilities, the formal standards and specifications for which have yet to be established specific to CM, may be yet another inflection point for the commercialization of CM.

Aseptic production also impacts the sterility required of raw material inputs. As previously mentioned, many individual amino acids are produced at food or feed-grade levels. More stringent aseptic conditions would require the creation of new supply chains resulting in increased raw material prices. It would also spell challenges for repurposing by-products from other agricultural industries, such as turkey egg shells or plant hydrolysates for scaffolding or media components[15,58,59].These studies and others that use non-pharmaceutical grade ingredients, such as microalgae extract[47–50], do provide proof of concept that cell growth is likely not negatively impacted by material purities lower than that of pharma-grade. However, it is still unclear how material grade impacts sterility, especially at production scale. As such, it will be valuable to track instances of contamination events as CM companies unveil large facilities around the world. In 2023 Eat Just was granted tacit approval by FDA for their cultured chicken product, grown in STRs (up to 1000 L), and demonstrated batch sterility in the six independent production runs performed for the approval. A survey of 15 CM manufacturers showed the majority currently used pharma-grade growth factors but planned to switch to food-grade, and of those who already used food-grade, none experienced increased contamination issues[60]. The bottom line is that the nature of aseptic production clearly impacts the cost, feasibility, and sustainability of the CM industry. Elucidating true aseptic requirements presents a significant challenge, as much remains uncertain until data or research on production-scale facilities are publicly shared, however, this is essential knowledge.

2.4 Chapter Summary

Climate and ecological health are inextricable with agriculture, and in combination with rising food demand, insist on novel and responsible protein production. Large-scale models of industrial CM production must be scrutinized to facilitate the scale-up of CM. Current CM TEAs adopt a variety of assumptions and processing approaches, which make comparisons challenging. Strikingly, for example, differences in media formulation and pricing lead to recombinant proteins and growth factors contributing >99% of the cost of media in one study[12], and a negligible contribution in another[7]. It is therefore essential to pursue increasingly relevant cell lines, media formulations, and bioreactor models in publicly available databases so industrial-scale modeling can improve in accuracy. It is also clear that these developments are necessary to bring CM to market, as the TEAs unanimously show that industrial production of CM without further scientific advancement could not compete meaningfully with conventional meat. A CM facility capable of producing ~1% of the US beef supply would require a massive investment with CAPEX estimates of $1.58-10.7 billion. Cost of production estimates also vary significantly ($400,000/kg[9] to $16/kg[6]) according to other critical assumptions, such as maximum bioreactor volume and CO_2 inhibition, use of perfusion culture, aseptic requirements, labor cost, financing, and overall potential for cost-reduction techniques. These reach the cost-competitive goal of $10/kg with significant additional assumptions of technological improvement. However, even in baseline scenarios, these industrial-scale models already make a serious assumption of technological achievement: relevant, immortalized animal cells with fast metabolisms (doubling time <24 hr) that can proliferate and perhaps even differentiate in suspension in >20,000 L bioreactors. Suspension culture has been explored to some extent in CM literature, but few studies have attempted to grow cells in STRs or ALRs. As such, TEAs and other models rely on data from less relevant cell types for growth characteristics. This is an essential opportunity for further research, as the behavior and tolerance of relevant cell types in suspension, as well as the development of relevant cell types, is understudied and unknown.

Some advancements the TEAs show to be a necessity are almost entirely missing from CM literature, such as serum-free differentiation in suspension, bioreactor and facility design, and media recycling and valorization. In summary, while varied in approach, TEAs on CM provide an invaluable lens to assess the potential of CM and directions for further research. Realistic, sustainable, and transformative approaches to food science and technology are paramount.

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CHAPTER 3: CIRCULAR CELL CULTURE TEA

3.1 Introduction

In pursuit of low-resource protein supply, cultivated meat (CM) presents a singular advantage but requires significant cost and efficiency gains to achieve widespread adoption. The TEAs analyzed in Chapter 2 assert that CM is especially contingent on the drastic cost reduction of basal medium macronutrients. Glucose and amino acids not only contribute the majority of CM media costs at scale but require scrutiny over environmental sustainability goals. Amino acids for cell culture are individually recombinantly produced and thus are considerably more resource and cost-intensive than, for example, complex, plant-based protein hydrolysates. Glucose is sourced directly from grain-based agriculture, which is fertilizer-dependent, vulnerable to climate change, and competes with other forms of food production[1,2]. As most established industries involving animal cell culture do not experience the same cost-limiting pressures as food products, CM is uniquely positioned (and likely required) to pioneer an unconventional and multidisciplinary reimagining of animal cell bioprocessing.

As summarized in Chapter 2, there are several promising examples of bench-scale CM studies that substitute conventional medium components with plant or microalgae-based compounds, such as fermented okara, microalgae *Auexnochlorella pyrenoidsa* extract, and microalgae-based antioxidants and flavonoids[3–6]. Each of these components was used to reduce serum dependence or improve growth in serum-free media. The only line of work exploring the replacement of basal media macronutrients such as glucose, amino acids, and vitamins, is a series of intriguing studies using live a microalgae culture in tandem with animal cell culture to recycle cell medium and entirely replace DMEM and FBS[1,7–10]. These papers are summarized in **Table 3.1**.

Table 3.1: Summary of a body of research from the Tokyo Women's Medical University and Waseda University towards using live microalgae cultures and microalgae extract to design a circular cell cultivation system with cost and resource-efficient media ingredients.

Authors, Year, & Primary Goal	Context	Animal & Microalgae Cell Type	Process	Results	Drawbacks
Okamoto et al., 2019 • Microalgae extract for sustainable, grain-free media	• Grain (for medium glucose) is associated with unsustainable and chemical-dependent agriculture and competes with other forms of food production. • Microalgae is more sustainably grown and produces most nutrients used in media.	• C2C12 • C. littorale, Stichococcus sp., C. vulgaris, E. gracilis, S. subsalsa, & A. platensis	• Extracted glucose and 18 out of 20 amino acids from microalgae biomass. • C2C12 cells were cultivated with glucose and amino-acid deficient medium either with or without microaalgae extracts for 2 days. • Compared 6 microalgae species and different nutrient extraction techniques.	• For the first time, mammalian cells were successfully grown with microalgae extracts, indicating that microalgae could function as a source of animal cell culture macronutrients. • Proposed optimal microalgae species, C. Vulgaris and C. Littorale., and optimal hydrolysis conditions for glucose and amino acid extraction. • The 8 vitamin types typically added in media were found in the microalgae extracts.	• The nutrient-deficient medium was the only control- did not compare microaalgae supplemented medium against normal DMEM.
Haraguchi et al., 2021 • Live microalgae for treating animal cell waste	• There is no other study to our knowledge on using microalgae for the treatment of mammalian cell culture spent medium.	• C2C12 • C. littorale & C. Vulgaris	• C2C12 cells were cultured for 3 days in DMEM and 10% FBS. • Microalgae were cultured C2C12 conditioned medium or with conventional microalgae medium. • Consumption analysis	• 80% and 26% of ammonia and 16% and 15% of phosphorus in the waste medium were consumed by C. littorale and C. vulgaris, respectively, also proliferating by 3.2 folds and 1.6 folds over a period of 7 days.	• Used FBS. • Unknown how microalgae growth and consumption characteristics are impacted by serum.
Okamoto et al., 2022 • Microalgae extract for replacing all basal medium nutrients	• Authors seek to demonstrate that C. vulgaris can provide vitamins as well, thus entirely replacing DMEM for BM cells.	• Bovine myoblast (BM) • C. vulgaris	• BMs were seeded on well plates and proliferated for 3 days in either a salt solution, a salt solution with microalgae extract and 10% FBS, or DMEM with or without 10% FBS. T • BMs were then differentiated for 3 days with the same medium groups with added horse serum.	• Demonstrates an animal-free, grain-independent replacement to DMEM for a more relevant CM cell type. • 14 out of 15 proteinogenic amino acids found in DMEM were extracted from the microalgae, including 5 additional proteinogenic amino acids. • Microalgae-based medium had higher BM cell viability than cells cultured with 10% DMEM but underperformed for differentiation.	• FBS used throughout. • Unclear why authors chose C. Vulgaris instead of C. Littorale.
Haraguchi et al., 2022 • Circular cell system with live microalgae culture and extract	• Animal-derived serum must be replaced. • Nutrients in culture media are not depleted equally, so discarding "used" media is wasteful. • A live microalgae and mammalian culture in tandem may allow media recycling, eliminating ammonia from solution while replenishing nutrients.	• C2C12, RL34 (rat hepatocytes) • C. littorale	• RL34 cells were cultivated with serum to create a conditioned medium with RL34 growth factors. • C2C12s were cultured for 48 hours in the RL34-conditioned medium. Then spent medium from that culture was used to culture microalgae. • Microalgae-conditioned medium was supplemented with microalgae extract and used to culture C2C12s.	• RL34-conditioned serum seemed a viable replacement for FBS. • C2C12 cells were able to proliferate when cultured in the microalgae waste from the first culture, and when supplemented with microalgae extracts, proliferated at a rate similar to 10% FBS and DMEM. This indicates that growth factors from the RL34 cells lasted more than one culture, and depleted nutrients were supplied by the microalgae. • C2C12 growth rate was improved by optimizing microalgae growth conditions.	• RL34 cells cultivated with serum.
Yamanaka et al., 2023 • Microalgae extract and feeder cells for serum and grain free media	• Authors seek to combine the microalgae-based circular cell culture and feeder cells with the more relevant CM cell type. • Must attempt to eliminate the serum reliance by the feeder RL34 cells.	• Bovine myoblasts • C. vulgaris	• RL34 cells were cultivated in a salt solution with microalgae extract to create a conditioned medium. • BMs were cultured in the conditioned medium with additional microalgae extract.	• RL34 cells proliferated significantly better in the DMEM media than in the microalgae experimental media • BM cells the experimental medium had the highest metabolic activity, statistically the same as cells in the unconditioned custom media with FBS and DMEM with FBS. • In the scaled-up conditions, the CM growth rate on the experimental medium was lower than the FBS-containing mediums.	• C. vulgaris extract is toxic to cells at high concentrations through an unknown mechanism, and the authors note that that limitation is evident.

In Haraguchi et al.'s[1] circular cell cultivation (CCC) system, the microalgae culture has two roles. *Chlorococcum littorale*, an autotrophic microalgae, is cultivated, hydrolyzed, and added to the animal cell culture medium at 5% volume/volume as a single complex ingredient to replace the glucose, amino acids, and vitamins typically found in basal medium. Secondly, a live *C. littorale* culture is grown on the spent medium to eliminate ammonia buildup and thus prepare the medium for reuse. In this way, the authors have not only presented the most transformational example of cell culture media formulation in CM literature but also introduced the first instance of CM media recycling.

Microalgae are incredibly diverse organisms and are used in a range of industries and applications, including biofuels and bioenergy, pharmaceuticals, aquaculture, phytoremediation, cosmetics, agro-based chemicals, nutritional products, animal feed, and carbon capture[2,11–14]. There are also several anticipatory life cycle analyses (LCAs) on CM production that assume macronutrient supply from cyanobacteria, a related but simpler organism to eukaryotic autotrophic microalgae such as *C. littorale*[15–17]. Tuomisto & Teixeira de Mattos[15] assumed the cyanobacteria was produced in open-pond systems and harvested at 50% efficiency. They found that CM significantly reduced (78-99%) greenhouse gas (GHG) emissions, land use, and water use, and had lower energy use than all meats but poultry[15]. A similar assessment that instead assumed cyanobacteria production in a PBR found that the CM energy use was on par with beef, while still maintaining an advantage in GHG and land use.[16] Both of these are relatively early and thus less robust CM LCAs, but they offer more positive results than other CM LCAs, even those that assume plant-based soy hydrolysates, and thus demonstrate the environmental efficiency of unicellular photosynthetic organisms as cell culture feed[18].

Motivated by these advancements, this study constructs an industrial-scale model of a theoretical CCC facility, translating some of the bench-scale data by Haraguchi et al.[1] into a novel CM TEA. The 2023 CM TEA by Negulescu et al.[19] is taken as a baseline model while building out the microalgae cultivation and media recycling facility areas to supply and process the medium inputs. A majority of available TEA literature on microalgae bioprocessing is on microalgae-based biofuel production facilities. The National Renewable Energy Laboratory (NREL) has published detailed TEAs in this field, on which this model heavily relies[11,20,21]. This is the first TEA of the simultaneous culture of animal cells and microalgae. While the additional infrastructure needed for microalgae cultivation and processing will likely add to the capital expenditures (CAPEX), the elimination of costly individual media components and the reuse of media may lead to a reduction in the overall cost of production (COP) and thus wholesale price of CM.

3.2 Methods

3.2.1 Model Overview

This TEA was simulated using the computer-aided software SuperPro Designer, Version 10 Build 3. The central CM cell production system is replicated from Negulescu et al.'s[19] publicly available SuperPro Designer files. They construct three different facility sizes based on the maximum size and type of the CM cell production bioreactors: a 42,000 L stirred tank bioreactor (STR), a 211,000 L STR, or a 262,000 L airlift bioreactor (ALR). A detailed description of the design choices made for this CM production system can be found in the original publication. Briefly, the authors set an industry target of 100,000,000 kg of CM a year, commonly selected in CM TEA studies as it is roughly equal to the size of the alternative protein market and the output of one US slaughterhouse[19,22]. Each facility includes a seed train with 5 or 6 steps of fed-batch STRs. The seed train feeds into 10 parallel production bioreactors in which the cells proliferate to the target density once more and then differentiate. The production bioreactors are staggered by a factor of 5, resulting in 50 production bioreactors total per facility. To reach the industry target output of CM, a total facility number of 25, 5, and 4 facilities, for the 42,000 L STR, 211,000 L STR, and 262,000 L ALR size models are needed, respectively. Negulescu et al.'s[19] model can be generally considered as a control for the microalgae-based CCC system modeled in this TEA.

This facility design consists of three general areas: the microalgae biomass (MAB) production system, the animal cell production system (from Negulescu et al.[19]), and the microalgae medium recycling (MAMR) system. **Figure 3.1** presents an overview of these bioprocesses.

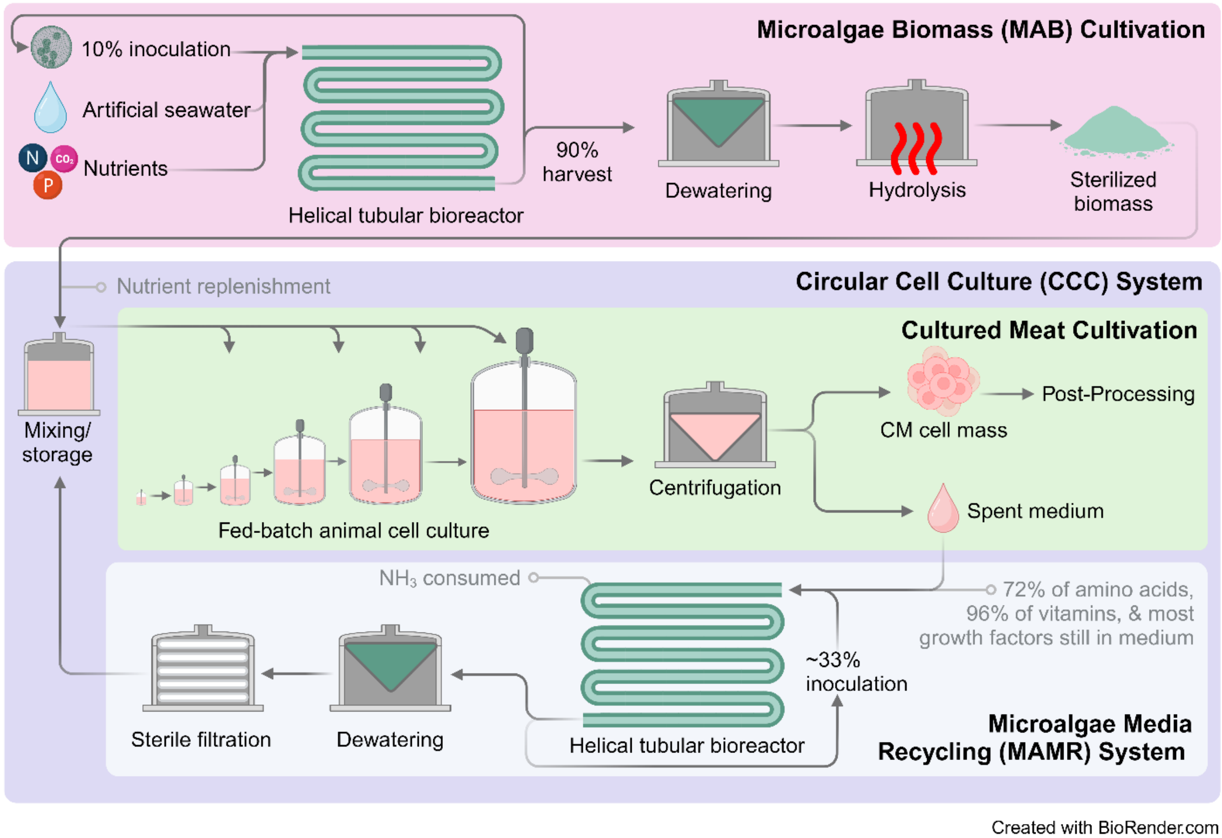

Figure 3.1: Overview of production systems for a CM production facility incorporating in-house production of macronutrients by MAB and media recycling through the MAMR system.

