

# Microalgae in lab-grown meat production

ARTURO NICKOLAY ROJAS-TAVARA<sup>1</sup>, ALBERTO JESUS DONAYRE-TORRES<sup>1,2\*</sup>

<sup>1</sup>Department of Bioengineering, University of Engineering and Technology (UTEC), Lima, Peru

<sup>2</sup>CentroBIO Research Center, University of Engineering and Technology (UTEC), Lima, Peru

\*Corresponding author: [adonayre@utec.edu.pe](mailto:adonayre@utec.edu.pe)

**Citation:** Rojas-Tavara A.N., Donayre-Torres A.J. (2023): Microalgae in lab-grown meat production. Czech J. Food Sci., 41: 406–418.

**Abstract:** Reports have shown that meat production operations today contribute to the climate crisis, facilitating the occurrence of infectious diseases, and contributing to environmental pollution. Consequently, the public demands alternatives to traditional meat, such as *in vitro* manufactured meat. Several authors have suggested that improvements should be made in the manufacturing of cell-cultured meat to make a more sustainable and scalable process. They recently proposed using microalgae as a sustainable system to produce important nutrients such as oxygen from cellular waste molecules of animal cultures such as ammonia and carbon dioxide. In this review, we discuss recent advances of different microalgae applications in the production of lab-grown meat, with special emphasis on their use as a replacement for fetal bovine serum (FBS) or culture media, as well as its applicability as a source of cell oxygenation and waste upcycling to extend the life of animal cell cultures. Also, we discuss the implementation and limitations of these algae systems in large-scale *in vitro* meat manufacturing.

**Keywords:** *chlorella vulgaris*; co-culture; fetal bovine serum replacement; *in vitro* meat

Livestock production consumes almost 10% of the world's water source and 70% of the agricultural land area (Steinfeld et al. 2006). While consumers are open to reducing meat consumption, technology offers meat produced in a laboratory and without using animals from farms (European Commission 2013; Vanhonacker et al. 2013; Graça et al. 2015; Sanchez-

Sabate et al. 2019). Specifically, it involves the production of meat in a culture vessel. The relative increased demand for this technology estimates by 2040, 35% of market meat could have a laboratory origin (Gerhart et al. 2020; Szejda et al. 2021).

It is assumed that producing 1 000 kg of lab meat would require only 1% of the land and 5% of the wa-

<https://doi.org/10.17221/69/2023-CJFS>

ter used to produce the same amount of conventional meat (Tuomisto and Teixeira de Mattos 2011). The key factor should be the price margins. It is crucial to reduce such margins, as Garrison et al. (2022) estimated that a large-scale production of *in vitro* meat would have a sale price for retailers of USD 63 per kg, in contrast with a USD 6.17 per kg price for lean beef in the U.S. Market (2022).

Recent estimations report that a million beef burgers could be produced from a single cow muscle biopsy through this system (Fang et al. 2017). An equivalent amount of the latter product would require slaughtering 500 cattle (Sans et al. 2017). These days, the development of *in vitro* propagation techniques makes a possible synthesis of tissue in lab conditions using animal cell culture systems. However, the fabrication of *in vitro* meat by culturing stem cells at a high scale still requires improvements. Lab meat production must overcome the following challenges before becoming available for consumers: (i) The system is typically provided with fetal bovine serum (FBS), an animal-derived component increasing the costs of cultures. (ii) *In vitro*-produced meat tissues typically experiment oxygen shortage issues that complicate the ability to create larger and multi-layered tissues in one vessel. (iii) The system produces continuous and numerous waste components from

culture medium that limit cell growth and increase fabrication costs.

In this review, we will discuss how microalgae can contribute to mitigate these problems. Microalgae are unicellular species of autotrophic photosynthetic microorganisms with multiple applications in the food, cosmetic, and pharmaceutical industries (Sathasivam et al. 2019, Pang et al. 2020). We emphasise the main applications of microalgae in the production of lab-grown meat, specifically for using microalgae extracts as a replacement for FBS or basal defined medium. Also, we discuss the co-culture of microalgae with mammalian cells to provide oxygen promoting larger tissues. The co-culture of microalgae with mammalian cells aims to recycle toxic compounds extending the lifespan of cultures. Finally, we argued the feasibility of implementing high-scale production of lab-grown meat *in vitro* and perspectives for future research.

## APPLICATIONS OF MICROALGAE IN CULTURED MEAT MANUFACTURING

The current process of *in vitro* meat production and the incorporation of microalgae in each manufacturing step is shown in Figure 1. The production of lab meat starts with the biopsy from bovine muscle for the isolation of muscle-derived stem cells (MDSCs); or mesen-

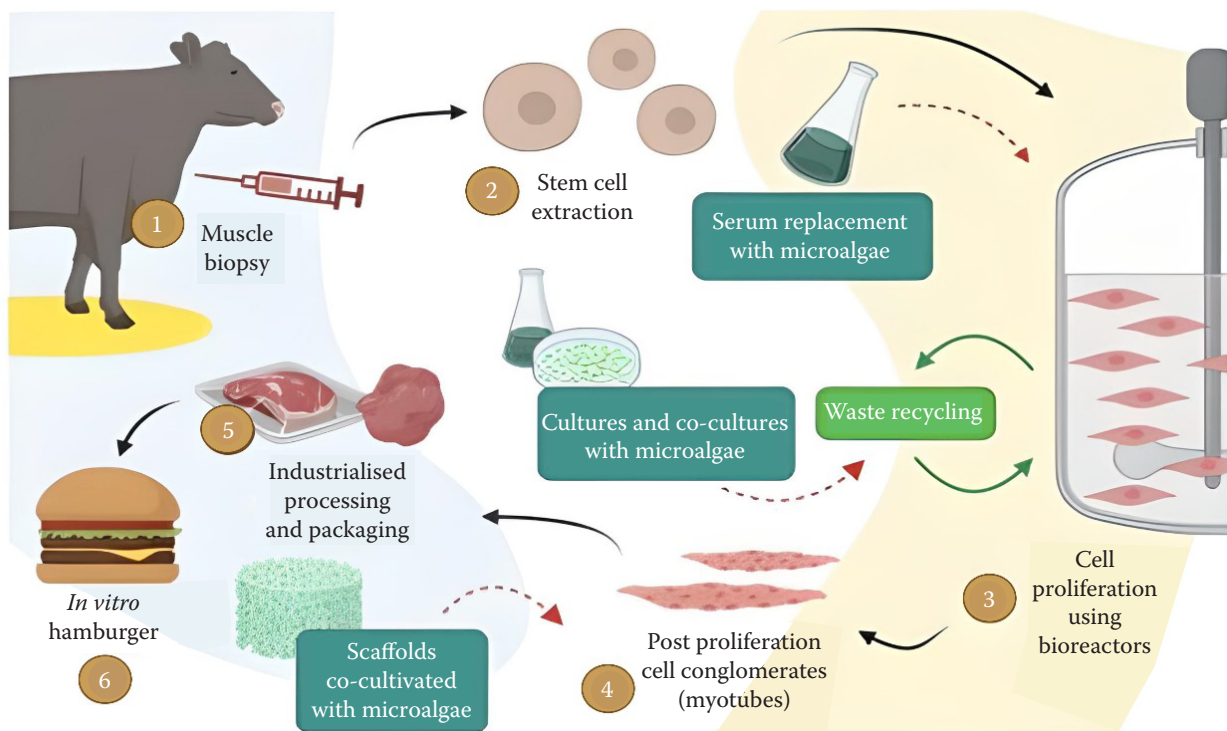


Figure 1. Production of lab-grown meat assisted by diverse applications of microalgae