In the MAB system, *C. littorale* is cultivated with conventional microalgae medium and dewatered with bioprocessing design and size similar to microalgae-based biofuel production facilities. The biomass is hydrolyzed and sterilized before being mixed into the CM medium as the sole macronutrient source for the CM cells. After animal cell cultivation, spent CM medium is separated from the cell product via centrifugation and sent to the MAMR system for conditioning by microalgae cells. This is followed by dewatering, sterile filtration, replenishment of macronutrients by the MAB system, and replenishment of a small amount of fresh medium to make up for lost volume.

3.2.2 Baseline Scenarios

This study presents two baseline scenarios in addition to the three facility designs based on maximum CM production bioreactor volume. Scenarios 1 and 2 represent high and low MAB demand, based on separate bench-scale data from Negulescu et al.[19] and Haraguchi et al.[1], respectively. Applying Negulescu et al.'s[19] C2C12 consumption data (calculations shown in section **3.2.4**) to calculate the MAB needed to supply the mammalian cell culture macronutrients results in a large microalgae extract demand in scenario 1: ~690 g *C. littorale* per liter of medium (accounting for 5% excess nutrients). Conversely, the experimental methodology used in the bench-scale CCC system studies by Haraguchi et al.[1] appears to require much less MAB per liter of medium. Haraguchi et al.[1] only supply their mammalian cells with a medium formula of 5% microalgae extract by volume. Further, it is possible that a portion of these macronutrients was not consumed: 72% of amino acids were present after one cycle of mammalian cell culture, but it is unclear if the remaining amino acids are usable (as opposed to unusable amino acids or proteins produced by the cells). Even assuming Haraguchi et al.'s[1] microalgae extract was 100% microalgae biomass (it is more likely <30%), this is a significant reduction in the demand estimated by scenario 1. Thus, scenario 2 adjusts the microalgae biomass cultivation system throughput to provide 5% microalgae extract by weight to the CCC medium.

3.2.3 Animal Culture Biological Assumptions

Most assumptions for the mammalian cell culture are kept consistent with Negulescu et al.'s[19] model. Their media consumption requirements and cell doubling time of 23 hours are experimentally derived from bench-scale cultivation of C2C12 cells in B8 medium[19,23,24]. Other cell biological assumptions are representative of general mammalian cells, including starting and ending density (20 g and 100 g fresh weight/L), cell weight (3×10^9 g fresh weight/cell), and size (17.7 μm diameter) which are unchanged in this study.

Each proliferation step has a culture time of 53.65 hours to reach the target end density, and the differentiation step takes 239 hours. The mature cells are then centrifuged into a product slurry of 30% dry cell mass.

3.2.4 Animal Cell Culture Stoichiometry & Media

Stoichiometry

The experimental cell culture medium consists of a simplified inorganic salt solution (ISS) proposed by Haraguchi et al.[1], hydrolyzed MAB, and recombinantly produced growth factors. The ISS composition can be found in **Appendix B**. In SuperPro Designer, the medium is aggregated into a single pure component termed "CCC medium" so the reaction stoichiometry used by Negulescu et al.[19] could be kept consistent. A slight increase in medium demand was required to maintain mass balance while adding ammonia waste to the CM culture products, resulting in equation (1):

$$52.44 \text{ CCC Medium} + 12.16O_2 \rightarrow 19.80CO_2 + 44.18CM + 0.62NH_3 \qquad \text{eq. (1)}$$

Ammonia production per mol of CM product was calculated using the work by Zeng et al.[25]. The authors found that ammonia production is generally associated with total amino acid consumption by a stoichiometric ratio that increases with excess glutamine concentration. As in Negulescu et al.[19], the medium in this study is designed to supply nutrients in 5% excess, resulting in a high residual glutamine concentration (>5 mmol/L). Thus, an NH_3 to total amino acid stoichiometric ratio of 0.44 mol/mol is selected.

Macronutrient Consumption and MAB Demand

In scenario 1, representative of high microalgae demand, experimental C2C12 data by Negulescu et al.[19] provides the CM cell consumption of the most significant macronutrients. MAB demand is calculated in **Table 3.2** using this data and the extraction efficiency of glucose and amino acids from *C. littorale* biomass[1,7]. L-glutamine is determined to be the limiting nutrient and ~690 g (dry weight) of microalgae must be produced per liter of medium.

Table 3.2: MAB demand calculations for scenario 1. The first and second columns (ingredients and media composition) are experimentally determined by Negulescu et al.[19] The third column is extraction efficiency as measured by Okamoto et al.[7] The second and third columns are multiplied to find the minimum required dry weight of microalgae. ~691 g of microalgae must be produced per liter of medium to supply sufficient L-glutamine and all other significant macronutrients. *Data points estimated using graphreader.com.

Ingredients significantly consumed by C2C12s	Media composition with 5% excess to achieve 100 g C2C12 per liter (g/L)	Extract per dry weight microalgae (g/g)*	Dry weight microalgae needed (g/L)	Nutrients supplied if microalgae biomass incorporated at 691 g/L (g/L)	Excess (g/L)
Carbohydrates	121.57	0.280	434.19	193.48	77.99
L-glutamine	16.79	0.024	690.95	16.79	0.84
L-arginine hydrochloride	6.79	0.011	622.48	7.53	1.09
L-isoleucine	0.82	0.003	247.61	2.28	1.50
L-leucine	0.89	0.010	87.70	6.98	6.14
L-methionine	0.26	0.002	112.43	1.59	1.34
L-serine	0.39	0.010	40.18	6.77	6.40
Total amino acids	26.37	0.166	159.14	114.50	89.45

Growth Factors

The stem cell media conditioning technique to replace serum-based growth factors in the bench-scale CCC system was not incorporated into this facility design. This is to isolate the impact of microalgae-based macronutrients and medium recycling, already two novel and distinct CM bioprocessing advancements. Instead, recombinantly produced growth factors are assumed to be produced outside facility boundaries and incorporated as a purchased raw material in the cell culture medium. The growth factor concentrations are based on the Beefy-9 formulation[23], also used by Negulescu et al.[19] The selection and concentration of recombinantly produced growth factors can be found in **Appendix C**. Experimental data of the CCC system suggests that most growth factors remain in the medium after at least two uses[1,9,10]. Due to volumetric losses in the facility model, ~17% of total media volume is supplemented each reuse cycle, and it is assumed this is enough to maintain effective growth factor concentration.

3.2.5 Microalgae Cell Culture Biological Assumptions

Microalgae Productivity

Productivity for industrial-scale microalgae cultivation systems is typically measured in terms of areal productivity. This “top-down” method considers yield as a factor of solar radiation and thus is typically more appropriate than extrapolating from lab scale data, as light is the primary limiting growth factor for autotrophic microalgae [11,20]. Clippinger & Davis[20] summarize current data on the productivity of helical tube bioreactors (**Appendix D**). They offer a reasonable best-case areal productivity of 42.5 g/m^2/day based on the expected productivity gains of industrial microalgae cultivation for biofuel production[11]. This value is selected to estimate productivity for the MAB system. Importantly, these productivity estimates grapple with the tradeoff between productivity and microalgae lipid content, the microalgae-derived ingredient used for biofuel production: Lipid content increases with culture time, but biomass productivity drops. However, protein content and productivity are both maximized with early harvest times and thus may more easily achieve these productivity targets[11].

Productivity is estimated differently for the MAMR system. Here, Haraguchi et al.'s[1] bench-scale growth rate data of *C. littorale* cultured on C2C12 waste (a doubling time of 35.6 hours) is used to estimate the time needed to consume nearly all of the ammonia in the recycling system. In addition to accounting for the experimental medium, this is done to optimize for different culture design goals explored later in the results.

3.2.6 Microalgae Cell Culture Stoichiometry & Media

The basal medium for the MAB culture is a simplified version of Diago artificial seawater, a conventional microalgae medium used with *C. littorale*[1,26–29], defined in **Appendix E**. As is common in microalgae bioprocessing models, ammonia and phosphorus nutrient demand is solved stoichiometrically[11,20,30,31] and is provided by bulk fertilizer ingredients, selected in this study as ammonia and dipotassium phosphate. The *C. littorale* stoichiometric equation is approximated using the Redfield ratio[21,31]. Equation (2) is the resulting stoichiometric equation for the MAB production system:

$$60NH_3 + 4K_2HPO_4 + 270H_2O + 424CO_2 \rightarrow 4C_{106}H_{181}O_{45}N_{15}P + 477O_2 + 4K_2 \quad \text{eq. (2)}$$

This stoichiometry is almost exactly maintained in the MAMR system as *C. littorale* consumes ammonia from the spent animal culture medium.

3.2.7 Engineering Assumptions

Animal Cell Culture

The engineering assumptions for the animal cell culture, like the model itself, are unchanged from Negulescu et al.'s model, and thus won't be explored in detail here[19]. Additional information on calculations for oxygen uptake rate, mixing and shear parameters, and power input for the seed and production bioreactors can be found in the supplementary material of the original publication[19]. This model also assumes that ~98% of the fresh cell culture medium can be sterilized with high temperature short time (HTST) sterilization, and the remaining more sensitive components such as growth factors must be sterilized via dead end filtration. Fresh batches of medium and any medium used for replenishment in the CCC system are prepared this way. However, the recycled medium must undergo filtration, as the components have already been mixed.

Microalgae Bioreactors

The range of bioreactors utilized or proposed for industrial-scale microalgae culture broadly fits into two categories. Open raceways, which involve simple open-system vessels such as concrete ponds, are more common and are used for low-value microalgae products or processes, such as biofuels, feed, biopolymers, and phycoremediation. Alternatively, photobioreactors (PBRs) are closed systems that offer increased control, yield, and sterility but at a higher cost and are therefore more appropriate for pharmaceutical, cosmetic, or food products[14,32,33]. PBRs are selected in this study based on the level of process control and sterility required for the MAB and MAMR systems. There are numerous (>30) PBR designs. Among them, tubular PBRs are the most commonly researched and used commercially[20,34]. This study selects the vertical helical tubular bioreactor proposed in the NREL report by Clippinger & Davis[20]. Vertical helical tubular PBRs support high-density culture (~2 g/L), are uniquely suited for clean-in-place (CIP) systems, and have a modular design that lends well to process intensification and ease of installation. The module design by Clippinger & Davis[20] consists of 400 meters of glass tubes (6.28 cm inner diameter) in a u-bend helix structure stacked 10 rows high, similar to the PBR shown in **Figure 3.11**. Propelled by a centrifugal pump, the microalgae circulate at 0.6 m/s through the tubes and a degassing column for oxygen and CO_2 transfer (gas sparged at 0.07 vvm).

Figure 3.11: A vertical helical tubular PBR. Image reproduced from Zhang[35].

The MAB and MAMR systems each include one vertical tubular PBR consisting of many 400 modules. The PBR size is determined by the culture volume required and the area-to-volume ratio of 35 m^2/m^3 provided by Clippinger & Davis[20]. Energy use (1644 kWh/ha/day) and PBR cost are estimated using the area footprint of the PBR. Size, single purchase cost, scaling factors, total purchase cost, electricity consumption, % contribution to total PEC, and related sources for each piece of equipment in each facility can be found in **Appendices F-K**.

For biofuel applications, microalgae tubular PBRs are assumed to stay sufficiently clean through mechanical scrubbing: Tubular PBRs are easily cleaned for negligible cost by sending small plugs or plastic particles known as "pigs" to scour the inside of the tubes[13,20]. One study modeling microalgae production at a pilot scale for pharmaceutical applications also included flushing the total volume of the tubular PBR with a 2% hypochlorite solution after cultivation[36]. These two cleaning methods are selected for the MAB and MAMR systems.

MAB Medium Preparation

The use of pre-sterilized water as a raw material input is unfeasible for large-scale microalgae cultivation[13]. In food or pharmaceutical applications, the medium must be therefore pasteurized[36–38]. Conventional boiler pasteurizers have significant energy demand, however. In scenario 1, pasteurization via boiler would increase OPEX by over 30% (data not shown). UV-C sterilization is a relatively new technology with less data availability but is expected to lower energy costs by orders of magnitude[39,40]. UV-C sterilization was selected in this study and others for high throughput medium sterilization[38]. Details on the associated energy use calculations can be found in **Appendix L**.

Dewatering & Nutrient Extraction

Dewatering, or the harvest of microalgae cells from their medium, is a considerable challenge in industrial-scale microalgae cultivation because the cells are small, dilute, and have a similar density to the culture medium. No one technique is optimal for all processing needs, as optimal dewatering methods can depend on cost, energy, or efficiency constraints, microalgae type, microalgae or medium end-use, and purity requirements[14]. Centrifugation effectively concentrates microalgae solutions to ~20-30% microalgae weight/volume and is likely the necessary ending step for dewatering in this model, as in many applications[14]. Centrifugation has a very high energy demand, however, and is typically paired with multiple dewatering steps to cut costs. Electro-flocculation is a relatively new dewatering method but was chosen as the primary dewatering step because it has high efficiency, low cost, and does not introduce impurities to the biomass or medium[31,41,42]. The design by Lee et al.[31,41], incorporating settling tanks, mixers, and electro-flocculation tanks, was used in both MAB and MAMR sections of this model. Equipment cost estimates, energy use (0.09 kWh/m^3), and aluminum consumption (0.0086 kg aluminum/ m^3 plus a calculated initial purchase amount) for the flocculation diode were calculated based on the methodology presented in their model. Settling, mixing, and electro-flocculation together result in a 90% harvest of microalgae biomass in an exit stream at 5% of the inlet volume[23]. This is followed by centrifugation, estimated to retain 95% of microalgae biomass at a concentration of 20% weight/volume, as per the NREL reports, which also supply associated energy demand estimates (0.389 kWh/m^3)[11,20].

In the MAMR system, centrifugation is followed by dead-end membrane filtration to maintain sterility and to remove ~100% of the microalgae biomass left in the medium before recirculation in the animal cell culture[11,19]. In the MAB system, centrifugation is followed by processing and sterilization of the biomass before mixing into the CCC medium. To extract amino acids and glucose, Haraguchi et al.[1] hydrolyze the MAB for 24 hours at 100° C with 0.5 M HCL followed by sodium hydroxide for neutralization. Because of the lack of data on industrial-scale microalgae nutrient extraction, this method is assumed to also be effective in this model. Acid hydrolysis is a common method to extract proteins, as is the 24-hour process time. Reflecting available literature, 0.5 M HCL is supplied at 1% weight for acid hydrolysis[43]. Next, HTST heat sterilization is applied for 30 minutes at 120° C to prepare the MAB for the animal cell culture, based on information from consultation with an industry expert in microalgae processing. Details on the energy demand calculations for acid hydrolysis (0.1 kWh/kg dry weight) and HTST (0.799 kWh/kg solution) can be found in **Appendix M**.

3.2.8 Economic Assumptions

Raw Material Costs

Appendix N lists all raw materials, cost per unit, and cost source. Growth factor costs are estimated with Humbird's[22] recombinant price volume correlation, reproduced here as Equation (3):

$$log\left(Price\,\frac{\$}{kg}\right) = -0.861log\left(Production\,Volume\left[\frac{MT}{yr}\right]\right) + 4.90 \qquad \text{eq. (3)}$$

Salts and other inorganic material cost per unit are estimated with bulk order pricing from Alibaba.com or made-in-china.com[19]. Ammonia and DAP pricing are from USDA statistics on market fertilizer prices[12].

Economic Model

An overview of the economic parameters used to estimate COP per kg of CM is provided in **Table 3.3**. The total capital investment (TCI) consists of the plant total direct cost (TDC) and total indirect cost (TIC), which are derived by applying Lang factor multipliers to bare equipment prices. Lang factors vary significantly by industry type and available equipment price, and equipment price estimates can represent anywhere from truly baseline costs of off-the-shelf, uninstalled equipment to customized, installed, fully jacketed equipment[44], making installation multipliers, for instance, redundant. Thus, for accurate TCI estimates, Lang factors should be tailored to individual equipment or equipment groups. This is especially relevant for this interdisciplinary facility design. The area-specific Lang factors are reproduced in **Table 3.4**. In the results section, **Figure 3.2** provides a facility process flow diagram labeled corresponding to these areas. See **Appendices F-K** for a detailed list of all process equipment in each facility.

Table 3.3: Contributing categories and relevant assumptions within OPEX, CAPEX, and annual operating expense. Capital charge factor (CCF) is calculated using Humbird's[22] methodology.

Parameter	Description
OPEX	
Media	Raw materials for microalgae biomass production (artificial seawater, ammonia, phosphorous, CO_2), microalgae harvest (hydrolysis), mammalian cell culture (recombinant proteins, inorganic salt solution), and media recycling (CO_2)
Consumables	Filters and electro-flocculation electrodes
Utilities	Electricity, heating and cooling agents, cleaning agents
Labor	Total labor cost (TLC) = basic labor rate × (1 + benefits(0.4) + supervision(0.2) + supplies(0.1) + administration(0.6)) Operator basic labor rate = $25/hour Operator TLC = $57.5/hour Time utilization = 60%
Facility-Dependent Costs	Maintenance = (4% TCI/yr) Insurance = (5% TCI/yr)
CAPEX	
Total Capital Investment (TCI)	Purchased equipment purchase cost + direct cost factors (piping, instrumentation, insulation, electrical facilities, buildings, yard improvement, auxiliary facilities) + indirect cost factors (engineering, construction) + other cost factors (contractor's fee, contingency)
Annual Operating Expense	
OPEX + Capital charge factor (CCF)	CCF = 9.809 % of TCI/year Payback period = 20 years Internal rate of return (IRR) = 7.5%

Table 3.4: Each TCI calculation component and area-specific lang factors. Lang factors applied to areas A100 and A300 are from Humbird and apply to CM bioprocessing. Lang factors in areas A200, A400, and A500 are from NREL TEA reports on microalgae-based biofuel facilities.

<table>
<tr><th>Total Capital Investment (TCI) components</th><th>Parameter</th><th>A100 & A300: cultivation</th><th>A100 & A300: other</th><th>A200</th><th>A400</th><th>A500</th></tr>
<tr><td rowspan="7">Plant Total Direct Costs (TDC):
For A200 & A400, applied to PEC
For A100, A300, A500, applied to installed cost</td><td>Purchased Equipment Cost (PEC)</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td></tr>
<tr><td>Installation</td><td>0</td><td>0</td><td>0.43</td><td>0.43</td><td>0</td></tr>
<tr><td>Process Piping</td><td>0</td><td>0.045</td><td>0.38</td><td>0.38</td><td>0</td></tr>
<tr><td>Intrumentation + Elec.</td><td>0</td><td>0</td><td>0.5</td><td>0.5</td><td>0</td></tr>
<tr><td>Buildings</td><td>0</td><td>0</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>Yard Improvement</td><td>0</td><td>0.09</td><td>0.15</td><td>0.15</td><td>0</td></tr>
<tr><td>Auxiliary Facilities</td><td>0.012</td><td>0.04</td><td>0.4</td><td>0.4</td><td>0</td></tr>
<tr><td rowspan="2">Plant Total Indirect Costs (TIC): applied to TDC</td><td>Engineering</td><td rowspan="2">0.314</td><td rowspan="2">0.6</td><td>0.25</td><td>0.25</td><td rowspan="2">0.14</td></tr>
<tr><td>Construction</td><td>0.35</td><td>0.35</td></tr>
<tr><td rowspan="2">Contractor's Fee and Contingency: applied to TDC + TIC</td><td>Contractor's Fee</td><td rowspan="2">Included in TIC</td><td rowspan="2">Included in TIC</td><td>0.05</td><td>0.05</td><td rowspan="2">Included in TIC</td></tr>
<tr><td>Contingency</td><td>0.1</td><td>0.1</td></tr>
</table>

NREL has published the most robust examples of large-scale microalgae TEA (and TEA generally) and divides the application of Lang factors to dewatering, microalgae cultivation, and outside battery limits (OSBL) facility areas[11,20]. This technique is used to estimate TCI costs of both MAB cultivation and processing areas in this study. The helical tubular bioreactor design and cost estimate used in this study is also from Clippinger & Davis[20] and includes the cost of installation, support structures, piping systems, valves and fittings, and field equipment. This is why, for instance, it is appropriate that many of the direct cost multipliers are zero.

For mammalian cell culture and media replenishment areas, Negulescu et al.'s[19] scaled price estimates were used for PEC. TCI was estimated with Lang factors for aseptic indoors bioprocessing, introduced in Petrides[45,46], reproduced by Humbird[44], and listed here in **Table 3.4**.

These Lang factors provide similar results to SuperPro Designer's TCI calculations, used by Negulescu et al.[19]

3.3 Results

Facility Outcomes

Figure 3.2 provides a detailed process diagram of the CCC facility design. The 42K STR facility is shown as an example, but the overall layout does not change significantly between the different facility sizes. Notably, this design differs from the bench-scale models by Haraguchi et al.[1] by separating MAB and MAMR into two systems. This is for several reasons. Most importantly, it was found that across all facility designs, the volume of CM cell culture medium available to be recycled is <4% of the volume of required MAB medium. In other words, sufficient MAB cannot be grown in the volume of spent medium without severely overshooting reasonable microalgae culture density limits (<6 g/L)[21]. Additionally, the different microalgae culture goals between the two systems are such that the microalgae culture techniques, processing, and cell characteristics have divergent optimal designs.

One major difference in optimal culture technique is the inoculation ratio. The MAB system design is derived almost entirely from microalgae-based biofuel and other types of conventional industrial microalgae facilities which are oriented around a similar goal: to extend the microalgae exponential growth phase, thus efficiently producing a maximal amount of biomass over a culture of many days[11,20,21,30,31,37,47]. This is enabled in part by low inoculation ratios, typically ~10%.

Conversely, the MAMR system is volume-limited: the microalgae must consume the mammalian cell ammonia waste within a few hours while remaining under a reasonable biomass density (< 6 g/L)[21] while suspended in only as much medium as each batch of CM culture requires. Turnaround time in both microalgae systems must be quick (<2.5 days for animal cell culture cycle time), but only the MAB system can compensate by increasing bioreactor volume. Uniquely, MAMR system can instead compensate with a high inoculation ratio. Only by using microalgae growth rate data (instead of area-based productivity estimates) is the benefit of the high inoculation ratio, optimized at 33% (see **Appendix O** for calculations), included in the model. A microalgae culture time based on areal productivity data, such as in the MAB system, would be far too slow: estimated ~140 days for the 42K STR facility (data not shown). Indeed, the MAMR system is unlike any large-scale microalgae bioprocessing system. It shares the waste-removal goal of microalgae-based water-treatment plants but must also accommodate acute time constraints and potentially pharmaceutical-level sterility. As a result, the MAMR system cannot be modeled with great certainty without additional bench and pilot-scale data.

MAB system should also differ from the MAMR system by optimizing culture conditions for high protein and carbohydrate content. Factors such as CO_2 concentration and culture time can be used to fine-tune microalgae biomass composition[20,29]. Desired impacts can be very sensitive to culture conditions, for example, between early and late cultivation stages Davis et al.[11] measured a drop in protein content from 34% to 9% in *Scenedesmus* and 40% to 13% in *Chlorella vulgaris*.

Microalgae composition can also be controlled by species selection. Protein content in different marine microalgae species varies at least from 28.6-70%, and *C. littorale* is not a particularly high protein microalgae[2]. While Okamoto et al.[7] investigated six microalgae species for compatibility with the CCC system, there are over 150,000 potential algae species. Further, there is additional room for species specialization if the two microalgae roles in the system are separated into MAB and MAMR systems. While the MAB microalgae should be optimized for nutrient contents, rapid growth, and low nutrient demand, the MAMR microalgae should be optimized for high-density culture and for the unique CCC medium it grows on. In fact, the two systems have at least one opposing specification: the MAB system keeps costs and environmental impacts down using a microalgae species with efficient ammonia and phosphorous use, but the MAMR system species should maximize nutrient demand to quickly consume the animal cell waste and recycle the CCC medium.

These intricacies revealed by the scaled CCC system advance the understanding of unknowns and opportunities for further research. The capital and operating expenses associated with the 42,000 L STR, 211,000 L STR, and 262,000 L ALR facilities in scenarios 1 and 2 are explored next in greater detail.

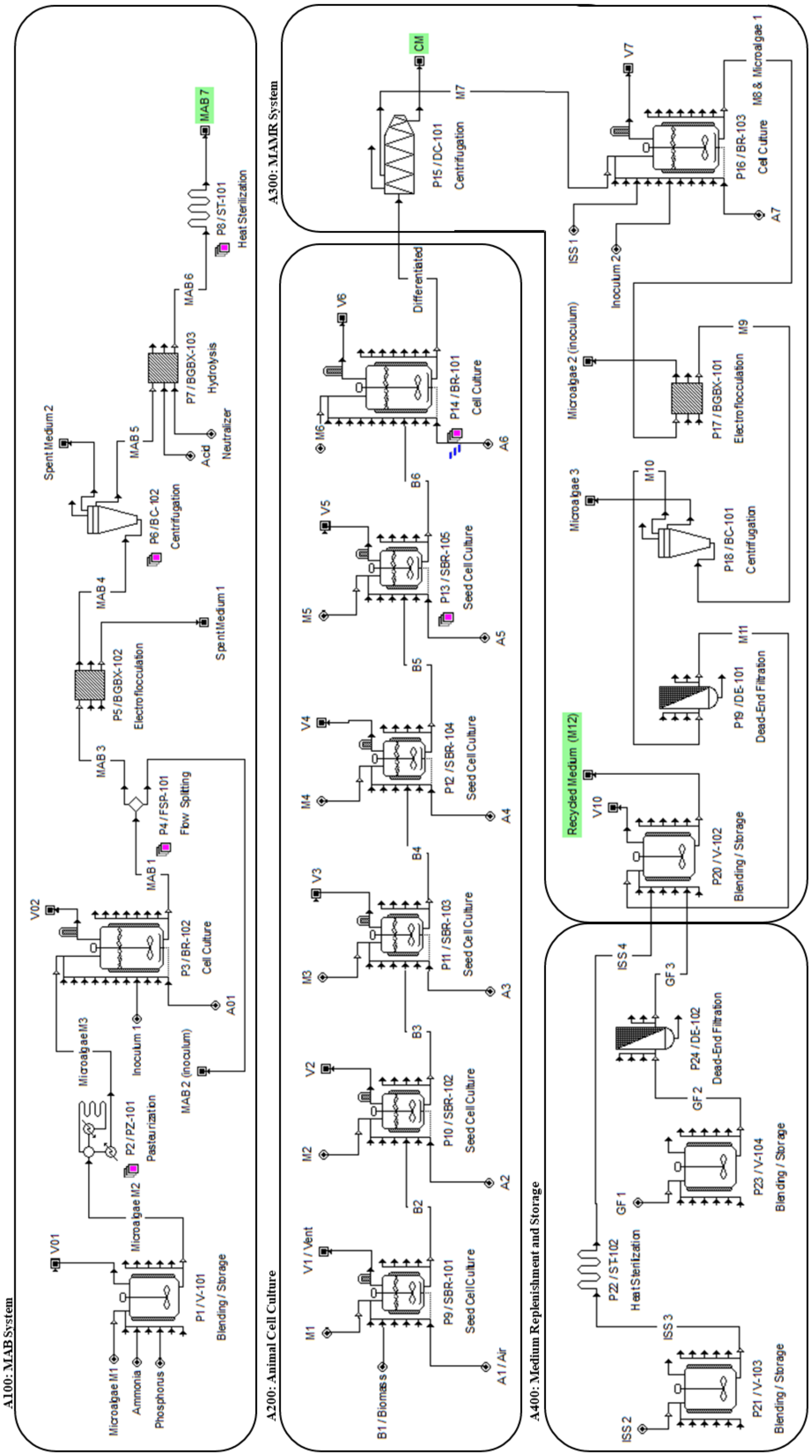

Figure 3.2: Process flow diagram of 42K STR CCC facility modeled in SuperPro Designer. Facility areas A100-A500 correspond to unique Lang factors as presented in **Table 3.4**.

Cost of CM by Weight

Table 3.5 summarizes the COP and cost of goods sold (COGS) for each facility scenario. COP is a more meaningful estimate of a wholesale price for CM as it incorporates payments on capital expenses, or annual capital charge, and OPEX. COP results range from $227/kg to $19/kg with an 80-90% reduction between scenarios 1 and 2 and decreases with increasing production bioreactor volume.

Table 3.5: Summary of economic cost parameters for each facility type in scenarios 1 and 2. Scenarios 1 and 2 represent high and low microalgae demand assumptions, respectively.

Cost Parameter	42K STR Sc. 1.	42K STR Sc. 2	211K STR Sc. 1.	211K STR Sc. 2	262K ALR Sc. 1.	262K ALR Sc. 2
COGS ($/kg)	150	25	131	18	127	13
COP ($/kg)	227	40	194	26	186	19

COGS, calculated only from OPEX, is included for sake of comparison with Negulescu et al.'s[19] results: Scenario 1 results are relatively high with a maximum of $150/kg, but scenario 2 COGS are equal to or slightly less than Negulescu et al.'s [19] values. Without depreciation, and in ascending order of production bioreactor volume, they estimate a COGS of $25/kg, $20/kg, and $16/kg[19]. Despite higher utility costs (increased by two orders of magnitude) and facility-related costs associated with the larger and more complex CCC facility design, scenario 2 COGS remains low by halving cell culture medium costs in comparison to Negulescu et al.'s [19] conventional medium.

Operating Costs

Industry-level operating costs for both scenarios and Negulescu et al.'s[19] model are visualized in **Figure 3.3**, displaying trends in OPEX and annual capital charge. Annual capital charge was not calculated by Negulescu et al. (and thus neither COP) but is estimated here using the same technique outlined in **Table 3.3**. As with COP and COGS, scenario 2 costs are less than or similar to Negulescu et al.'s results, and scenario 1 is an order of magnitude more expensive. **Figure 3.3** also demonstrates that cost efficiency scales with production bioreactor size.

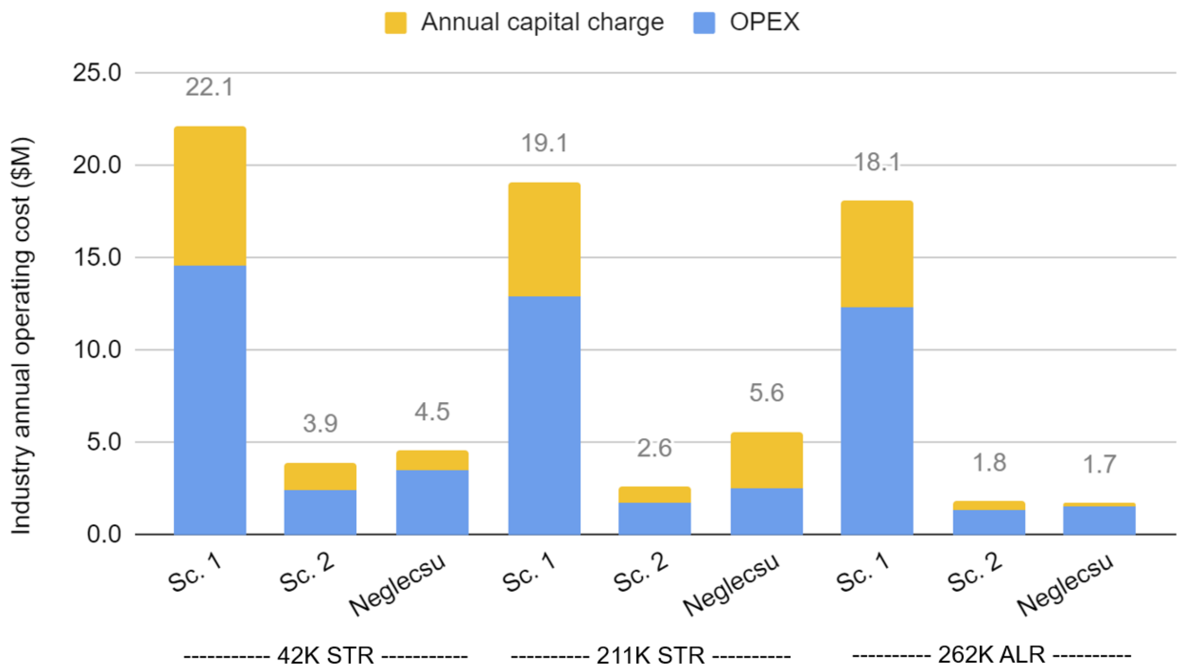

Figure 3.3: Industry annual cost (annual capital charge + OPEX) for each facility design in this study and by Negulescu et al.[19] Annual capital charge for Negulescu et al.[19] is estimated using their reported OPEX and a capital charge factor calculated with an internal rate of return of 7.5% and payback period of 20 years.