chymal cell lines and adipogenic progenitors to create *in vitro* meat (steps 1 and 2) (Kumar et al. 2021; Reiss et al. 2021). These cells are cultured in a bioreactor to produce a biomass of 50 billion cells per kilogram of meat approximately (step 3) (Post 2014). At this stage, it is possible to replace FBS or the basal medium with microalgae extracts (Song et al. 2012; Okamoto et al. 2019; Ng et al. 2020; Jeong et al. 2021). Furthermore, microalgae can be introduced into the system to mitigate toxic compounds and to provide oxygen (Haraguchi et al. 2017; Haraguchi and Shimizu 2021a, b; Marko et al. 2022). Later cells are transferred to scaffolds, which are built using gelatine or collagen by means of 3D printing or solvent casting fabrication (Kumar et al. 2022; Lee et al. 2022). The scaffold mimics the conditions provided by the extracellular matrix of support and nutrition, promoting cell maturation and proliferation allowing cell differentiation in the required morphology, creating a multilayer tissue originating the lab meat (Hopfner et al. 2014). These scaffolds can also enhance oxygenation of the cells through microalgae co-culture (Hopfner et al. 2014; Lode et al. 2015; Maharjan et al. 2021). During the final stages of the process (steps 5 and 6), tissue obtained is composed by muscle cells infused with flavourings and vitamins and undergoes additional treatments before being packaged and prepared for customer presentation (Post 2014).

#### NEED FOR AN ALTERNATIVE TO FETAL BOVINE SERUM (FBS)

Animal cells cultivated *in vitro* are nourished with a culture medium, which are formulations to ensure sustained proliferation. Nutritional requirements of culture mammalian cells are considered in culture medium providing a formulated variation in composition and supplements (O'Neill et al. 2020). Typically, nutrients are composed of glucose, amino acids, vitamins, inorganic salts, and growth factors (Yao and Asayama 2017).

The FBS is an additive to the animal culture medium at 2–10% concentrations. It provides nutrients and hormones for *in vitro* cultured cells, and also factors for protecting cells from endotoxins and pH alterations (Puri et al. 2015). FBS is obtained from the blood of unborn fetuses in the final months of bovine pregnancy. Around 800 000 L of FBS are consumed annually for *in vitro* cell culture. Consequently, two million unborn calves are used to fulfil FBS demand (Jochems et al. 2002). The production of one kilogram of *in vit-*

*ro* cultivated meat involves the use of approximately 50 L of FBS (Brindley et al. 2012; Post 2014). Due to this fact, the process of lab meat production remains contradictory as potential consumers demand a product free of animal suffering in the manufacturing process (Jochems et al. 2002; Van der Valk and Gstraunthaler 2017; Sanchez-Sabate et al. 2019). Furthermore, the high demand constantly creates considerable fluctuations in the prices of FBS. In 2015, the cost of a half-litre of FBS increased from EUR 80 to EUR 1 200 (Van der Valk and Gstraunthaler 2017). In addition, batches of FBS exhibit slight variations in the concentration of components, causing alterations in the quality of *in vitro*-produced muscle, and modifying characteristics such as the speed of muscle contraction (Jochems et al. 2002, Khodabukus and Baar 2014; Usta et al. 2014; Stephens et al. 2018; Post et al. 2020).

Microalgae offer a good alternative as an FBS substitute due to their high protein content and nutritional values. Some efforts have demonstrated the use of microalgae in promoting the viability and proliferation of mammalian cell lines. The mesenchymal stem cells and C2C12 cell line are proven to mature in skeletal muscle and cardiac muscle cells (McMahon et al. 1994; Okamoto et al. 2019; Ng et al. 2020). Even though, microalgae have also been used to replace nutrients from basal defined media in these mammalian cell cultures (Okamoto et al. 2019).

#### COMPATIBILITY OF MICROALGAE EXTRACTS IN MAMMALIAN CULTURE MEDIA

Using microalgae extracts requires the isolation of nutrients by means of the biomass extraction process. The two main procedures are: acid/alkaline hydrolysis (Sekine et al. 2011); and hot water (100 °C) and high-pressure extraction (Song et al. 2012; Okamoto et al. 2019; Ng et al. 2020; Jeong et al. 2021). These required a high biomass from microalgae, preferably lyophilised and diluted in distilled water to a desired concentration.

The extraction in hot water comprises lyophilisation of microalgae biomass followed by boiling for a few minutes and immediately freezing. Later, centrifugation removes debris, and supernatant containing bioactive compounds is collected. The extraction in hot water is the most commonly used method because it is economically affordable (Song et al. 2012; Ng et al. 2020; Jeong et al. 2021). In contrast, sulfuric acid and high temperature are used in acid/alkaline

<https://doi.org/10.17221/69/2023-CJFS>

hydrolysis to extract glucose and amino acids from biomass. The chemical analysis of hydrolysis extracts showed the absence of glutamine and the presence of glutamic acid instead due to the hydrolytic conditions that facilitate the hydrolysis process of glutamine into glutamic acid and other constituent amino acids. At a concentration of 4 mM, glutamic acid could replace glutamine in mammalian cell cultures, although higher concentrations are needed to maintain cell viability in contrast to glutamine (Okamoto et al. 2019). A standardised system is yet to be implemented, including microalgae extracts in the formulation, establishing an exact concentration in reproducible studies for replacement of FBS.

The previously described manufacturing processes of microalgae extracts have shown high levels of protein and carbohydrates in composition. Independent studies reported a rate of 61–67% protein, 19–27% carbohydrates, and 4–5% ash. These analyses were conducted after acid/alkaline hydrolysis in hot water using *Chlorella vulgaris* biomass (Song et al. 2012; Ng et al. 2020). A similar report, compared the composition of commercial FBS and the extract from the cyanobacteria

*Spirulina maxima*, showing analogous concentrations of protein and carbohydrates. The commercial FBS contains 85% protein and 8.08% carbohydrates, whereas *Spirulina maxima* extract, 79.2% and 13.2%, respectively (Jeong et al. 2021). Table 1 summarises the nutrient composition of extracts in different reports.