Table 3.6 provides a breakdown of specific operating costs for each facility with color grading to help identify the most impactful variables. **Figure 3.4** simplifies this data and presents cost categories as a percentage of facility operating cost. Fixed operating costs make up the majority of operating costs in every facility scenario. Of these, maintenance, insurance, and capital charge factor are calculated with a Lang factor multiplier against CAPEX, and thus are especially high in scenario 1 facilities. Raw material OPEX is kept low by the CCC design, but overall OPEX is significantly impacted by the high purchase cost of MAB production equipment. Electricity costs are also relatively impactful due to the power-intensive requirements of microalgae mixing, aeration, cooling, and dewatering. This is consistent with biofuel TEA and other microalgae literature[11,14,20,41,48]. CO_2 is the largest variable operating cost in all of the facility scenarios, consistent with microalgae biofuel TEAs[11,20]. The impact of reducing CO_2 OPEX will be explored in more detail later.

Table 3.6: Operating costs for each facility under scenarios 1 and 2. Color grading applied to visualize the relative size of contributing costs.

Annual Operating Costs	42K STR Sc. 1.	42K STR Sc. 2	211K STR Sc. 1.	211K STR Sc. 2	262K ALR Sc. 1.	262K ALR Sc. 2
Variable Operating Costs						
Media production and recycling						
MAB: artificial seawater	$46.4K	$2.8K	$204.8K	$17.1K	$253.9K	$15.5K
MAB: ammonia & dipotassium phosphate	$5.5K	$0.4K	$22.9K	$1.7K	$28.4K	$2K
CO2	$173.4K	$13.2K	$766.6K	$61.6K	$950.5K	$72.8K
Hydrolysis (raw materials)	$0K	$0K	$0.2K	$0K	$2.2K	$0K
CM culture: recombinant proteins	$9K	$9K	$57.4K	$57.4K	$71.6K	$71.6K
CM culture: ISS	$0.5K	$0.5K	$11K	$11K	$13.5K	$13.5K
Consumables						
Filters & electrodes	$4.4K	$4.1K	$1.9K	$0.3K	$2.3K	$0.3K
Utilities						
Electricity	$34.3K	$2.6K	$153.7K	$14.2K	$188.8K	$10.5K
Heating & cooling	$19.3K	$2.7K	$170.1K	$11.9K	$190.5K	$14.1K
Cleaning agents	$14.4K	$3.4K	$54.1K	$8K	$66.5K	$9.1K
Fixed Operating Costs						
Labor	$2.3K	$2.3K	$1.8K	$1.8K	$1.8K	$1.8K
Maintenance	$122.6K	$24.6K	$505.7K	$71.2K	$586.6K	$52.3K
Insurance	$153.2K	$30.8K	$632.2K	$89K	$733.3K	$65.4K
Capital charge factor (CCF)	$300.6K	$60.4K	$1240.2K	$174.6K	$1438.6K	$128.4K
Facility Annual Operating Costs	$885.8K	$156.8K	$3822.5K	$519.9K	$4528.6K	$457.4K
Industry Annual Operating Costs	$22146K	$3919.2K	$19112.6K	$2599.4K	$18114.5K	$1829.7K

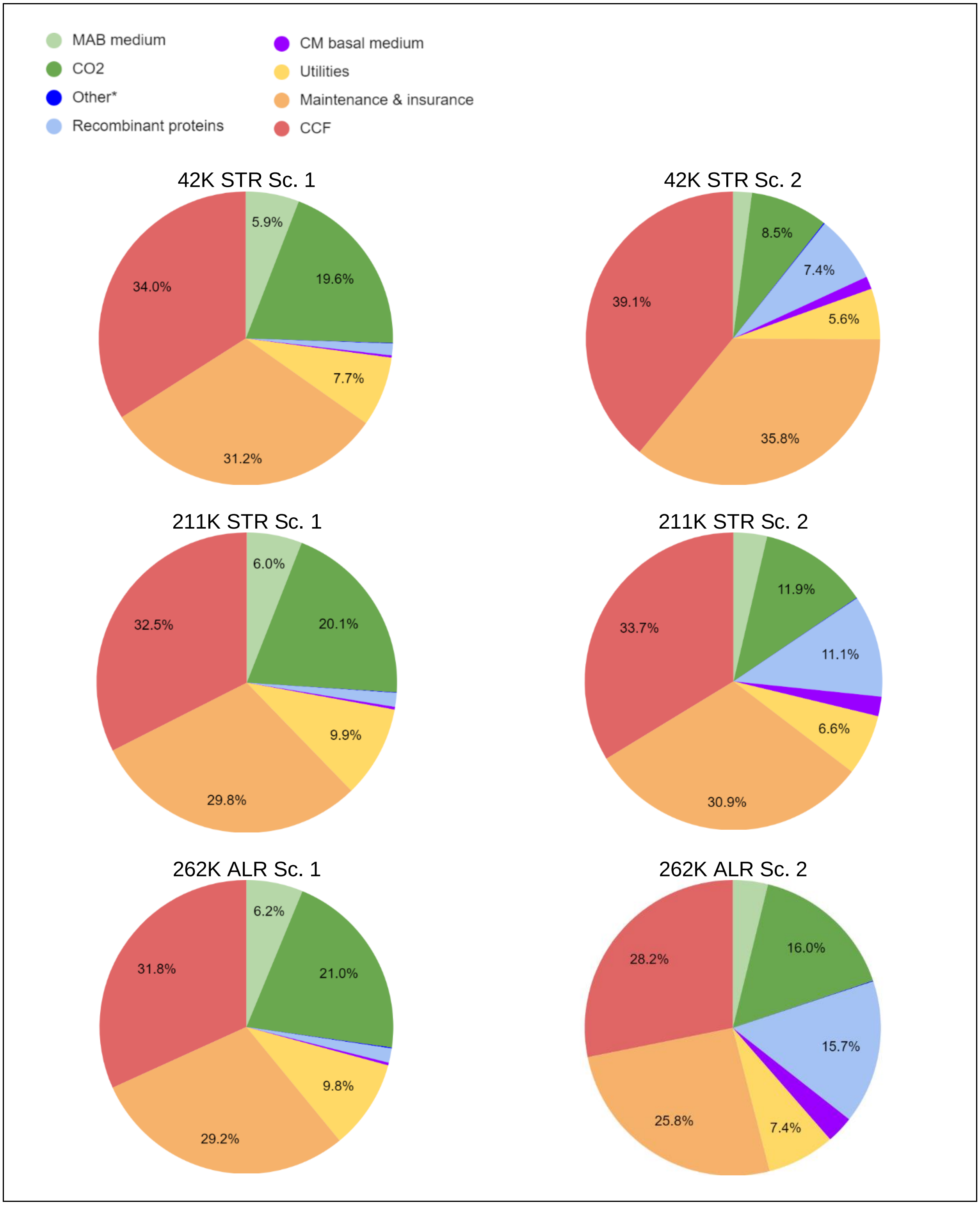

Figure 3.4: Facility operating costs for each facility scenario. *Labor, raw materials used for MAB hydrolysis, and consumables including filters and electro-flocculation electrodes.

Capital Costs

Table 3.7: Capital costs for each facility under scenarios 1 and 2. Color grading is applied to visualize the relative size of contributing costs.

Capital Costs	42K STR Sc. 1.	42K STR Sc. 2	211K STR Sc. 1.	211K STR Sc. 2	262K ALR Sc. 1.	262K ALR Sc. 2
Direct Costs: Purchased Equipment						
MAB: helical tubular bioreactor	$805M	$58M	$3615M	$268M	$4445M	$319M
MAB: dewatering	$349M	$22M	$1539M	$117M	$1923M	$138M
MAB: other	$486M	$27M	$2095M	$80M	$2575M	$150M
CM culture: seed bioreactors	$3M	$3M	$9M	$9M	$11M	$11M
CM culture: production bioreactors	$53M	$53M	$117M	$117M	$15M	$15M
Media recycling, replenishment, storage, other	$9M	$10M	$34M	$33M	$41M	$40M
Direct Costs: Other						
Installation	$26M	$26M	$59M	$59M	$17M	$17M
Piping, instrumentation, electrical	$90M	$56M	$285M	$134M	$236M	$50M
Buildings & yard improvement	$143M	$76M	$485M	$184M	$447M	$76M
Auxiliary facilities	$67M	$27M	$244M	$69M	$249M	$33M
Total direct cost (TDC)	$2031M	$359M	$8483M	$1071M	$9960M	$849M
Indirect Costs						
Engineering and construction	$976M	$200M	$4033M	$581M	$4669M	$422M
Fees and contingencies	$57M	$57M	$128M	$128M	$38M	$38M
Facility total capital investment (TCI)	$3064M	$616M	$12644M	$1780M	$14666M	$1309M
Industry TCI	$76608M	$15398M	$63218M	$8900M	$58663M	$5234M

Table 3.7 provides a breakdown of facility total capital investment (TCI), in this study considered equal to CAPEX, for each scenario. In scenario 1, direct costs are overwhelmingly attributed to MAB production equipment, particularly the microalgae tubular helical bioreactor. The MAB helical tubular bioreactor in scenario 1 facilities is of comparable footprint (1540 acres, 6908 acres, and 8496 acres for 42K STR, 211K STR, and 262K STR facilities, respectively) to NREL's microalgae biofuel bioreactor with 5,000 acres of cultivation area[20]. Purchase costs for all other equipment, including the mammalian cell seed and production bioreactors, helical tubular bioreactor and dewatering equipment associated with the MAMR

system, and medium replenishment and storage equipment are 0.8-4% of the cost of the MAB equipment.

"Other" direct costs as listed in **Table 3.7**, such as installation and buildings, are relatively constant over the facility types, as the purchase equipment cost of the MAB production equipment already includes these additional costs. Indirect costs are calculated by multiplying Lang factors to total direct cost (TDC), and thus trend with the significant cost of the MAB equipment. In scenario 2, reduced MAB demand leads to a significant reduction in MAB equipment size and therefore cost, and overall CAPEX is more evenly distributed across production areas. This is visualized below in **Figure 3.5**, showing the distribution of facility CAPEX costs across scenarios.

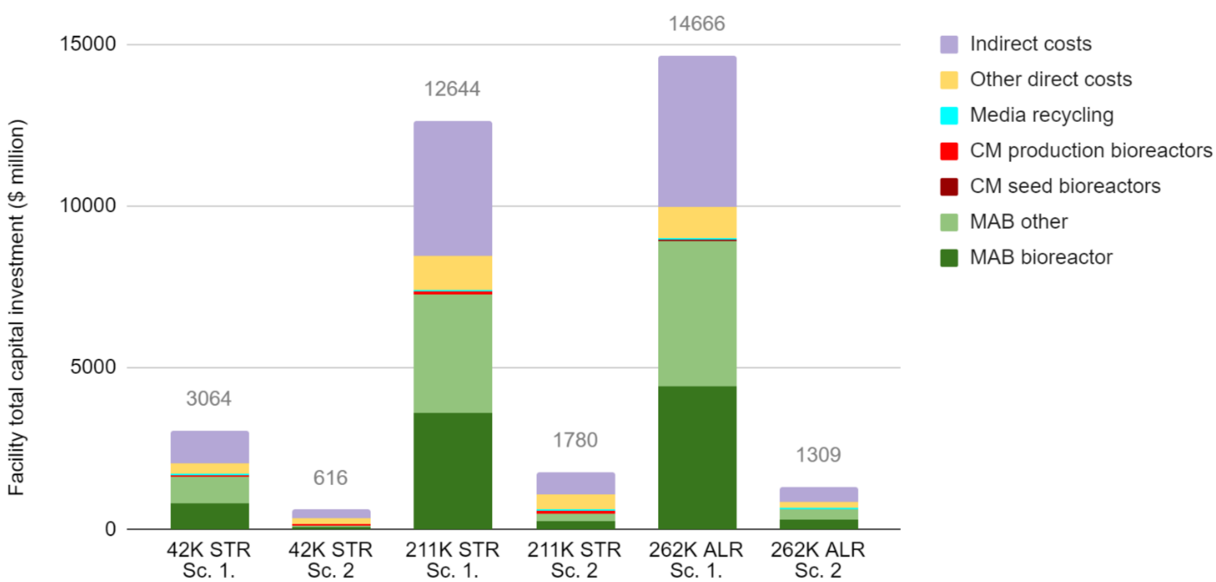

Figure 3.5: Breakdown of contributions to facility CAPEX across all facility scenarios. "Other" direct costs include installation, piping, instrumentation, electrical infrastructure, buildings, yard improvement, and auxiliary facilities.

Scenario 2 brings down facility CAPEX by >80% for each facility size, bringing CAPEX to the same order of magnitude as results by Negulescu et al.[19] Facility CAPEX increases with maximum production bioreactor size, as expected, but when normalized by an industry-wide annual output of 1 million kg of CM, the larger facilities are increasingly cost-efficient. Industry CAPEX by Negulescu et al.[19] and scenarios 1 and 2 is presented below in **Figure 3.6**.

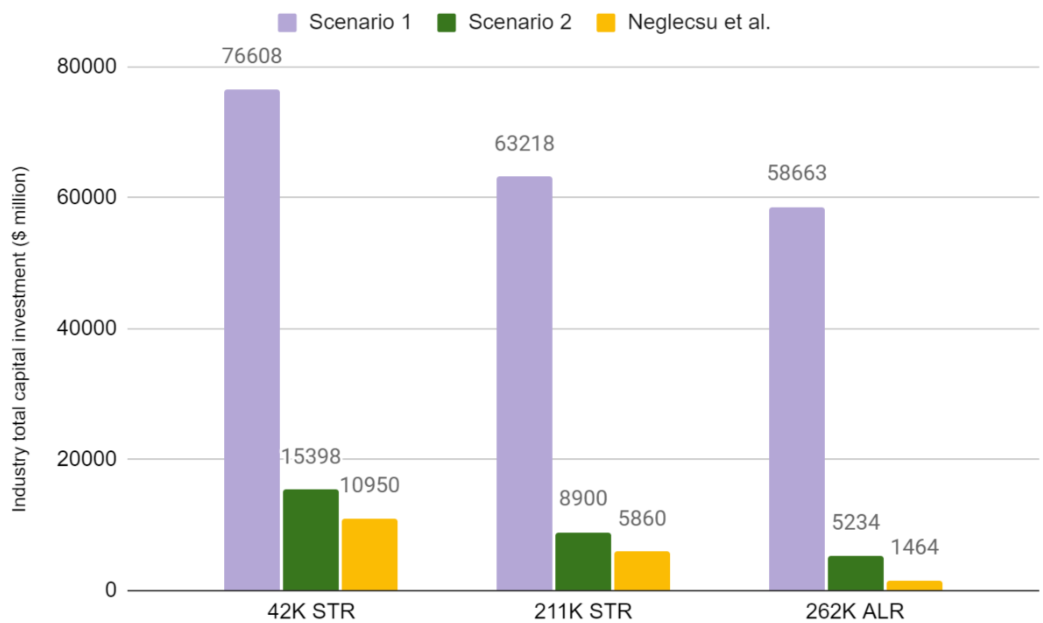

Figure 3.6: Industry CAPEX for each facility design in this study and by Negulescu et al.

Media Cost

This CM TEA is unique in that the facility design is expanded to include in-house production of CM media macronutrients. The cost of media, therefore, is calculated not only as a sum of purchased raw material medium ingredients but also utilities, labor, consumables, cleaning agents, and other raw materials used in media production. Media production includes MAB cultivation, dewatering, and purification, and MAMR system medium recycling and replenishment. This means media cost per liter varies with facility size and scenario. Media cost decreases slightly with facility size due to more efficient use of variable operating costs. Media is produced at~$7/L in scenario 1 facilities, dropping below $1/L in scenario 2. Scenario 2 redefines the medium composition by lowering the demand of MAB per liter of media by >92%, and as MAB production costs account for most of the variable operating costs, this translates to a reduction in media cost by >87%. Scenario 2 facilities find the lowest baseline scenario medium cost amongst the other CM TEAs (Humbird's[22] estimate is not in cost per liter and is not plotted), as shown in **Figure 3.7**. Note that the CM TEAs' assumptions and methodologies, especially when calculating the cost of media, vary significantly and thus are not directly comparable but can be taken to contextualize media cost predictions within CM literature.