As depicted in Table 1, the fabrication method is the procedure to extract nutrients from microalgae species. *C. vulgaris* and *S. maxima* (cyanobacteria) were the main sources for nutrient extraction assays. The concentration of crude protein, carbohydrates, lipids, and ash is shown.

Animal culture supplements must meet commercial certifications. Therefore, some efforts have focused on developing a chemically defined medium. A precise composition medium for animal cell culture could allow a rapid transition to marketing. However, up to date, these formulations or extracts have only been able to replace FBS in a limited number of mammalian cell lines. Some of such formulations are reported in databases like the FCS-free database (<https://fcs-free.org/fcs-database>), where there is no report of a microalgae-based culture media formulation. Ex-

Table 1. Composition of microalgae extracts and fabrication methods: A comparison of fetal bovine serum (FBS) and microalgae for medium replacement in animal cell cultures

Fabrication method	Species	Extract composition (%)				Observations	Reference
		protein	carbohydrates	lipids	ash		
Acid/alkaline hydrolysis	<i>Chlorella vulgaris</i>	ND	ND	ND	ND	The authors evaluated other 5 microalgae species but <i>C. vulgaris</i> had better amino acid yield. Glutamine was not present in microalgae extract, but substituted by Glutamic acid. Glutamic acid could effectively replace Glutamine in cell culture, but at higher concentrations.	Okamoto et al. 2019
Hot water extraction	<i>Chlorella vulgaris</i>	67.10	27.40	0.00	5.70	ND	Yao et al. 2020
Hot water extraction	<i>Spirulina maxima</i>	79.20	13.20	4.40	ND	<i>Spirulina maxima</i> is a photosynthetic cyanobacteria. Extracts differed greatly in potassium (0.15% for <i>S. maxima</i> and 0.01% for FBS), and phosphorus (1.31% for <i>S. maxima</i> and 0.03% for FBS).	Jeong et al. 2021
	FBS	85.00	8.08	1.75	ND		
Hot water extraction	<i>Chlorella vulgaris</i>	64.58	20.17	0.00	14.98	ND	Song et al. 2012

ND – values were not determined



tracts from microalgae are an excellent alternative for specific mammalian cell lines, after a comprehensive case-by-case evaluation considering specific nutritional requirements. We review several reports on this topic in the next sections.

### MICROALGAE-BASED SUPPLEMENTS PARTIALLY REPLACE FBS AND BASAL MEDIUM IN MAMMALIAN CULTURES

The impact of microalgae extracts as a replacement of culture medium nutrients or FBS is determined mainly by evaluating cell viability. The number of viable living cells (capable of cell division) is evaluated estimating the percentage of these relative to the total cell population. The calculation is expressed in percentage or relative cell viability seeking 100% or value '1', respectively. Cell viability is rapidly assessed by colourimetric assays using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) or 2,3-bis(2-methoxy-4-ni-

tro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) as chemical agents. Only viable cells can reduce formazan salt producing a purple colour in samples. Viability tests are commonly conducted 24 h following the microalgae extract application or after starvation or fasting periods. In the latter, cells are grown with the regular medium with components to be replaced (FBS of culture medium) for a period of time. Subsequently, cultures are transferred to a medium without supplements using microalgae extracts in a certain proportion instead. At this point, daily cell viability testing is conducted (Okamoto et al. 2019; Ng et al. 2020). Microalgae extract replacements are evaluated to yield a concentration in which cell viability is maintained at 100% across the study. Extracts using photosynthetic cells such as microalgae and cyanobacteria are preferred for FBS or culture media replacements. To date, microalgae extracts cannot fully replace FBS or medium, only a percentage is tolerated by animal cells *in vitro*. Table 2 comprehensively reviews efforts at-

Table 2. Summary of studies showing the effects of using cellular extracts from microalgae and cyanobacteria as a replacement for fetal bovine serum (FBS) in mammalian cultures *in vitro*

Microalgae species	Cell viability	Mammalian cell line	Reference
<i>Chlorococcum littorale</i> <i>Stichococcus</i> sp. <i>Chlorella vulgaris</i> <i>Euglena gracilis</i> <i>Spirulina subsalsa</i> <i>Arthrospira platensis</i>	A 10% of microalgae extract was used to obtain cell viability of 1.4–1.8 in 90% DMEM medium without glucose and aminoacids with <i>A. platensis</i> and <i>C. vulgaris</i> . The highest was obtained using <i>C. littorale</i> : cell viability increase of 3.8 in 90% DMEM with no glucose and 10% microalgae extract.	C2C12 mouse myoblasts	Okamoto et al. 2019
<i>Chlorella vulgaris</i>	A 70% recovery in cell viability using 0.001 g·L <sup>-1</sup> of microalgae extract with 0% serum, compared with 400% microalgae extract and 5–10% FBS serum. Cell viability is affected using levels greater than 0.25–1 g·L <sup>-1</sup> of microalgae extract, results are relative to the cell line.	Chinese hamster ovary cells mesenchymal stem cells differentiated into HDF, HaCaT, hMSC, and ADSC	Ng et al. 2020
<i>Spirulina maxima</i>	Cell viability is greater than 100% (optimal) for three days. A 50% replacement of the volume of FBS in the medium, was achieved.	human lung cancer cell lines H460	Jeong et al. 2021
<i>Chlorella vulgaris</i>	Increase in cell viability to 124, 135.4, and 155% using concentrations of 12.5, 25, and 50 µg·mL <sup>-1</sup> from microalgae extract for a 24 h treatment. Cell viability does not decrease in the period of culture.	rat intestinal epithelial cell line IEC-6 human hepatic cell line WRL68	Song et al. 2012

A list of microalgae species used for FBS replacement studies is shown; cell viability is represented in percentage regarding viability of the mammalian cells in culture; the mammalian cell lines show variable response according to the extracts; DMEM – Dulbecco's Modified Eagle Medium; HDF – human dermal fibroblast; HaCaT – human epidermal keratinocytes; hMSC – human mesenchymal stem cells; ADSC – adipose-derived stem cells

<https://doi.org/10.17221/69/2023-CJFS>

tempting culture medium and FBS replacements using photosynthetic cell extracts. A summary of cell extraction procedures using microalgae species for mammalian cell line medium supplements are presented. In all cases, a successful assay is determined by cell viability of 100% followed by colourimetric staining; most reports aim for an FBS replacement.

Several studies showed that microalgae can replace only 20% of the volume of FBS or culture medium. Increasing the amount of extract affects cell viability due to toxicity (Song et al. 2012; Okamoto et al. 2019; Ng et al. 2020; Jeong et al. 2021). Okamoto and colleagues (2019) showed the performance of C2C12 mouse myoblast exposed to microalgae extracts such as *C. vulgaris*, *Chlorococcum littorale*, *Stichococcus* sp., *Euglena gracilis*, cyanobacteria *Spirulina subsalsa* and *Arthrospira platensis*. These experiments aimed a replacement for amino acids and glucose from the basal medium. The C2C12 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) basal medium in the absence of glucose and amino acids; supplemented with microalgae extracts instead. The reported relative cell viability was 1.4–1.8, for a 5–10% volume of DMEM replaced using *A. platensis* and *Chlorella vulgaris* extracts. A robust relative cell viability of 3.8 was found using *C. littorale* replacing 5–10% of glucose volume in DMEM medium (Okamoto et al. 2019).