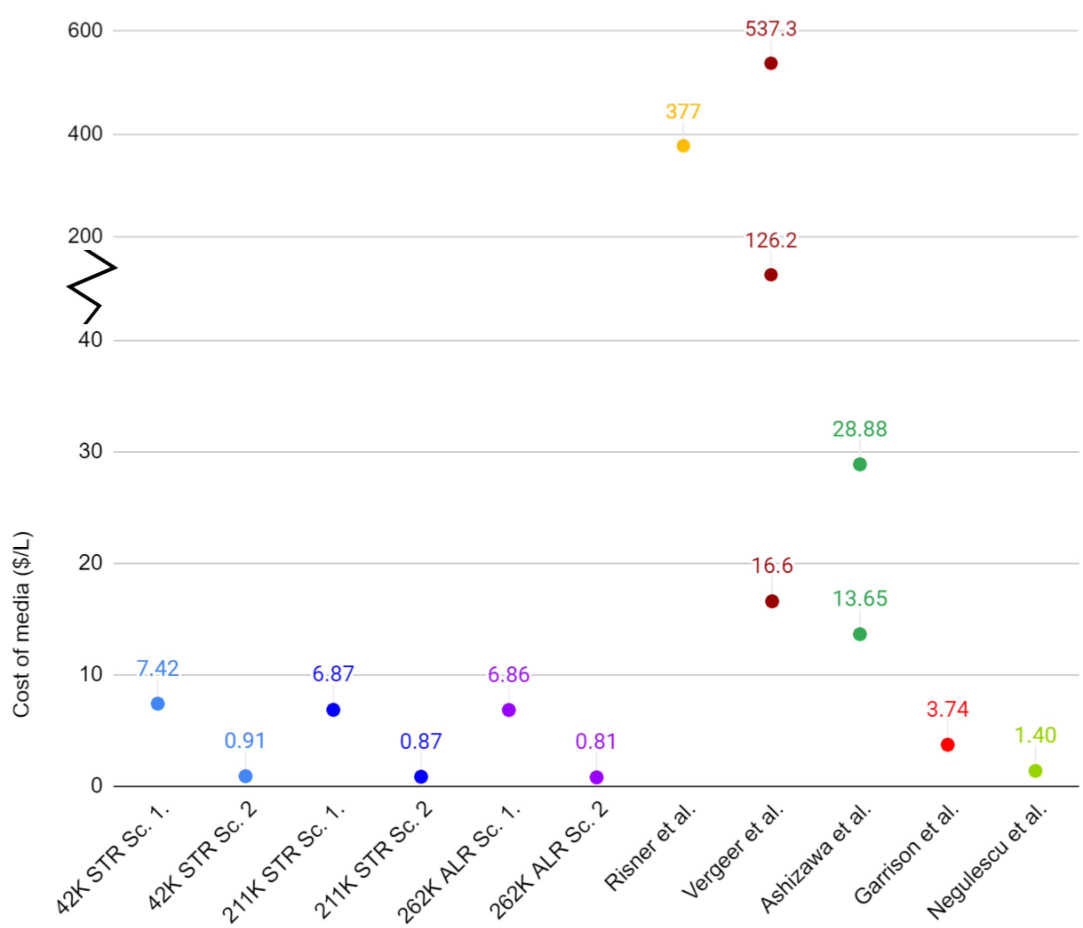

Figure 3.7: Cost of media for each facility design in this study and by 5 other CM TEAs.

Media Recycling

To reduce the costs and environmental impacts of CM cell medium, this TEA presents a hypothetical CM facility design for medium recycling. It is interesting to consider the impact of medium recycling on variable OPEX and ultimately COP.

Haraguchi et al.[1] demonstrate the efficacy of the bench-scale CCC design for one recycling loop. Without additional experimental data, it is difficult to predict the maximum number of times a batch of medium may be safely and effectively recycled. In the CCC facility models, however, it was found that there is a logistical maximum to the amount of medium that can be recycled in a production year. Due to the staggered scheduling design of the scaled CCC facility models, 11 or 12 batches worth of CCC medium are circulating within the CM bioreactors in the 42K STR or 211K STR and 262K ALR facilities, respectively, after the facilities have fully started up. When CM medium needs to be replaced at the start of the production year or after the medium has reached a maximum reuse number (if made necessary by safety requirements), a minimum of 11 or 12 batches worth of fresh medium is needed for the facility start-up. With 123 total batches in a year, this initial medium volume can be reused a maximum of 11 or 10 additional times in the 42K STR or 211K STR and 262K ALR facilities, respectively. See **Appendix P** for further explanation of these scheduling details.

Media cost per liter must therefore be determined by averaging media-related OPEX over the course of 123 batches. Total fresh and recycled batch count is calculated based on a selected maximum reuse parameter (1-12) and the scheduling considerations outlined above. Variable operating costs associated with the MAMR system and preparation and replenishment of make-up medium or preparation of an entire fresh batch volume of media adjusted accordingly to each batch. All previous economic results presented in this study were calculated assuming no recycling.

Ultimately, media batch reuse count makes a negligible impact on media cost across facility types and baseline scenarios. This is for several reasons. For a CM industry size of 1 million kg of CM per year, media cost is dominated by macronutrient demand when assuming that recombinant protein and growth factor costs decrease drastically with scale[19,22,49]. Because 100% consumption of MAB-sourced macronutrients is assumed, the most expensive media component is replenished at the same rate regardless of media recycling. Instead, recycling reduces demand for the CM basal medium, consisting of purified water and salts, and growth factors.

However, it is valuable to take a closer look at the assumptions surrounding growth factor cost used in this study and in other CM TEAs[19,22,44,49]. **Figure 3.4** shows that in scenario 2, without medium recycling, recombinant proteins make up 5.9-15.7% of annual operating costs, a non-significant contribution that one might reasonably expect to reveal cost benefits under recycling conditions. Instead, despite reducing industry-wide demand for growth factors by >80%, recycling only allows a ~20% reduction in growth factor OPEX and almost no impact on media cost because the demand reduction also leads to an increase in estimated growth factor cost per weight by >420%. This relationship between industry demand, cost per weight, and industry expenditure for recombinantly produced insulin over media reuse count is graphed in **Figure 3.8**. Industries of scale are a useful consideration to demonstrate the potential of a mature CM market. But it is counter-intuitive that a bioprocessing technique that considerably reduces the need for raw materials such as TGF-β with current vendor prices at >$11 million/mg would not be strongly incentivized.

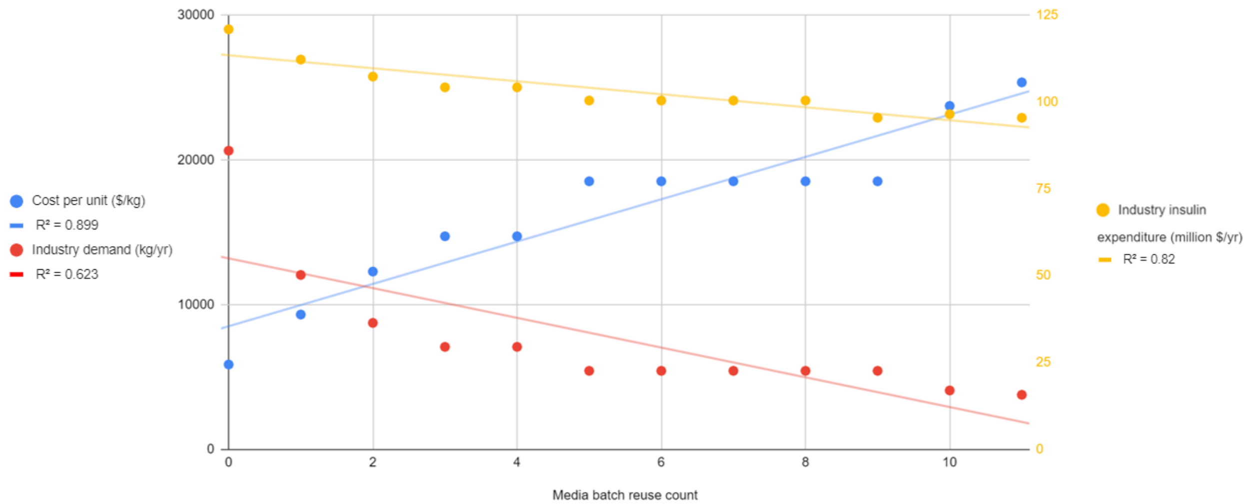

Figure 3.8: The price volume correlation of insulin and resulting industry-wide insulin expenditure as a function of the number of times medium batches are reused. Data is an example by the 42K STR facility but does not change significantly from other facility sizes.

Further, there is significant reason to doubt that growth factor production will scale and costs decrease substantially as the price-volume calculations suggest[44]. If 2024 vendor prices for recombinantly produced growth factors (listed in **Appendix C**) are utilized for medium cost estimates, media batch reuse count begins to have a meaningful impact not only on medium cost per liter, as shown in **Figure 3.9**, but overall COP for CM, shown in Figure **3.10**. In the case that growth factor suppliers do not completely scale, for example, in the case that scientific advancements eliminate the need for most but not all required growth factors, media recycling systems as proposed in this model may offer needed cost savings.

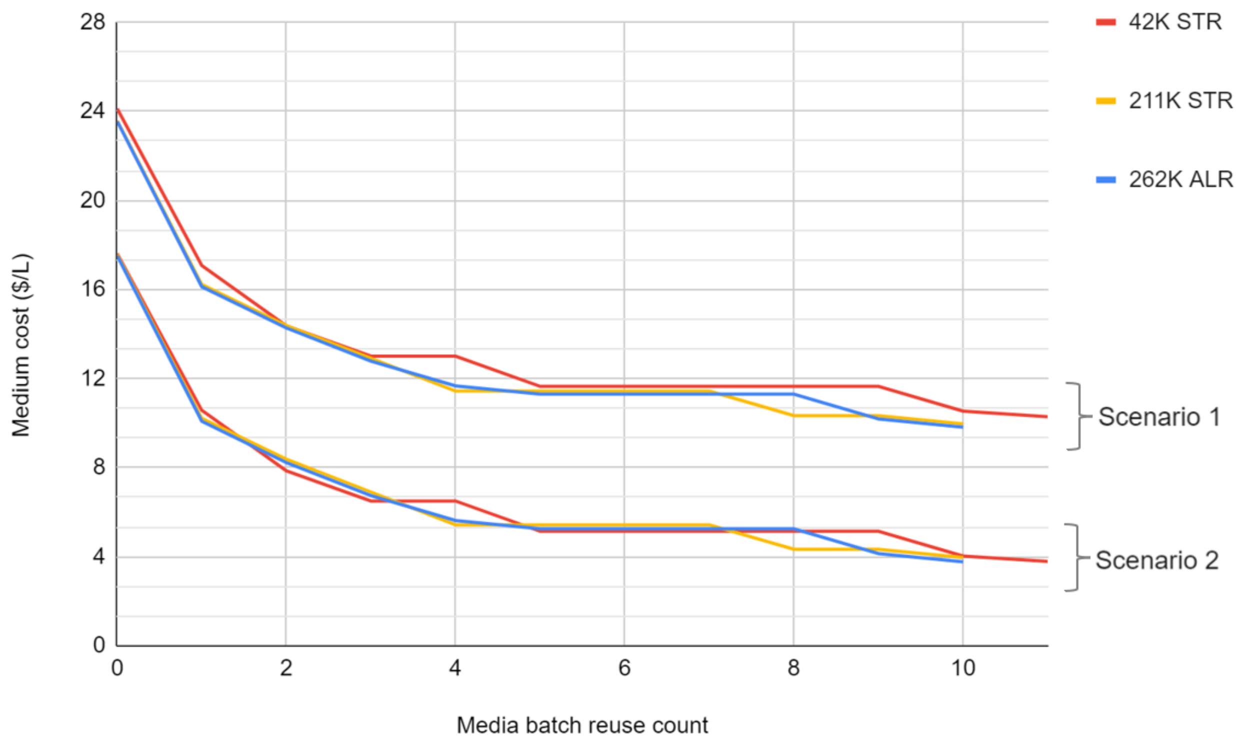

Figure 3.9: Medium cost per liter for each facility design as a function of the number of times medium baches are reused, assuming that recombinantly produced growth factors are purchased at vendor prices.

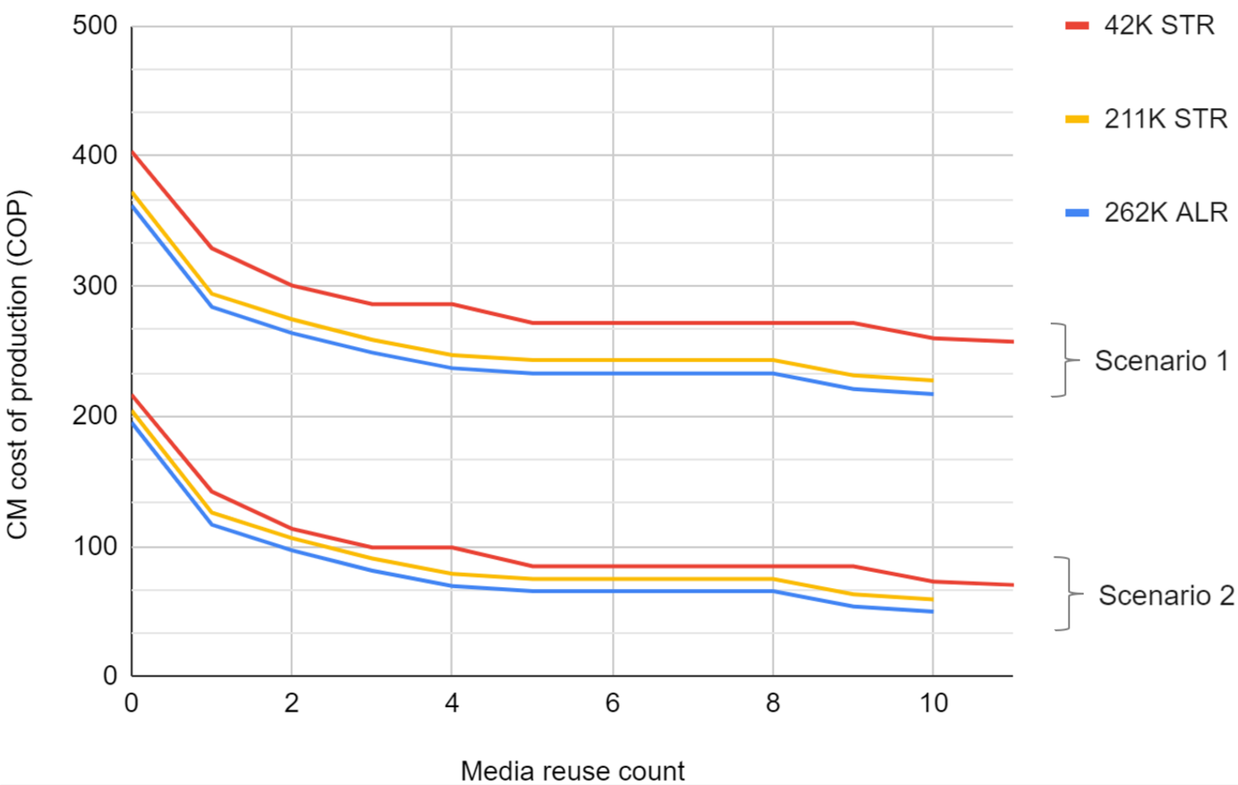

Figure 3.10: COP for each facility design as a function of the number of times medium baches are reused, assuming that recombinantly produced growth factors are purchased at vendor prices.

Alternative scenario: CO_2 cost reduction

In the baseline scenarios for this TEA, CO_2 is modeled as a purchased raw material, supplied by flue gas concentrated outside of the facility boundary by carbon capture technology, transported into facility bounds, and stored in a pressurized tank as outlined in the NREL microalgae biofuel TEAs[11,20]. They estimated that the purification, compression, and transportation of CO_2 in 2022 cost $45/metric ton of CO_2, also used in this model, and represents the largest variable operating cost in MAB production. Alternatively, if the facility is co-constructed with an energy plant utilizing carbon capture technology, CO_2 OPEX is ~$0[11,20]. This better-case scenario reduces COP by 8-21%, as seen in **Table 3.8**, with a new minimum COP of $15.7/kg of CM by the 262K ALR facility in scenario 2. Co-construction is a possible but optimistic assumption, as it adds to the complexity of facility location requirements[11,20,21]. Locating 25 of such co-construction opportunities, as required in the 42K STR facility scenarios, would be challenging.

Table 3.8: New cost of production for each facility scenario assuming co-construction with a carbon capture facility and percent reduction from the baseline COP estimates.