Additional efforts were also focused on the replacement of FBS. Extracts from *C. vulgaris* were used as a supplement for human cell lines *in vitro* (Ng et al. 2020). FBS substitution in Chinese hamster ovary cells (CHO) and mesenchymal stem cells (MSCs) was evaluated after the differentiation to human dermal fibroblast (HDF), human epidermal keratinocytes (HaCaT), human mesenchymal stem cells (hMSC), and adipose-derived stem cells (ADSC) cell lines. These differentiated cells were exposed to 24 h FBS starvation conditions. Later, microalgae extract was added to a final concentration of 0.001 g·L<sup>-1</sup> with concentrations of 0–10% of FBS in the medium, calculating recovery in cell viability under starvation conditions. *C. vulgaris* extract under a complete absence of FBS fails to recover 100% cell viability during 7 days post starvation. Conversely, in a culture medium supplemented with 5 to 10% FBS, cells reach up to 400% viability relative to initial values (Ng et al. 2020). These results highlight that microalgae extracts act as a functional supplement enhancing the nutritional properties of mammalian culture media. Microalgae extract concentrations ranging from 0.21 to 1.0 g·L<sup>-1</sup> ex-

hibit detrimental cytotoxic effects, obtaining cell viability values under 100% (Ng et al. 2020). Similar reports found microalgae extracts promote an increase in mammalian cell viability (Song et al. 2012; Jeong et al. 2021). An increment in cell viability was obtained after a 24 h treatment of mouse intestinal epithelial cells (IEC-6) using an extract from *C. vulgaris*. The increased values were 124, 135, and 155%, corresponding to 12.5, 25, and 50 µg·mL<sup>-1</sup> extract concentration, respectively (Song et al. 2012; Jeong et al. 2021). Remarkably, an extract of the cyanobacteria *S. maxima* is capable of replacing up to 50% of the FBS volume without affecting cell viability in lung cancer cell line H460, after three days of incubation (Jeong et al. 2021).

Previous studies focused on evaluating *C. vulgaris* as the 'model' microalgae species to conduct studies with mammalian cells. However, *C. vulgaris* extract has shown toxic effects when used as a replacement for DMEM medium or FBS, exceeding the limit of 20% relative to the final volume (Okamoto et al. 2019; Ng et al. 2020). In contrast, species such as *C. littorale* and *A. platensis* showed better responses promoting cell viability as a replacement for amino acids and glucose-complementing DMEM. Despite promising results, the latter microalgae species have not been further explored as FBS or culture medium replacement candidates. Non-model species from microalgae and cyanobacteria are gaining attention since recent advances showed better performance as alternative sources for mammalian culture and FBS replacements (Okamoto et al. 2019). Future studies should lead to a more profound analysis considering non-model species. Some of these alternatives are commercially available for testing in lab procedures. The South Korean company SeaWith is using microalgae as an FBS replacement for manufacturing lab-grown meat (Allan et al. 2019). The company raised USD 7 million in venture capital investment to achieve manufacture lab meat at a reduced price of USD 3 by 2030 (Lee 2021).

#### APPLICATION OF MICROALGAE AND MAMMALIAN CELLS CO-CULTURES FOR OXYGEN PROVISION AND WASTE REMOVAL

There are still few attempts to evaluate the co-culture of mammalian cell lines in combination with microalgae cells for *in vitro* meat production. In a co-culture, microalgae cohabit with mammalian cells in a mutu-

alistic relationship. A complex culture system is established in a specialised bioreactor supporting both species types (Wu et al. 2010; Bachus and Fussenegger 2013; Goers et al. 2014; Okamoto et al. 2019). The application of co-culture formats should provide two important advantages: Providing a permanent source of oxygen to promote thicker tissues; and increase the removal and recycling of metabolic waste byproducts to reduce maintenance costs. In the following sections, we analyse achievements and drawbacks of testing these strategies.

### MAMMALIAN CELLS *IN VITRO* REQUIRE A CONSTANT PROVISION OF OXYGEN

Manufacturing meat products in a laboratory demands a massive proliferation of mammalian cells by *in vitro* culture systems (Figure 1). Increasing exponentially the biomass increases tissues *in vitro*, consequently, oxygen demands are increased. However, the oxygen supply *in vitro* is a complex task in tissue with an increasing biomass. Results have shown that tissues farther than 100–200 µm from an oxygen source induce programmed cell death (Rowkema et al. 2008; Sekine et al. 2011; Kolesky et al. 2016). Low oxygen perfusion induces severe tissue damage such as muscle atrophy, lack of cell differentiation, proliferation, and maturation (Haraguchi and Shimizu 2021b). Although it has been demonstrated that a multilayer tissue is successfully produced under supra-physiological oxygen levels, cellular energy metabolism fails to be efficient and cellular distribution is not uniform within cellular scaffolds, affecting the quality of tissues *in vitro* (Levorson et al. 2014).

Attempts of co-cultivation of microalgae cells with mammalian cultures *in vitro* have been explored. Microalgae or cyanobacteria cells produce oxygen by means of photosynthesis as a permanent source to animal cells, improving cell survival and increasing multilayer tissue thickness for lab-cultured meat demands (Hopfner et al. 2014; Lode et al. 2015; Haraguchi et al. 2017; Haraguchi and Shimizu 2021a, b; Maharjan et al. 2021).

To quantify the effect of microalgae on tissue oxygen levels, the concentration in samples is measured through an electronic oxygen sensor in real-time, as well as the metabolic indicator of the lactate/glucose ratio (L/G). Monitoring the L/G ratio shows the cell's predominance of aerobic or anaerobic respiration. The parameter of L/G is based on Cori's Cycle, where during anaerobic respiration, one molecule of glu-

cose produces two molecules of pyruvate through the process of glycolysis, which later is converted into two molecules of lactate, yielding a lactate/glucose ratio equal to 2. However, under aerobic respiration, pyruvate is oxidised through the Krebs cycle and the amount converted to lactate is reduced, obtaining a ratio of L/G value that is less than 2. Therefore, a tissue with predominant aerobic respiration (in the presence of oxygen), will exhibit an L/G value considerably less than 2 (Fraga et al. 2009).

Oxygen levels can also be determined by indicators such as hypoxia-inducible factor 1α (HIF-1α), and creatine kinase. Expression of HIF-1α can be monitored by enzyme-linked immunosorbent assay (ELISA) assays. HIF-1α is a transcription factor that regulates oxygen homeostasis; increasing levels indicate a dysregulation of aerobic enzymes (Fraga et al. 2009). Similarly, the release of the enzyme creatine kinase is an indicator of cell death in muscle and cardiac cells (Haraguchi et al. 2017).