Parameter	42K STR		211K STR		262K ALR	
	Sc. 1	Sc. 2	Sc. 1	Sc. 2	Sc. 1	Sc. 2
COP ($/kg)	182.5	36.8	155.4	23.3	146.8	15.8
Reduction in baseline COP	20%	8%	20%	12%	21%	16%

3.4 Chapter Summary

Translating the novel CCC design by Haraguchi et al.[1] to an industrial scale reveals the strengths of the concept, its complexities, and areas where improvement is still necessary to bring CM to market. This is the first CM TEA that models the in-house production of cell medium macronutrients. Considering the scale of equipment and energy involved in the production and processing of amino acids and glucose that falls outside of the scope of previous CM TEAs, it is significant that this model results in COP estimates that are on par with previous results. Under scenario 2, assuming low MAB demand, COP ranges from $40-19/kg, at or below results from the baseline case by Negulescu et al.[19] By sourcing basal medium nutrients from microalgae, simply and efficiently grown organisms, raw material costs are cut considerably. Scenario 2 facilities optimistically predict media costs below $1/L. Additionally, microalgae may enable medium recycling, further reducing raw material demand. This is shown to become impactful, allowing a ~2x reduction in COP, if the growth factor industries remain at their current production level. As there is a significant risk growth factor that industries will not develop at the necessary sterility, speed, and scale, medium recycling may be an important facet of CM cost reduction. The CCC facility model also demonstrates that the MAB system and MAMR systems most likely must be separated due to drastic differences in medium demand. This diverges from the bench-scale models but clarifies how the systems can therefore be optimized independently, including the optimization of microalgae species growth characteristics, nutrient composition, extraction efficiency, and culture conditions.

This model demonstrates that the success of a microalgae-based CM medium is highly variable depending on several crucial assumptions. This is in addition to the assumptions shared by other CM TEAs, including the development of relevant myogenic animal cell species with improved metabolism, the ability to proliferate and differentiate in suspension, and reduced CO_2 inhibition, and the adequacy of food-grade aseptic conditions. Most significantly, the CCC facility appears feasible only under MAB demand far reduced from scenario 1 assumptions. Scenario 1 requires the highest total industry CAPEX out of the CM TEAs, ranging from $766-$58.7 billion. This is unrealistically massive, on par with theoretical biofuel production facilities[11,20]. Ultimately, the cost and footprint of each facility design, especially those in scenario 1, are dominated by the production of MAB production. Without additional data, it is difficult to reconcile the drastic differences in MAB demand predicted by scenarios 1 and 2.

Consumption analysis and other growth characteristics for *C. littorale* or other relevant microalgae species at scale in select PBRs are essential in improving the accuracy of productivity estimates and thus minimum facility sizes and associated costs. For example, Davis et al.[11] modeled a 40% decrease in productivity between an early-stage biomass harvest (before nutrient depletion) and a late-stage biomass harvest (6-9 day culture), but found that productivity gains from early harvest were offset by the increase in nutrient costs to maintain high nutrient concentration. These significant sensitivities to culture conditions are lost in the generalized areal productivity and bench-scale data used in this study. Additionally, due to the novel and interdisciplinary nature of this model, it is difficult to predict the adequacy of the various bioprocessing design selections, for example, whether the CIP and mechanical scrubbing of the MAMR microalgae bioreactor is sufficient to maintain sterility in the recycled medium. It is also possible, for example, that the MAB system could adopt a less process-intensive PBR model, such as hanging bags or horizontal tubes, or perhaps even an open-pond design. Culturing, dewatering, and MAB processing methods would likely develop with pilot-scale experimentation. Overall, there are significant opportunities for process improvement in the CCC system.

Finally, it is out of the scope of this study to evaluate the environmental impact of the CCC medium in comparison to conventional CM production models. However, there is a significant body of literature on the environmental impact of microalgae as a novel food source[50]. While it is understood that microalgae provide unique potential benefits, such as high productivity on otherwise nonarable land and the valorization of waste streams, these benefits are contingent on an array of factors. Facility location, microalgae species, cultivation vessel, and dewatering techniques impact energy demand, driven higher with process-intensive components like tubular bioreactors and centrifuges, as used in this study. Nutrients ammonia and phosphorus introduce significant variability in environmental impact as they can be sourced from waste streams (as in the MAMR system) or, for example, derived from fossil fuel-based fertilizers (as in the MAB system). It is additionally possible that heterotrophic microalgae could be better suited and less environmentally impactful as the MAB source[50]. These factors should drive the CCC system design as intensely as economic concerns do, for the ultimate goal of CM is to develop an abundant and sustainable protein supply.

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CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

This thesis is amongst agricultural biotechnology and systems engineering efforts that seek to provide sustainable and abundant nutrition in response to global environmental pressures. CM is a form of cellular agriculture that grows real animal tissue in bioreactors, a slaughter-free food product that may drastically reduce meat's severe resource demands. This thesis investigates paths to scale-up CM and offers a cross-disciplinary production design that incorporates plant-based technology and circular bioprocessing for a novel, sustainable alternative to industrial animal agriculture.

Presented in Chapter 2, a literature review on current large-scale CM production models concludes that under the current technological paradigm, CM is unlikely to achieve widespread adoption. LCAs and TEAs till date that predict the environmental benefits and economic competitiveness of CM make substantial assumptions in their baseline models which have not yet been achieved, including the availability of immortalized cells of relevant species with ideal metabolisms, able to proliferate and perhaps differentiate in suspension in large (>20,000 L) bioreactors. Assumptions such as plant or microalgae-based medium and food-grade aseptic techniques also improve these results. Still, current TEAs (forgoing very ambitious assumptions) do not present a cost per kilogram lower than that of choice-grade ground beef. This review offers essential research directions, such as suspension culture and plant-based cell medium, and critiques areas of research less important to achieving wide-scale impact. One of the most pressing challenges is reformulating cell culture medium, the most environmentally and economically expensive component of CM at scale, as it is typically composed of tens of individually manufactured components. This analysis establishes the appropriate gravity and impact-driven perspective for more meaningful research in CM and other food biotechnology.

In Chapter 3, a large-scale production model that incorporates these ideas is raised. Cell culture medium macronutrients are replaced with a singular component, produced in-house: microalgae biomass. A second live microalgae culture is used to recycle spent medium, enabling repeated use of this valuable material. A TEA reveals that this system achieves the lowest base-case estimate of medium cost (<$1/L) compared to other CM TEAs, contingent on the metabolic demands of the animal cells. Also encouragingly, the estimated cost of CM per kg is on par with conventional models. Challenges to reducing capital expenditures remain, as well as significant unknowns about cellular metabolism and aseptic technique. This circular cell culture system rethinks the model of CM production to reflect trends in cellular agriculture, relying on the efficiency of microorganisms to reduce the complexity and resource intensity of bioprocess systems. By leveraging microalgae-based technology alongside animal cell culture, this work boldly proposes a CM manufacturing design towards a more sustainable ideal.

4.2 Future Directions

There are several clear ways by which the conclusions of this thesis may be strengthened and extended. Chapter 3 presents a facility design for a microalgae-based production of CM. The interdisciplinary design is primarily informed by several bench-scale primary studies of the CCC system, biofuel literature, and previous CM TEAs. There is no available data on the consumption rate and mass balance of CM cells on microalgae extract, resulting in the division of the microalgae demand estimate into scenarios 1 and 2. These scenarios provide drastically different results in the size, CAPEX, and OPEX of the facilities and ultimately the cost and feasibility of

CM. Primary studies could greatly clarify this unknown with direct measures of CM cell growth characteristics on microalgae-based medium. Primary studies could also investigate medium recycling for more than one reuse, as is currently available in literature. As stated throughout this analysis, CM TEA should be improved with additional data from liter-scale suspension culture with relevant media and cell types.

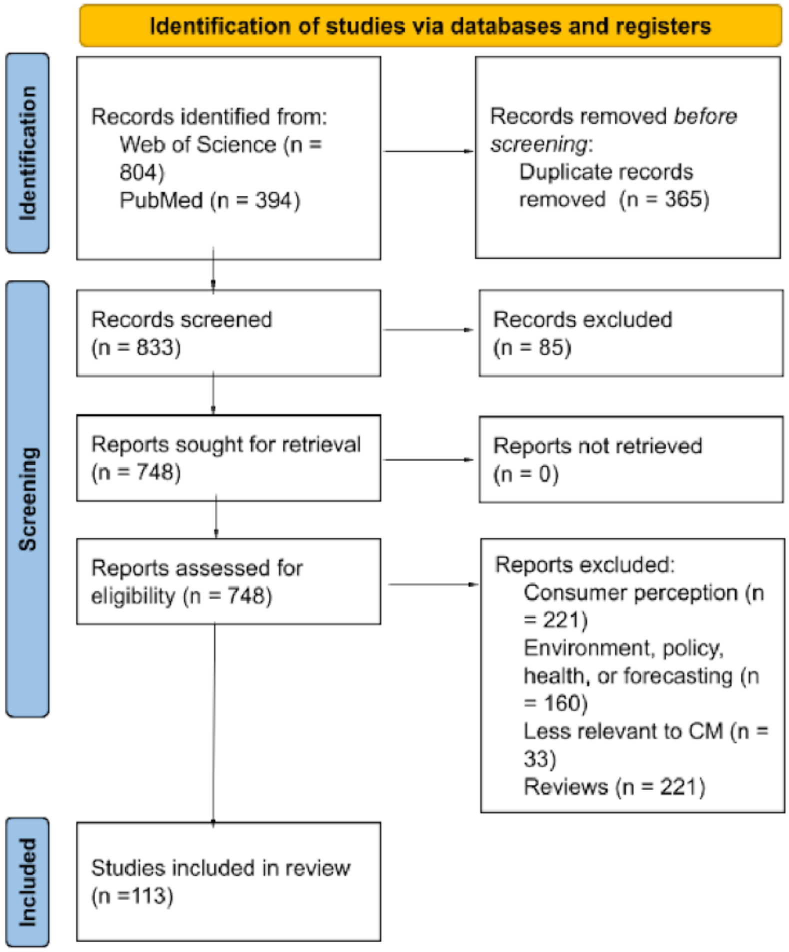
An obvious next step also includes an LCA of the CCC system. Though the system demonstrated potential economic benefits, the in-house production of microalgae-based medium may also provide significant environmental benefits in comparison to conventional CM production models. Assuming that growth factors have a negligible cost at scale, media recycling provided no economic benefit but may relieve impacts such as eutrophication from discarded media and other impacts related to growth factor production. While the CCC is on par with the economic benefits of other CM TEAs, it may perform strongly according to an LCA.

There are many potential adjustments to make to the CCC facility design. Advancements should focus on reducing CAPEX. For example, it is not certain that the microalgae grown for medium macronutrients, the output of the MAB system, must be grown in PBRs. PBRs, closed system bioreactors, provide additional controls and sterility at a substantially higher purchase and operating cost. Because the MAB system is responsible for the majority of the COP, additional scenarios of the CCC facility should include open pond MAB cultivation similar to microalgae-based biofuel facilities.

Finally, in evaluating future research directions and collaborations, the food system as a whole must be considered. At the time of writing (August 2024), there are hundreds of CM companies globally, a handful with products for human or animal consumption approved for sale, and at least one selling to customers in Singapore. This and the growing body of CM literature demonstrate, of course, that CM is technologically feasible. More advancements are undoubtably underway. But CM must still be proven scalable. Despite being incentivized for growth, many start-up based products end up oriented toward small niche markets. This is evident within the CM industry as companies continually unveil high-end novelty products. As such, it is essential that academic research and government-funded initiatives persist in the goal towards accessible, popular, and sustainable protein. Non-profit work, beholden to internal standards, must also stay true to these goals. Further, there are significant economic, political, and other structural barriers in achieving sustainability and equitability in US and global food systems. One innovative technology or product is unable to address these crises alone. CM does not inherently provide a solution for, for instance, food waste, monocropping, fossil fuel dependency, unjust agricultural working conditions, or unhealthy diets. CM has potential to be a part of the resolution to these challenges, as media macronutrients could be supplied from diverse plant groups, CM factories are suited for electrification, and CM nutritional characteristics may be tailored to better suit our needs. But none of these achievements are guaranteed. Neither is CM's primary claim of sustainability, and proponents must avoid the pitfalls of greenwashing. A massive collaboration between government, community, researchers, industry, and other innovators will be necessary to realize the benefits of CM and other components of a more livable future. Members of the CM field are urged to remain connected to the greater context of food system change.

APPENDICES

Appendix A: Scoping review PRISMA chart



A scoping review of primary CM literature was performed according to PRISMA guidelines, summarized in this flow chart. The purpose, characteristics, and main findings of each study were recorded to understand the distribution of efforts within CM research and the full range of more granular topics, summarized in Table S1. The scoping review also led to the identification of the six CM TEA publications upon which Chapter 2 focuses.

Appendix B: Composition of simplified inorganic salt solution (ISS) for CCC animal cell culture basal medium

Ingredient		mg/L
NaHCO3	sodium bicarbonate	3700
NaCl	sodium chloride	6400
H2O	water	1000000

Based on ISS recipe by Yamanaka et al. Ingredients <0.1% of solution weight are excluded.

Appendix C: Recombinantly produced growth factors and concentration in CCC medium with 2024 vendor prices

Recombinant Growth Factor	Concentration (mg/L)	Vendor price ($/kg)	Vendor/Source
FGF-2	20	6780000000	Sigma
Insulin	20	24500	Humbird, 2020
NRG1	0.005	11300000000	Sigma
TGF-β	0.0001	34700000000	Sigma
Transferrin	0.0001	594000	Sigma
Rapeseed	800	3.21	Stout et al., 2023

Concentration based on Beefy-R formula by Stout et al.

Appendix D: Reported areal productivity of microalgae culture in helical tube bioreactors

Areal productivity ($g/m^2/day$)
19.4
24.2
12.6
15.6
38.5
38.5

Adopted from Clippinger & Davis[1].

Appendix E: Composition of simplified Diago's artificial seawater for MAB cultivation

Recombinant Growth Factor	Concentration (mg/L)	Vendor price ($/kg)	Vendor/Source
FGF-2	20	6780000000	Sigma
Insulin	20	24500	Humbird, 2020
NRG1	0.005	11300000000	Sigma
TGF-β	0.0001	34700000000	Sigma
Transferrin	0.0001	594000	Sigma
Rapeseed	800	3.21	Stout et al., 2023