### MICROALGAE CO-CULTURES INCREASE CELL OXYGENATION AND PRODUCE THICKER TISSUES

Reports showed co-cultures of microalgae and mammalian cells in 3D configurations (Hopfner et al. 2014; Lode et al. 2015; Haraguchi et al. 2017; Haraguchi and Shimizu 2021a, b; Maharjan et al. 2021) and in bioreactors (Marko et al. 2022). The *in vitro* 3D culture using microalgae has been tested with or without scaffold structures. In scaffold-aided 3D cultures, microalgae were used to create a printable bioink material to manufacture the latter scaffold (Maharjan et al. 2021) or cultivated in a commercial scaffold with mammalian cells (Hopfner et al. 2014). In the 3D cultures without scaffolds, a technique stacked layers of muscle cells on top of layers with microalgae as the oxygen source (Haraguchi et al. 2017; Haraguchi and Shimizu 2021a). Modified hollow-fiber bioreactors have also been used, where microalgae and the culture medium circulate, nourishing the animal cells located above the *in vitro* containers (Marko et al. 2022).

In all cases, the culture systems tested in bioreactors or 3D *in vitro* configurations have demonstrated an increment in oxygen levels using microalgae. Haraguchi et al. (2017) tested the distribution of stacked layers of cells using *Chlamydomonas reinhardtii* and *C. littorale* on C2C12 myoblast and mouse cardiac cells, obtaining a 22–29% decrease in L/G ratio. A decrease of more than 80% in creatine kinase release was

<https://doi.org/10.17221/69/2023-CJFS>

also obtained compared to cultures without microalgae. Also, these co-cultures successfully increased thickness of tissue up to 160  $\mu\text{m}$ , around 2 times the previously reported thickness of 40–80  $\mu\text{m}$  in mouse cardiac cells cultivated in a medium depleted of dissolved oxygen (Haraguchi et al. 2017). To date, a breakthrough was obtained cultivating mouse C2C12 in a three-dimensional co-culture with *C. vulgaris* obtaining a thicker tissue of 200–400  $\mu\text{m}$ . Additionally, lactate dehydrogenase release and ammonia levels were reduced, compared to control cultures of mammalian cells without microalgae, reducing a potential tissue damage (Haraguchi and Shimizu 2021a).

The use of scaffolds with *C. reinhardtii* managed to increase the oxygen concentration by 26% in mouse embryonic fibroblast cells (NIH-3T3), as well as 84% reduction of HIF-1 $\alpha$  transcription factor (Hopfner et al. 2014). The co-cultivation of the human cell line SaOS-2 (human sarcome osteogenic cells) along with *C. reinhardtii* successfully increased oxygen concentration from 0.06  $\text{mg}\cdot\text{L}^{-1}$  to 1.16  $\text{m}\cdot\text{L}^{-1}$  on the first day of growth (Lode et al. 2015). *C. reinhardtii* was also used to implement a bioink for a scaffold in co-cultures of C2C12 myoblast and HepG2 (hepatocellular carcinoma cell line) tumor liver cells. Increased cell viability of the mammalian lines and a reduction in HIF-1 $\alpha$  expression were achieved. Optimal results were obtained upon *C. reinhardtii* biomass digestion with cellulase, obtaining 90% oxygenation promoting active vascular formation within HepG2 liver tissue attached to microchannels of gelatin methacryloyl (GelMA) hydrogels. The latter *in vitro* culture configuration was enhanced using GelMa hydrogel inoculated with human umbilical vein endothelial cells (HUVECs) (Maharjan et al. 2021). Similar results were reported testing a hollow-fiber bioreactor holding co-cultivation of *C. reinhardtii* in combination with PSBC (primary bovine satellite cells) blood cells, showing a qualitative improvement in dissolved oxygen with an increased concentration in the culture medium (Marko et al. 2022).

## MICROALGAE REMOVING TOXINS FROM ANIMAL CULTURES

*In vitro* cell cultures in active proliferation normally produce ammonia and lactic acid as waste metabolites. Such compounds are highly toxic to cells, forcing up to fifteen changes in fresh culture medium as a part of the manufacturing process (Post et al. 2020). Rou-

tine replacements of medium increase substantially the costs of obtaining multilayer meat cuts from muscle cells produced *in vitro* (Stephens et al. 2018). The lab-grown meat industry consumes 45 L of culture medium per kg of meat manufactured. This makes the process economically challenging, considering commercial production requires a continuous escalation to satisfy market demands (Post 2014; Richelle and Lewis 2017). In this sense, new methodologies ensuring the removal and reuse of toxic byproducts need to be implemented (Mattick et al. 2015; Allan et al. 2019).

The toxic effects of lactic acid and ammonia are evident at concentrations greater than 20–30 mM and 2–3 mM, respectively (Schneider et al. 1996; Haraguchi et al. 2017). These effects are harmful to cells, affecting the density and metabolism of cellular energy (Schneider et al. 1996). Microalgae such as *C. vulgaris* are considered extremophile organisms (Pang et al. 2020). Microalgae and cyanobacteria tolerate high concentrations of ammonia, being able to metabolise these molecules with glutamate to synthesise glutamine (Haraguchi and Shimizu 2021b). Since oxygen provision in tissues and aerobic respiration of mammalian cells is promoted by microalgae co-cultures, the conversion to lactate from pyruvate is reduced, consequently reducing the concentration of lactic acid in cultures (Hopfner et al. 2014; Lode et al. 2015; Haraguchi et al. 2017; Haraguchi and Shimizu 2021a, b; Maharjan et al. 2021).

Cultures of *C. vulgaris* and *C. littorale* with C2C12 mouse myoblast cells were evaluated for ammonia recycling. A consumption of 26% and 80% of total ammonia levels were reported in the cultures with *C. vulgaris* and *C. littorale*, respectively (Haraguchi and Shimizu 2021b). Similar results showed co-cultivation of *C. littorale* in combination with C2C12 mouse myoblasts cells and mouse heart cells without scaffolds, obtaining a reduction of up to 88% content of ammonia (Haraguchi et al. 2017). The co-cultivation of *C. vulgaris* and C2C12 cells, resulted in increased utilisation of ammonia up to 91%, without scaffold. Also, levels of lactic acid and lactate are decreased by microalgae. A reduction of 29% in the L/G rate was detected, meaning lower levels of toxic lactate relative to glucose molecules synthesised (Haraguchi et al. 2017; Haraguchi and Shimizu 2021a). A summary of the results on applications of microalgae as an oxygen source and for removal of toxic compounds from mammalian cultures can be found in Table 3.



Table 3. The use of microalgae as source of oxygen and waste recycling in co-cultures with mammalian cells

Applications of microalgae	Microalgae species	Mammalian cell line	Results	Reference
Oxygen source (in scaffolds)	<i>Chlamydomonas reinhardtii</i>	fibroblast cell line 3T3	increase of 26.1% in oxygen concentration	Hopfner et al. 2014
Oxygen source (in bioreactor)	<i>Chlamydomonas reinhardtii</i>	primary bovine satellite cells	co-cultures showed greater dissolved oxygen	Marko et al. 2022
Oxygen source (in scaffolds)	<i>Chlamydomonas reinhardtii</i>	human liver cancer cell line HepG2 C2C12 mouse myoblasts human umbilical vein endothelial cells (HUVECs)	increased cell viability in cells treated with microalgae in a 7-day culture	Maharjan et al. 2021
Oxygen source (in scaffolds)	<i>Chlamydomonas reinhardtii</i>	human osteosarcoma cell line SaOS-2	dissolved oxygen concentration increased from 0.06 mg·L <sup>-1</sup> to 1.16 mg·L <sup>-1</sup> in 24 h cultures	Lode et al. 2015
Oxygen source and removal of toxic compounds	<i>Chlorella vulgaris</i>	C2C12 mouse myoblasts	Thicker lab-meat tissue is obtained (200–400 µm) without tissue damage. Ammonia levels were reduced by 35–91%.	Haraguchi and Shimizu 2021a
Oxygen source and removal of toxic compounds	<i>Chlorococcum littorale</i>	mouse cardiac cell line C2C12 mouse myoblasts	Thicker lab-meat tissue is obtained (160 µm). Levels of ammonia reduced by 85–88%.	Haraguchi et al. 2017
Removal of toxic compounds	<i>Chlorococcum littorale</i> <i>Chlorella vulgaris</i>	C2C12 mouse myoblasts	<i>C. vulgaris</i> depleted 26% ammonia in co-cultures, whereas <i>C. littorale</i> 80%	Haraguchi and Shimizu 2021b

Microalgae was evaluated as a source of oxygen and its potential for removal of toxic compounds in co-cultures with different mammalian cell lines; results are presented as increased levels of oxygen, reduction of ammonia, tissue stratification, and cell viability

## ECONOMIC CONSIDERATIONS FOR MICROALGAE AND LAB MEAT PRODUCTION

Microalgae biomass production systems are under technological exploration. Exploitation of microalgae is economically sustainable, producing high-value products for low-demand markets. Several companies originally created for biofuels transitioned to these industries. Carotenoids, polyunsaturated fatty acids and immunostimulatory polysaccharides make microalgae production systems affordable for investors (Barsanti and Gualtieri 2018). Soybean production is USD 1 per kg, beef is USD 3 per kg, and microalgae biomass is around USD 3 per kg. Com-

panies such as Unilever and Nestlé collaborate with startups to develop novel products in microalgae systems (Khalil 2021). Microalgae is still characterised by low energy conversion in aerobic photosynthetic process. The goal is that genetic modifications of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) in novel strains with high biomass will promote the incorporation of microalgae in the market. Also, the optimisation of photobioreactors for efficient light transmission is expected to increase biomass yields to 30 g per m<sup>2</sup> per day and fatty acid accumulation by up to 40%, to be economically viable. According to recent reports, 10 years of research and development are required to reach microalgae economic sustainability (Barsanti and Gualtieri 2018). Nevertheless,

<https://doi.org/10.17221/69/2023-CJFS>

algae biodiesel and bioethanol are commercially available at USD 5 per gallon and USD 2.95 per gallon, respectively. Companies like Algenol sustain industrial production of biodiesel, costing up to 85 cents per liter (Khan et al. 2018). Lab meat faces similar constraints; the cells must perform at a high metabolic rate to intensify the production with a drastic reduction of culture medium cost (Humbird 2021).

Lab meat technology underscores the urgent need for alternative sustainable products. This technology demands a complete reconstruction of agricultural systems, ensuring social and economic sustainability. Nevertheless, an important challenge involves overcoming financial risk liability for investors (Tuomisto and Teixeira de Mattos 2011). Only few examples of economic value are available for lab-meat endeavors. Startup initiatives normally do not release financial expectations data (Li et al. 2022). The business model demands the selection of two types of products: unstructured cell biomass or structured tissue. The former should involve harvesting, enzymatic processing for texturing, for obtaining a ground meat-like product ready for extrusion. The latter requires an edible scaffold of nursing cells, shaping a beef steak-like tissue. The unstructured animal cell biomass could be used in a great variety of meat substitutes and so would be economically more feasible (Humbird 2021). Lab meat affordability is the main concern for investors. Technological improvements are required for a significant reduction in manufacturing costs. Several feasibility studies show that lowering costs in culture medium for animal cells make this meat's high-scale production affordable. More than 25 companies are dedicating efforts in replacing animal culture medium with alternative, cost-effective components derived from plants and microalgae (Li et al. 2022).

Considering the latter, lab-meat and meat-like projects raise enthusiasm among venture capital investors. Additional dedication should be applied on these projects, especially public initiatives funding on research and development (R&D) to implement a profitable product. A growing expansion business is the market for plants and microalgae as feedstock in meat-like products (Brevel, Chunk, Redefine Meat) and implementing lab-grown meat (Mewery, SuperMeat, Aleph farms). Recently, the company Mewery announced the incorporation of microalgae to manufacturing lab-grown meat pork. The latter and innovative entrepreneurs such as Upside Foods rely on the pending authorisation of these products. Singapore and the United States are the only countries with author-

ised commercialisation under the 'novel food' category (Barsanti and Gualtieri 2018).

## CONCLUSION

In this review, we present a critical analysis of the incorporation of microalgae in the manufacturing of lab-grown meat. According to recent data, biomass extracts from photosynthetic species can partially substitute not only the use of FBS but also the macro- and micronutrients from the basal defined medium. Future research efforts should evaluate if this partial replacement significantly reduces the cost of lab-cultured meat production. Co-culture assays using microalgae nourishing mammalian cells can also enhance oxygen concentration and stimulate aerobic respiration to create meat tissues at least twice as thick as previously achieved. Moreover, this system has made possible the reutilisation of ammonia and lower lactate to non-toxic levels, expecting to reduce the costs relative to medium replacement during cultured meat manufacture. The most suitable configuration integrating microalgae towards industrial *in vitro* meat production remains a subject for future studies. Additional research is necessary to improve aspects of production, such as affordable medium costs and metabolic improvement of animal and microalgae cells to yield cost-effective lab meat products. As reviewed, the active collaboration between academia and industry has been quite successful in lab-meat manufacture. To date, emerging companies are using this technology and have received significant investment funds as financing, so a greater transfer of new solutions to the market can be expected in the medium term. The field of research on the use of microalgae in cultured meat production is also expected to grow considerably in the following years.