Appendix F: Infrastructure for 42K STR Scenario 1 Facility

42K STR: Scenario 1 Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost Quote ($)	Year of Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	1	1	6530000	2022	2242912	L	172708263	L	0.6	0.978	86489452	86489452	0	5.07			Negulescu et al.[19]
P2	Pasteurizer	24	1			SuperPro Designer Built in Model		98951	L/hr	N/A	N/A	15528000	372672000	497377	21.85	SuperPro Designer example file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	1539	acre	N/A	1.385	805340260	805340260	1827113	47.22			Clippinger & Davis[20]
P5	Electroflocculation - Electroflocculation Tanks	134	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	186537849		10.94			
P5	Electroflocculation - Hydrolic Mixer	384	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	61978368	2360	3.63			Roles et al.[31]; Lee et al.[42]
P5	Electroflocculation - Settler	138	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	87832722		5.15			
P5	Electroflocculation - Auminum Diode	1	1	2.3	2024	N/A	$/kg	62303	kg	N/A	1.000	143296	143296		0.01	statista.com[56]	Roles et al.[31]; Lee et al.[42]	
P6	Bowl Centrifuge	4	1	2242500	2013	463	m3/hr	445	m3/hr	0.6	1.406	3077252	12309008	2746	0.72	Davis et al.[21]	Roles et al.[31]	
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	983	dry kg/hr	0.6	1.528	8400940	8400940	2360	0.49	Humbird et al.[55]	Carvalho et al.[58]	
P8	High temperature short time (HTST) sterilizer	22	1	755000	2022	42060	L/hr	50000	L/hr	0.6	0.978	818760	18012720	439291	1.06	teknoice.com[57]		Negulescu et al.[19]
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	28000	2022	125	L	125	L	0.6	0.978	27372	27372	3	0.00			Negulescu et al.[19]
P10	STR seed bioreactor	1	1	73000	2022	631	L	631	L	0.6	0.978	71363	71363	13	0.00			Negulescu et al.[19]
P11	STR seed bioreactor	1	1	193000	2022	3200	L	3201	L	0.6	0.978	188707	188707	68	0.01			Negulescu et al.[19]
P12	STR seed bioreactor	1	1	512000	2022	16254	L	16256	L	0.6	0.978	500555	500555	347	0.03			Negulescu et al.[19]
P13	STR seed bioreactor	5	1	517000	2022	16500	L	16502	L	0.6	0.978	505442	2527210	1056	0.15			Negulescu et al.[19]
P14	STR production bioreactor	10	6	904000	2022	41901	L	41904	L	0.6	0.978	883764	53025840	5828	3.11			Negulescu et al.[19]
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	990000	2022	83002	L/hr	83506	L/hr	0.6	0.978	971319	971319	300	0.06			Clippinger & Davis[20]
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	3	acre	0.6	1.385	1470281	1470281	4435	0.09			
P17	Electroflocculation - Electroflocculation Tanks	0.3	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	417622.05		0.02			
P17	Electroflocculation - Hydrolic Mixer	2	1	850	2013	N/A	Installed cost/m2	70	m2	N/A	1.406	83665	167330	27	0.01			Roles et al.[31]; Lee et al.[42]
P17	Electroflocculation - Settler	1	1	2630	1998	N/A	N/A	377	m2	0.678	1.924	282573	282573		0.02			
P5	Electroflocculation - Auminum Diode	1	1	2.3	2024	N/A	$/kg	144	kg	N/A	1.000	331	331		0.00	statista.com[56]	Roles et al.[31]; Lee et al.[42]	
P18	Bowl Centrifuge	1	1	2242500	2013	463	m3/hr	84	m3/hr	0.6	1.406	1131939	1131939	130	0.07	Davis et al.[21]	Roles et al.[31]	
P19	Dead-end filtrator	1	1	40000	2022	10	m2	340	m2	0.6	0.978	324412	324412	0	0.02			Negulescu et al.[19]
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	2188000	2022	362513	L	372771		0.6	0.978	2175043	2175043	0	0.13			Negulescu et al.[19]
P21	Blending/storage tank	1	1	2188000	2022	362513	L	372771		0.6	0.978	2175043	2175043	0	0.13			Negulescu et al.[19]
P22	High temperature short time (HTST) sterilizer	1	1	755000	2022	42060	L/hr	12426	L/hr	0.6	0.978	355114	355114	9 4962	0.02 0.00	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39], Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	315	L	0.6	0.978	31189	31189	0	0.00			Negulescu et al.[19]
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.00			Negulescu et al.[19]
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	37370	t microalgae/yr	0.6	1.385	731099	731099	0	0.04	Davis et al.[11]		

Appendix G: Infrastructure for 42K STR Scenario 2 Facility

Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost Quote ($)	Year of Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	1	1	6530000	2022	2242912	L	12472733	L	0.6	0.978	17871140	17871140	0	10.32	Negulescu et al.[19]		
P2	Pasteurizer	2	1	SuperPro Designer Built-in Model				85753	L/hr	N/A	N/A	3214000	6428000	33961	3.71	SuperPro Designer ex. file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	111	acre	N/A	1.385	58278047	58278047	132218	33.65	Clippinger & Davis[20]		
P5	Electroflocculation- Electroflocculation Tanks	10	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	9280490	196	5.36	Roles et al.[31]; Lee et al.[42]		
P5	Electroflocculation- Hydrolic Mixer	28	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	4519256		2.61			
P5	Electroflocculation- Settler	10	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	6364690		3.68			
P5	Electroflocculation- Auminum Diode	1	1	2.3	2024	N/A	$/kg	4442	kg	N/A	1.000	10217	10217		0.01	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P6	Bowl Centrifuge	1	1	2242500	2013	463	m3/hr	127	m3/hr	0.6	1.406	1448204	1448204	195	0.84	Davis et al.[21]		Roles et al.[31]
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	70	dry kg/hr	0.6	1.528	1720969	1720969	168	0.99	Humbird et al.[55]		Carvalho et al.[58]
P8	High temperature short time (HTST) sterilizer	2	1	755000	2022	42060	L/hr	37607	L/hr	0.6	0.978	690134	1380268	30037	0.80	teknoice.com[57]	Negulescu et al.[19]	
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	28000	2022	125	L	125	L	0.6	0.978	27372	27372	3	0.02	Negulescu et al.[19]		
P10	STR seed bioreactor	1	1	73000	2022	631	L	631	L	0.6	0.978	71363	71363	13	0.04	Negulescu et al.[19]		
P11	STR seed bioreactor	1	1	193000	2022	3200	L	3201	L	0.6	0.978	188707	188707	68	0.11	Negulescu et al.[19]		
P12	STR seed bioreactor	1	1	512000	2022	16254	L	16256	L	0.6	0.978	500555	500555	347	0.29	Negulescu et al.[19]		
P13	STR seed bioreactor	5	1	517000	2022	16500	L	16502	L	0.6	0.978	505442	2527210	1056	1.46	Negulescu et al.[19]		
P14	STR production bioreactor	10	6	904000	2022	41901	L	41904	L	0.6	0.978	883764	53025840	5828	30.62	Negulescu et al.[19]		
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	990000	2022	83002	L/hr	83506	L/hr	0.6	0.978	971319	971319	300	0.56	Negulescu et al.[19]		SuperPro Designer V10
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	3	acre	0.6	1.385	1470281	1470281	4435	0.85	Clippinger & Davis[20]		
P17	Electroflocculation- Electroflocculation Tanks	0.3	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	278414.7	27	0.16	Roles et al.[31]; Lee et al.[42]		
P17	Electroflocculation- Hydrolic Mixer	2	1	850	2013	N/A	Installed cost/m2	70	m2	N/A	1.406	83665	167330		0.10			
P17	Electroflocculation- Settler	1	1	2630	1998	N/A	N/A	377	m2	0.678	1.924	282573	282573		0.16			
P5	Electroflocculation- Auminum Diode	1	1	2.3	2024	N/A	$/kg	144	kg	N/A	1.000	331	331		0.00	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P18	Bowl Centrifuge	1	1	2242500	2013	463	m3/hr	84	m3/hr	0.6	1.406	1131939	1131939	130	0.65	Davis et al.[21]		Roles et al.[31]
P19	Dead-end filtrator	1	1	40000	2022	10	m2	340	m2	0.6	0.978	324412	324412	0	0.19	Negulescu et al.[19]		
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	2188000	2022	362513	L	372771		0.6	0.978	2175043	2175043	0	1.26	Negulescu et al.[19]		
P21	Blending/storage tank	1	1	2188000	2022	362513	L	372771		0.6	0.978	2175043	2175043	0	1.26	Negulescu et al.[19]		
P22	High temperature short time (HTST) sterilizer	1	1	755000	2022	42060	L/hr	12426	L/hr	0.6	0.978	355114	355114	9	0.21	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39], Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	315	L	0.6	0.978	31189	31189	0	0.02	Negulescu et al.[19]		
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.02	Negulescu et al.[19]		
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	2044	t microalgae/yr	0.6	1.385	127881	127881	0	0.07	Davis et al.[11]		

Appendix H: Infrastructure for 211K STR Scenario 1 Facility

Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost Quote ($)	Year of Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	4	1	6530000	2022	2242912	L	191811294	L	0.6	0.978	92108515	368434060	0	4.97	Negulescu et al.[19]		
P2	Pasteurizer	106	1	SuperPro Designer Built in Model				99528	L/hr	N/A	N/A	15528000	1645968000	2277937	22.21	SuperPro Designer ex. file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	6908	acre	N/A	1.385	3614830240	3614830240	8201134	48.79	Clippinger & Davis[20]		
P5	Electroflocculation-Electroflocculation Tanks	592	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	824107512	12193	11.12	Roles et al.[31]; Lee et al.[42]		
P5	Electroflocculation-Hydrolic Mixer	1693	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	273253586		3.69			
P5	Electroflocculation-Settler	609	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	387609621		5.23			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	275181	kg	N/A	1.000	632916	632916		0.01	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P6	Bowl Centrifuge	17	1	2242500	2013	463	m3/hr	462	m3/hr	0.6	1.406	3149414	53540038	25113	0.72	Davis et al.[21]		Roles et al.[31]
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	325	dry kg/hr	0.6	1.528	4320957	4320957	10425	0.06	Humbird et al.[55]		Carvalho et al.[58]
P8	High temperature short time (HTST) sterilizer	94	1	755000	2022	42060	L/hr	49645	L/hr	0.6	0.978	815266	76635004	1863642	1.03	teknoice.com[57]	Negulescu et al.[19]	
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	27000	2022	124	L	125	L	0.6	0.978	26522	26522	3	0.00	Negulescu et al.[19]		
P10	STR seed bioreactor	1	1	73000	2022	626	L	627	L	0.6	0.978	71431	71431	13	0.00	Negulescu et al.[19]		
P11	STR seed bioreactor	1	1	192000	2022	3177	L	3178	L	0.6	0.978	187730	187730	68	0.00	Negulescu et al.[19]		
P12	STR seed bioreactor	1	1	510000	2022	16133	L	16135	L	0.6	0.978	498600	498600	345	0.01	Negulescu et al.[19]		
P13A	STR seed bioreactor	2	1	892000	2022	40940	L	40943	L	0.6	0.978	872034	1744068	1311	0.02	Negulescu et al.[19]		
P13B	STR seed bioreactor	5	1	1364000	2022	83161	L	82872	L	0.6	0.978	1330628	6653140	5318	0.09	Negulescu et al.[19]		
P14	STR production bioreactor	10	5	2386000	2022	211109	L	210790	L	0.6	0.978	2330375	116518750	45160	1.57	Negulescu et al.[19]		
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	4250000	2022	418187	L/hr	420727	L/hr	0.6	0.978	4169807	4169807	573	0.06	Negulescu et al.[19]		SuperPro Designer V10
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	14	acre	0.6	1.385	7409602	7409602	22353	0.10	Clippinger & Davis[20]		
P17	Electroflocculation-Electroflocculation Tanks	2	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	2784147	134	0.04	Roles et al.[31]; Lee et al.[42]		
P17	Electroflocculation-Hydrolic Mixer	5	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	807010		0.01			
P17	Electroflocculation-Settler	2	1	2630	1998	N/A	N/A	950	m2	0.678	1.924	528407	1056814		0.01			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	682	kg	N/A	1.000	1568	1568		0.00	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P18	Bowl Centrifuge	1	1	2242500	2013	463	m3/hr	422	m3/hr	0.6	1.406	2984402	2984402	652	0.04	Davis et al.[21]		Roles et al.[31]
P19	Dead-end filtrator	1	1	71000	2022	20	m2	1680	m2	0.6	0.978	990776	990776	0	0.01	Negulescu et al.[19]		
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	6530000	2022	2242912	L	1879497		0.6	0.978	5741168	5741168	0	0.08	Negulescu et al.[19]		
P21	Blending/storage tank	1	1	6530000	2022	2242912	L	1879497		0.6	0.978	5741168	5741168	0	0.08	Negulescu et al.[19]		
P22	High temperature short time (HTST) sterilizer	1	1	755000	2022	42060	L/hr	62650	L/hr	0.6	0.978	937404	937404	38	0.01	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39], Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	1565	L	0.6	0.978	81621	81621	0	0.00	Negulescu et al.[19]		
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.00	Negulescu et al.[19]		
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	152170	t microalgae/yr	0.6	1.385	1697708	1697708	0	0.02	Davis et al.[11]		

Appendix I: Infrastructure for 211K STR Scenario 2 Facility

Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost of Quote ($)	Year Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	1	1	6530000	2022	2242912	L	57350104	L	0.6	0.978	44637082	44637082	0	7.15	Negulescu et al.[19]		
P2	Pasteurizer	8	1	SuperPro Designer Built in Model				98574	L/hr	N/A	N/A	3214000	25712000	192549	4.12	SuperPro Designer ex. file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	512	acre	N/A	1.385	268048527	268048527	722714	42.92	Clippinger & Davis[20]		
P5	Electroflocculation-Electroflocculation Tanks	45	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	62643307.5	1074	10.03	Roles et al.[31]; Lee et al.[42]		
P5	Electroflocculation-Hydrolic Mixer	127	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	20498054		3.28			
P5	Electroflocculation-Settler	46	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	29277574		4.69			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	20576	kg	N/A	1.000	47326	47326		0.01	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P6	Bowl Centrifuge	2	1	2242500	2013	463	m3/hr	294	m3/hr	0.6	1.406	2399041	4798082	907	0.77	Davis et al.[21]		Roles et al.[31]
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	325	dry kg/hr	0.6	1.528	4320957	4320957	779	0.69	Humbird et al.[55]		Carvalho et al.[58]
P8	High temperature short time (HTST) sterilizer	7	1	755000	2022	42060	L/hr	49837	L/hr	0.6	0.978	817158	5720106	139319	0.92	teknoice.com[57]	Negulescu et al.[19]	
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	27000	2022	124	L	125	L	0.6	0.978	26522	26522	3	0.00	Negulescu et al.[19]		
P10	STR seed bioreactor	1	1	73000	2022	626	L	627	L	0.6	0.978	71431	71431	13	0.01	Negulescu et al.[19]		
P11	STR seed bioreactor	1	1	192000	2022	3177	L	3178	L	0.6	0.978	187730	187730	68	0.03	Negulescu et al.[19]		
P12	STR seed bioreactor	1	1	510000	2022	16133	L	16135	L	0.6	0.978	498600	498600	345	0.08	Negulescu et al.[19]		
P13A	STR seed bioreactor	2	1	892000	2022	40940	L	40943	L	0.6	0.978	872034	1744068	1311	0.28	Negulescu et al.[19]		
P13B	STR seed bioreactor	5	1	1364000	2022	83161	L	82872	L	0.6	0.978	1330628	6653140	5318	1.07	Negulescu et al.[19]		
P14	STR production bioreactor	10	5	2386000	2022	211109	L	210790	L	0.6	0.978	2330375	116518750	45160	18.66	Negulescu et al.[19]		
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	4250000	2022	418187	L/hr	420727	L/hr	0.6	0.978	4169807	4169807	573	0.67	Negulescu et al.[19]		SuperPro Designer V10
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	14	acre	0.6	1.385	7409602	7409602	22353	1.19	Clippinger & Davis[20]		
P17	Electroflocculation-Electroflocculation Tanks	2	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	2784147	134	0.45	Roles et al.[31]; Lee et al.[42]		
P17	Electroflocculation-Hydrolic Mixer	5	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	807010		0.13			
P17	Electroflocculation-Settler	2	1	2630	1998	N/A	N/A	950	m2	0.678	1.924	528407	1056814		0.17			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	682	kg	N/A	1.000	1568	1568		0.00	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P18	Bowl Centrifuge	1	1	2242500	2013	463	m3/hr	422	m3/hr	0.6	1.406	2984402	2984402	652	0.48	Davis et al.[21]		Roles et al.[31]
P19	Dead-end filtrator	1	1	71000	2022	20	m2	1680	m2	0.6	0.978	990776	990776	0	0.16	Negulescu et al.[19]		
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	6530000	2022	2242912	L	1879497		0.6	0.978	5741168	5741168	0	0.92	Negulescu et al.[19]		
P21	Blending/storage tank	1	1	6530000	2022	2242912	L	1879497		0.6	0.978	5741168	5741168	0	0.92	Negulescu et al.[19]		
P22	High temperature short time (HTST) sterilizer	1	1	755000	2022	42060	L/hr	62650	L/hr	0.6	0.978	937404	937404	38	0.15	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39], Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	1565	L	0.6	0.978	81621	81621	0	0.01	Negulescu et al.[19]		
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.01	Negulescu et al.[19]		
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	10743	t microalgae/yr	0.6	1.385	346058	346058	0	0.06	Davis et al.[11]		