**Acknowledgement.** The authors would like to thank Nancy Refulio Ph.D. for her comments and assistance during the writing process of the manuscript, as well to the Department of Bioengineering of University of Engineering and Technology (UTEC) and Julio Valdivia Ph.D. for overall support throughout the project.

## REFERENCES

- Allan S., De Bank P., Ellis M. (2019): Bioprocess design considerations for cultured meat production with a focus on the expansion bioreactor. *Frontiers in Sustainable Food Systems*, 3: 44.

- Bacchus W., Fussenegger M. (2013): Engineering of synthetic intercellular communication systems. *Metabolic Engineering*, 16: 33–41.
- Barsanti L., Gualtieri P. (2018): Is exploitation of microalgae economically and energetically sustainable? *Algal Research*, 31: 107–115.
- Brindley D., Davie N., Culme-Seymour E., Mason C., Smith D., Rowley J. (2012): Peak serum: Implications of serum supply for cell therapy manufacturing. *Regenerative Medicine*, 7: 7–13.
- European Commission (2013): Attitudes of Europeans towards building the single market for green products. Available at <https://europa.eu/eurobarometer/surveys/detail/1048> (accessed Apr 3, 2022).
- Fang J., Wei Y., Teng X., Zhao S., Hua J. (2017): Immortalization of canine adipose-derived mesenchymal stem cells and their seminiferous tubule transplantation. *Journal of Cellular Biochemistry*, 119: 3663–3670.
- Fraga A., Ribeiro R., Medeiros R. (2009): Hipoxia tumoral: Papel del factor inducible por hipoxia. *Actas Urológicas Españolas*, 33: 9. (in Spanish)
- Garrison G.L., Biermacher J.T., Brorsen B.W. (2022): How much will large-scale production of cell-cultured meat cost? *Journal of Agriculture and Food Research*, 10: 100358.
- Gerhart C., Warschun M., Donnan D., Ziemssen F. (2020): When consumers go vegan, how much meat will be left on the table for agribusiness? Kearney. Available at <https://www. Kearney.com/consumer-retail/article?/a/when-consumers-go-vegan-how-much-meat-will-be-left-on-the-table-for-agribusiness> (accessed Apr 8, 2022).
- Goers L., Freemont P., Polizzi K. (2014): Co-culture systems and technologies: Taking synthetic biology to the next level. *Journal of The Royal Society Interface*, 11: 201400652.
- Graça J., Oliveira A., Calheiros M. (2015): Meat, beyond the plate. Data-driven hypotheses for understanding consumer willingness to adopt a more plant-based diet. *Appetite*, 90: 80–90.
- Haraguchi Y., Kagawa Y., Sakaguchi K., Matsuura K., Shimizu T., Okano T. (2017): Thicker three-dimensional tissue from a 'symbiotic recycling system' combining mammalian cells and algae. *Scientific Reports*, 7: 41594.
- Haraguchi Y., Shimizu T. (2021a): Three-dimensional tissue fabrication system by co-culture of microalgae and animal cells for production of thicker and healthy cultured food. *Biotechnology Letters*, 43: 1117–1129.
- Haraguchi Y., Shimizu T. (2021b): Microalgal culture in animal cell waste medium for sustainable 'cultured food' production. *Archives of Microbiology*, 203: 5525–5532.
- Harris R.A. (2021): Energy metabolism | Gluconeogenesis. In: Jez J. (ed.): *Encyclopedia of Biological Chemistry III*. Amsterdam, Elsevier: 170–186.
- Hopfner U., Schenck T., Chávez M., Machens H., Böhne A., Nickelsen J., Giunta R., Egaña J. (2014): Development of photosynthetic biomaterials for *in vitro* tissue engineering. *Acta Biomaterialia*, 10: 2712–2717.
- Humbird D. (2021): Scale-up economics for cultured meat. *Biotechnology and Bioengineering*, 118: 3239–3250.
- Jeong Y., Choi W., Park A., Lee Y., Lee Y., Park G., Lee S., Lee W., Ryu Y., Kang D. (2021): Marine cyanobacterium *Spirulina maxima* as an alternate to the animal cell culture medium supplement. *Scientific Reports*, 11: 4906.
- Jochems C., van der Valk J., Stafleu F., Baumans V. (2002): The use of fetal bovine serum: Ethical or scientific problem? *Alternatives to Laboratory Animals*, 30: 219–227.
- Khalil Y. (2021): Steak expectations for alternative protein sources. *Nature Portfolio News Feature*. Available at <https://www.nature.com/articles/d42859-021-00061-8> (accessed Oct 26, 2022).
- Khan M., Shin J., Kim J. (2018): The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17: 36.
- Khodabukus A., Baar K. (2014): The effect of serum origin on tissue engineered skeletal muscle function. *Journal of Cellular Biochemistry*, 115: 2198–2207.
- Kolesky D., Homan K., Skylar-Scott M., Lewis J. (2016): Three-dimensional bioprinting of thick vascularized tissues. *Proceedings of the National Academy of Sciences*, 113: 3179–3184.
- Kumar P., Sharma N., Sharma S., Mehta N., Verma A., Chennamalar S., Sazili A. (2021): *In-vitro* meat: A promising solution for sustainability of meat sector. *Journal of Animal Science Technology*, 63: 693–724.
- Kumar A., Sood A., Han S.S. (2022): Technological and structural aspects of scaffold manufacturing for cultured meat: Recent advances, challenges, and opportunities. *Critical Reviews in Food Science and Nutrition*, 63: 1–28.
- Lee H. (2021): Three is the magic number: South Korean's Seawith targets cultured steak at US\$3/kg by 2030. *Food Navigator Asia*. Available at <https://www.foodnavigator-asia.com/Article/2021/08/02/Three-is-the-magic-number-South-Korean-s-Seawith-targets-cultured-steak-at-US-3-kg-by-2030#> (accessed Apr 10, 2022).
- Lee D.Y., Lee S.Y., Jung J.W., Kim J.H., Oh D.H., Kim H.W., Kang J.H., Choi J.S., Kim G.D., Joo S.T., Hur S.J. (2022): Review of technology and materials for the development of cultured meat. *Critical Reviews in Food Science and Nutrition*, 63: 8591–8615.
- Levorson E., Santoro M., Kurtis Kasper F., Mikos A. (2014): Direct and indirect co-culture of chondrocytes and mesenchymal stem cells for the generation of polymer/extracellular matrix hybrid constructs. *Acta Biomaterialia*, 10: 1824–1835.

<https://doi.org/10.17221/69/2023-CJFS>

- Li X., Athira N., Luang-In V., Ling N. (2022): Lab-based meat the future food. *Environmental Advances*, 10: 100315.
- Lode A., Krujatz F., Brüggemeier S., Quade M., Schütz K., Knaack S., Weber J., Bley T., Gelinsky M. (2015): Green bioprinting: Fabrication of photosynthetic algae-laden hydrogel scaffolds for biotechnological and medical applications. *Engineering in Life Sciences*, 15: 177–183.
- Maharjan S., Alva J., Cámara C., Rubio A., Hernández D., Delavaux C., Correa E., Romo M., Bonilla D., Santiago M., Li W., Cheng F., Ying G., Zhang Y. (2021): Symbiotic photosynthetic oxygenation within 3d-bioprinted vascularized tissues. *Matter*, 4: 217–240.
- Marko J., Forauer J., Shih J., Berry T. (2022): Designing an algal co-culture system for increased sustainability in cellular agriculture. 13<sup>th</sup> Annual Sustainability Project Competition. Available at <https://wp.wpi.edu/sustainability-competition13/designing-an-algal-co-culture-system-for-increased-sustainability-in-cellular-agriculture/> (accessed May 10, 2022).
- Mattick C., Landis A., Allenby B., Genovese N. (2015): Anticipatory life cycle analysis of *in vitro* biomass cultivation for cultured meat production in the United States. *Environmental Science & Technology*, 49: 11941–11949.
- McMahon D., Anderson P., Nassar R., Bunting J., Saba Z., Oakeley A., Malouf N. (1994): C2C12 Cells: Biophysical, biochemical, and immunocytochemical properties. *American Journal of Physiology-Cell Physiology*, 266: C1795–C1802.
- Ng J., Chua M., Zhang C., Hong S., Kumar Y., Gokhale R., Ee P. (2020): *Chlorella vulgaris* extract as a serum replacement that enhances mammalian cell growth and protein expression. *Frontiers in Bioengineering and Biotechnology*, 8: 564667.
- Nitsos C., Filali R., Taidi B., Lemaire J. (2020): Current and novel approaches to downstream processing of microalgae: A review. *Biotechnology Advances*, 45: 107650.
- O'Neill E., Cosenza Z., Baar K., Block D. (2020): Considerations for the development of cost-effective cell culture media for cultivated meat production. *Comprehensive Reviews in Food Science and Food Safety*, 20: 686–709.
- Okamoto Y., Haraguchi Y., Sawamura N., Asahi T., Shimizu T. (2019): Mammalian cell cultivation using nutrients extracted from microalgae. *Biotechnology Progress*, 36: e2941.
- Pang N., Bergeron A.D., Gu X., Fu X., Dong T., Yao Y., Chen S. (2020): Recycling of nutrients from dairy wastewater by extremophilic microalgae with high ammonia tolerance. *Environmental Science & Technology*, 54: 15366–15375.
- Post M. (2014): Cultured beef: Medical technology to produce food. *Journal of the Science of Food and Agriculture*, 94: 1039–1041.
- Post M., Levenberg S., Kaplan D., Genovese N., Fu J., Bryant C., Negowetti N., Verzijden K., Moutsatsou P. (2020): Scientific, sustainability and regulatory challenges of cultured meat. *Nature Food*, 1: 403–415.
- Puri G., Chaudhary S.S., Singh V.K., Sharma A.K. (2015): Effects of fetal bovine serum and estrus buffalo serum on maturation of buffalo (*Bubalus bubalis*) oocytes *in vitro*. *Vet World*, 8: 143–146.
- Reiss J., Robertson S., Suzuki M. (2021): Cell sources for cultivated meat: Applications and considerations throughout the production workflow. *International Journal of Molecular Sciences*, 22: 7513.
- Richelle A., Lewis N. (2017): Improvements in protein production in mammalian cells from targeted metabolic engineering. *Current Opinion in Systems Biology*, 6: 1–6.
- Rouwkema J., Rivron N., van Blitterswijk C. (2008): Vascularization in tissue engineering. *Trends in Biotechnology*, 26: 434–441.
- Sanchez-Sabate R., Sabaté J. (2019): Consumer attitudes towards environmental concerns of meat consumption: A systematic review. *International Journal of Environmental Research and Public Health*, 16: 1220.
- Sans P., Combris P. (2015): World meat consumption patterns: An overview of the last fifty years (1961–2011). *Meat Science*, 109: 106–111.
- Sathasivam R., Radhakrishnan R., Hashem A., Abd Allah E.F. (2019). Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences*, 26: 709–722.
- Schneider M., Marison I., von Stockar U. (1996): The importance of ammonia in mammalian cell culture. *Journal of Biotechnology*, 46: 161–185.
- Sekine W., Haraguchi Y., Shimizu T., Umezawa A., Okano T. (2011): Thickness limitation and cell viability of multi-layered cell sheets and overcoming the diffusion limit by a porous-membrane culture insert. *Journal of Biochips & Tissue Chips*, 1: 7.
- Sinke P., Odegard I. (2021): LCA of cultivated meat: Future projections for different scenarios. CE Delft: Delft, 2021.
- Song S., Kim I., Nam T. (2012): Effect of a hot water extract of *Chlorella vulgaris* on proliferation of iec-6 cells. *International Journal of Molecular Medicine*, 29: 741–746.
- Steinfeld H., Gerber P., Wassenaar T., Castel V., Rosales M., Haan C. (2006): Livestock's long shadow: Environmental issues and options. Food and Agriculture Organization of the United Nations. Available at <https://www.fao.org/3/a0701e/a0701e.pdf> (accessed Apr 8, 2022).
- Stephens N., Di Silvio L., Dunsford I., Ellis M., Glencross A., Sexton A. (2018): Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture. *Trends in Food Science & Technology*, 78: 155–166.
- Szejda K., Bryant C.J., Urbanovich T. (2021): US and UK consumer adoption of cultivated meat: A Segmentation Study. *Foods*, 10: 1050.



<https://doi.org/10.17221/69/2023-CJFS>

- Treich N. (2021): Cultured meat: Promises and challenges. *Environmental and Resource Economics*, 79: 33–61.
- Tuomisto H., Teixeira de Mattos M. (2011): Environmental impacts of cultured meat production. *Environmental Science & Technology*, 45: 6117–6123
- Usta S., Scharer C., Xu J., Frey T., Nash R. (2014): Chemically defined serum-free and xeno-free media for multiple cell lineages. *Annals of Translational Medicine*, 2: 97.
- Van der Valk J., Gstraunthaler G. (2017): Fetal Bovine Serum (FBS) – A pain in the dish? *Alternatives to Laboratory Animals*, 45: 329–332.
- Vanhonacker F., Van Loo E., Gellynck X., Verbeke W. (2013): Flemish consumer attitudes towards more sustainable food choices. *Appetite*, 62: 7–16.
- Wu M., Huang S., Lee G. (2010): Microfluidic cell culture systems for drug research. *Lab on a Chip*, 10: 939–956.
- Yao T., Asayama Y. (2017): Animal-cell culture media: History, characteristics, and current issues. *Reproductive Medicine and Biology*, 16: 99–117.

Received: May 8, 2023

Accepted: November 27, 2023

Published online: December 20, 2023