Appendix J: Infrastructure for 262K ALR Scenario 1 Facility

Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost Quote ($)	Year of Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	5	1	6530000	2022	2242912	L	189022012	L	0.6	0.978	91302507	456512535	0	5.07	Negulescu et al.[19]		
P2	Pasteurizer	130	1	SuperPro Designer Built in Model				98951	L/hr	N/A	N/A	15528000	2018640000	2817790	22.40	SuperPro Designer ex. file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	8495	acre	N/A	1.385	4445031429	4445031429	10084650	49.33	Clippinger & Davis[20]		
P5	Electroflocculation-Electroflocculation Tanks	739	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	1028742317	15118	11.42	Roles et al.[31]; Lee et al.[42]		
P5	Electroflocculation-Hydrolic Mixer	2113	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	341042426		3.79			
P5	Electroflocculation-Settler	761	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	484352909		5.38			
P5	Electroflocculation-Aluminum Diode	1	1	2.3	2024	N/A	$/kg	343353	kg	N/A	1.000	789713	789713		0.01	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P6	Bowl Centrifuge	22	1	2242500	2013	463	m3/hr	445	m3/hr	0.6	1.406	3077252	67699544	31138	0.75	Davis et al.[21]		Roles et al.[31]
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	383	dry kg/hr	0.6	1.528	4768256	4768256	12926	0.05	Humbird et al.[55]		Carvalho et al.[58]
P8	High temperature short time (HTST) sterilizer	116	1	755000	2022	42060	L/hr	49881	L/hr	0.6	0.978	817587	94840092	2310733	1.05	teknoice.com[57]	Negulescu et al.[19]	
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	31000	2022	154	L	154	L	0.6	0.978	30305	30305	3	0.00	Negulescu et al.[19]		
P10	STR seed bioreactor	1	1	83000	2022	776	L	776	L	0.6	0.978	81139	81139	17	0.00	Negulescu et al.[19]		
P11	STR seed bioreactor	1	1	219000	2022	3940	L	3939	L	0.6	0.978	214056	214056			Negulescu et al.[19]		
P12	STR seed bioreactor	1	1	580000	2022	20003	L	20004	L	0.6	0.978	567010	567010	84	0.00	Negulescu et al.[19]		
P13A	STR seed bioreactor	2	1	1014000	2022	50761	L	50765	L	0.6	0.978	991306	1982612	1626	0.02	Negulescu et al.[19]		
P13B	STR seed bioreactor	6	1	1391000	2022	85926	L	85628	L	0.6	0.978	1356973	8141838	6411	0.09	Negulescu et al.[19]		
P14	ALR production bioreactor	10	5	313000	2022	261755	L	260847	L	0.6	0.978	305343	15267150	5561	0.17	Negulescu et al.[19]		
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	5226000	2022	518515	L/hr	518515	L/hr	0.6	0.978	5108799	5108799	625	0.06	Negulescu et al.[19]		SuperPro Designer V10
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	18	acre	0.6	1.385	9187101	9187101	27715	0.10	Clippinger & Davis[20]		
P17	Electroflocculation-Electroflocculation Tanks	2	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	2784147	196	0.03	Roles et al.[31]; Lee et al.[42]		
P17	Electroflocculation-Hydrolic Mixer	7	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	1129814		0.01			
P17	Electroflocculation-Settler	2	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	1272938		0.01			
P5	Electroflocculation-Aluminum Diode	1	1	2.3	2024	N/A	$/kg	992	kg	N/A	1.000	2281	2281		0.00	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P18	Bowl Centrifuge	2	1	2242500	2013	463	m3/hr	262	m3/hr	0.6	1.406	2242792	4485584	796	0.05	Davis et al.[21]		Roles et al.[31]
P19	Dead-end filtrator	1	1	71000	2022	20	m2	2090	m2	0.6	0.978	1129477	1129477	0	0.01	Negulescu et al.[19]		
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	6530000	2022	2242912	L	2328653		0.6	0.978	6528871	6528871	0	0.07	Negulescu et al.[19]		
P21	Blending/storage tank	1	1	6530000	2022	2242912	L	2328653		0.6	0.978	6528871	6528871	0	0.07	Negulescu et al.[19]		
P22	High temperature short time (HTST) sterilizer	1	1	755000	2022	42060	L/hr	77622	L/hr	0.6	0.978	1066019	1066019	34	0.01	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39]; Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	1925	L	0.6	0.978	92401	92401	0	0.00	Negulescu et al.[19]		
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.00	Negulescu et al.[19]		
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	187118	t microalgae/yr	0.6	1.385	1921919	1921919	0	0.02	Davis et al.[11]		

Appendix K: Infrastructure for 262K ALR Scenario 2 Facility

Equipment Unit	Equipment Type	Number of Parallel Equipment	Stagger Factor	Single Purchase Cost Quote ($)	Year of Quote	Quoted Capacity	Unit	Capacity	Unit	Capacity Scaling Exponent	Year Scaling Factor	Single Scaled Purchase Cost ($)	Total Purchase Cost of all Equipment ($)	Electricity Consumption (kWh/batch)	% of Total PEC	Capacity Source	Cost Estimate Source	Electricity Consumption Source
A100: Biomass Production																		
P1	Blending/storage tank	1	1	6530000	2022	2242912	L	68175310	L	0.6	0.978	49516683	49516683	0	7.36	Negulescu et al.[19]		
P2	Pasteurizer	10	1	SuperPro Designer Built in Model				93744	L/hr	N/A	N/A	8892000	88920000	192549	13.22	SuperPro Designer ex. file "Omega3Oils_v14a"		Ward et al.[51]
P3	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	609	acre	N/A	1.385	318552118	318552118	421725	47.37	Clippinger & Davis[20]		
P5	Electroflocculation-Electroflocculation Tanks	53	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	73779895.5	1074	10.97	Roles et al.[31]; Lee et al.[42]		
P5	Electroflocculation-Hydrolic Mixer	150	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	24210300		3.60			
P5	Electroflocculation-Settler	54	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	34369326		5.11			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	24246	kg	N/A	1.000	55766	55766		0.01	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P6	Bowl Centrifuge	2	1	2242500	2013	463	m3/hr	346	m3/hr	0.6	1.406	2647395	5294790	1053	0.79	Davis et al.[21]		Roles et al.[31]
P7	Hydrolysis	1	1	19812400	2009	8333	dry kg/hr	383	dry kg/hr	0.6	1.528	4768256	4768256	918	0.71	Humbird et al.[55]		Carvalho et al.[58]
P8	HTST sterilizer	9	1	755000	2022	42060	L/hr	45677	L/hr	0.6	0.978	775519	6979671	164172	1.04	teknoice.com[57]	Negulescu et al.[19]	
A200: Cultivated Meat Culture																		
P9	STR seed bioreactor	1	1	31000	2022	154	L	154	L	0.6	0.978	30305	30305	3	0.00	Negulescu et al.[19]		
P10	STR seed bioreactor	1	1	83000	2022	776	L	776	L	0.6	0.978	81139	81139	17	0.01	Negulescu et al.[19]		
P11	STR seed bioreactor	1	1	219000	2022	3940	L	3939	L	0.6	0.978	214056	214056	84	0.03	Negulescu et al.[19]		
P12	STR seed bioreactor	1	1	580000	2022	20003	L	20004	L	0.6	0.978	567010	567010	427	0.08	Negulescu et al.[19]		
P13A	STR seed bioreactor	2	1	1014000	2022	50761	L	50765	L	0.6	0.978	991306	1982612	1626	0.29	Negulescu et al.[19]		
P13B	STR seed bioreactor	6	1	1391000	2022	85926	L	85628	L	0.6	0.978	1356973	8141838	6411	1.21	Negulescu et al.[19]		
P14	ALR production bioreactor	10	5	313000	2022	261755	L	260847	L	0.6	0.978	305343	15267150	5561	2.27	Negulescu et al.[19]		
A300: Medium Recycling																		
P15	Decanter centrifuge	1	1	5226000	2022	518515	L/hr	518515	L/hr	0.6	0.978	5108799	5108799	625	0.76	Negulescu et al.[19]		SuperPro Designer V10
P16	Helical tubular photobioreactor	1	1	377892	2014	N/A	Installed cost/acre	18	acre	0.6	1.385	9187101	9187101	27715	1.37	Clippinger & Davis[20]		
P17	Electroflocculation-Electroflocculation Tanks	2	1	660000	2013	25.92	m3	26	m3	0.6	1.406	928049	2784147	196	0.41	Roles et al.[31]; Lee et al.[42]		
P17	Electroflocculation-Hydrolic Mixer	7	1	850	2013	N/A	Installed cost/m2	135	m2	N/A	1.406	161402	1129814		0.17			
P17	Electroflocculation-Settler	2	1	2630	1998	N/A	N/A	1250	m2	0.678	1.924	636469	1272938		0.19			
P5	Electroflocculation-Auminum Diode	1	1	2.3	2024	N/A	$/kg	992	kg	N/A	1.000	2281	2281		0.00	statista.com[56]		Roles et al.[31]; Lee et al.[42]
P18	Bowl Centrifuge	2	1	2242500	2013	463	m3/hr	262	m3/hr	0.6	1.406	2242792	4485584	796	0.67	Davis et al.[21]		Roles et al.[31]
P19	Dead-end filtrator	1	1	71000	2022	20	m2	2090	m2	0.6	0.978	1129477	1129477	0	0.17	Negulescu et al.[19]		
A400: Medium Replenishment and Storage																		
P20	Blending/storage tank	1	1	6530000	2022	2242912	L	2328653		0.6	0.978	6528871	6528871	0	0.97	Negulescu et al.[19]		
P21	Blending/storage tank	1	1	6530000	2022	2242912	L	2328653		0.6	0.978	6528871	6528871	0	0.97	Negulescu et al.[19]		
P22	HTST sterilizer	1	1	755000	2022	42060	L/hr	77622	L/hr	0.6	0.978	1066019	1066019	34	0.16	teknoice.com[57]	Negulescu et al.[19]	Pasteurization estimate method: Singh et al.[39], Neoklaus et al.[40]
P23	Blending/storage tank	1	1	212000	2022	7398	L	1925	L	0.6	0.978	92401	92401	0	0.01	Negulescu et al.[19]		
P24	Dead-end filtrator	1	1	40000	2022	10	m2	10	m2	0.6	0.978	39103	39103	0	0.01	Negulescu et al.[19]		
A500: Other																		
CO2 Storage Sphere		1	1	1400800	2014	190000	t microalgae/yr	12771	t microalgae/yr	0.6	1.385	383888	383888	0	0.06	Davis et al.[11]		

Appendix L: MAB Medium Pasteurization Energy Demand

Energy efficiency for UV-treated products is commonly measured by electrical energy per order (E_{EO}), representing the total energy (kWh) needed to reduce contaminants by one order of magnitude per m^3 of fluid. EEO is calculated using Equation 4:

$$E_{EO} = P_{UV}/[F \times (\frac{C_0}{C_t}) \qquad \text{eq (4)}$$

Where P_{uv} is the power of the UV lamp (kW), F is the volumetric flow rate (m^3/h), C_0 is the initial contaminant concentration (CFU/mL), and C_t is the final contaminant concentration (CFU/mL)[51].

Ward et al. [51] reported an E_{EO} of 0.172 kWh/m^3 log for the inactivation of Escherichia coli from milk. This value, along with Negulescu et al.'s[19] estimate of an appropriate contamination reduction ratio, is used to calculate the energy consumption of the UV-C pasteurizers used to sterilize the microalgae biomass production system media. An example for the 40K STR facility is supplied below, assuming 1 contamination event per 50 years, in which 6150 batches take place:

$C_0 = 10^6$ spores/mL

C_t = (1 spore / 6150 batches) / (138166610 L / batch) = $1.18 * 10^{-15}$

$\log(C_0/C_t)$ = 20.93 log

Total energy use = 0.172 kWh/m^3/log * (138166.61 m^3 / batch) * 20.93 log = 497377 kWh/batch

Appendix M: MAB Acid Hydrolysis and HTST Sterilization Energy Demand

It is difficult to predict the energy demand for bulk acid hydrolysis based on the little available literature. There are values available for the hydrolysis of plant cell matter, such as 0.22 kWh per 100 g of lignocellulosic biomass, but these would result in a significant overestimate for

MAB hydrolysis because HTST microalgae lack a cell wall.[52] One source reviews a number of microalgae cell disruption methods and estimates their energy usage, describing methods using 6-0.23 kWh/kg of dry weight as “energy intensive.” Hydrolysis is listed as having “low” energy demand without a specific value. Thus, a value of 0.1 kWh/kg of dry weight will be chosen as an estimate for acid hydrolysis in this study. Microalgae dry weight is assumed to be 10% of total wet weight[53].

HTST energy demand is approximated using data reported by conventional boiler pasteurizers. A pasteurizer with a capacity of 20,000 L/hr was found to process 111.78 kg of milk a day while consuming 3.73 kW of electricity[39,40]. This translates to ~0.799 kWh/kg of solution pasteurized. This is also used to estimate the energy demand of the HTST sterilization of fresh animal cell culture medium before entry into the CCC system.

Appendix N: Raw materials and costs

*Example values from 42K STR facility scenario 1 with no medium recycling. Assuming maximal recycling can increase price per weight by an order of magnitude due to decreased industry demand.

Total Raw Material Demand, Recycling Incorporated				
Raw Material	Cost per Mass	Unit	Notes	Cost Source
Utilities/Other				
Labor	57.5	$/labor-hr		Negulescu et al.[19]
Carbon Dioxide	0.045	$/kg	Projected in 2016 for a 2022 facility.	Davis et al.[11]
Air	0.0			Negulescu et al.[19]
Purified Water	0.055	$/L		Negulescu et al.[19]

Potable Water	0.0004	$/L		Negulescu et al.[19]
Filter Cartridge	1000	$/unit		SuperPro Designer
Electricity	0.100	$/kWh		Negulescu et al.[19]
Steam	12	$/MT		Negulescu et al.[19]
Cooling Water	0.05	$/MT		Negulescu et al.[19]
Chilled Water	0.40	$/MT		Negulescu et al.[19]
Inorganic Salt Solution (Basal Mammalian Media)				
Water	0.055	$/L		Negulescu et al.[19]
Sodium bicarbonate	0.292	$/kg		Alibaba.com
Sodium chloride	0.055	$/kg		Made-in-china.com
Artificial Seawater (Basal Algae Medium)				
Potable Water	0.000	$/L		Negulescu et al.[19]
Calcium chloride dihydrate	0.176	$/kg		Alibaba.com
Magnesium dichloride hexahydrate	0.083	$/kg		Alibaba.com
Sodium chloride	0.055	$/kg		Made-in-china.com
Sodium sulfate	0.072	$/kg		Alibaba.com
Algae Medium Supplement				
Ammonia	0.921	$/kg	Average of USDA data from 2011-2014 (most recent year available), indexed to 2024	Clippinger & Davis[20]; USDA[54]
Dipotassium phosphate	0.752	$/kg		
*Growth Factors**				
FGF-2	7402001	$/kg	Price volume correlation for industry size of 100,000,000 kg CM/year	Humbird[22]
Insulin	5861	$/kg		
NRG1	214857130	$/kg		
TGF-B	214857130	$/kg		

Transferrin	5861	$/kg		
Rapeseed	3.21	$/kg		Stout et al.[23]
Electroflocculation				
Aluminum (MAB)	2.705	$/kg		Roles et al.[31]
Aluminum (MAMR)	2.705	$/kg		Roles et al.[31]
Hydrolysis				
32% HCL	0.110	$/kg		Made-in-china.com
Water	0.055	$/L		Negulescu et al.[19]
Neutralizer				
NaOH	0.331	$/kg		Made-in-china.com
Clean in Place				
Citric Acid	0.180			Alibaba.com
Water	0.055	$/L		Negulescu et al.[19]
NaOH	0.331	$/kg		Made-in-china.com
Water	0.055	$/L		Negulescu et al.[19]
70% Hypochlorite	0.882	$/kg		Made-in-china.com
Potable Water	0.000	$/L		Negulescu et al.[19]

Appendix O: MAMR inoculation ratio calculations

Given that a new batch of spent medium enters the MAMR system every 59.68 hours, the maximum allowable microalgae culture time in the MAMR system while allowing for a CIP procedure and liquid transfer is ~57 hours. Using the *C. littorale* doubling time of 35.6 hours[1], the following growth rate formula is used to determine the maximum inoculation ratio is determined by the growth rate formula presented in Equation (5):

$$\log(inoculation\ ratio) = \frac{\Delta t \times log\ (2)}{T_d} \qquad \text{eq (5)}$$

Where Δt is the maximum allowable microalgae culture time and T_d is the doubling time. The given values result in an inoculation ratio of ~33%.

Appendix P: Medium recycling scheduling

The minimum volume of fresh CM medium needed to be created at a time and the maximum times that volume can be reused is limited by the scheduling design of the CM production and medium recycling systems. The MAMR system was designed to recycle a batch of medium in ~2.5 days, equal to the cycle time in the CM production line. Thus, for example, in the 42K STR system (with a batch time of 24.01 days), medium batch 1 starts fresh at time 0, enters the MAMR system at 24.01 days, and is first available for reuse at 26.94 days (24.01 + 2.5), or just before medium batch 12 is initiated. In that time, 11 total batches have been initiated and each can only be supplied with fresh medium, as recycled medium is not yet available. Batches 12-22 represent the first reuse of the total medium, batches 23-33 represent the second reuse of total medium, etc., until all 123 batches in the year have been completed. The total set of 11 initial batches can be used a maximum of 11 times, with batches 1 and 2 reused an additional 12th time, to fulfill 123 annual batches. The 211K STR and 262K ALR facilities have a slightly longer batch time of 26.30 days and thus have 12 batches circulating at once that can be used a maximum of 11 times in a year (batches 1-3 used a total of 11 times and batches 4-12 used 10 times).