Environmental Impacts of Serum-free Food-grade and Complex Culture Medium Production for Cultivated Meat

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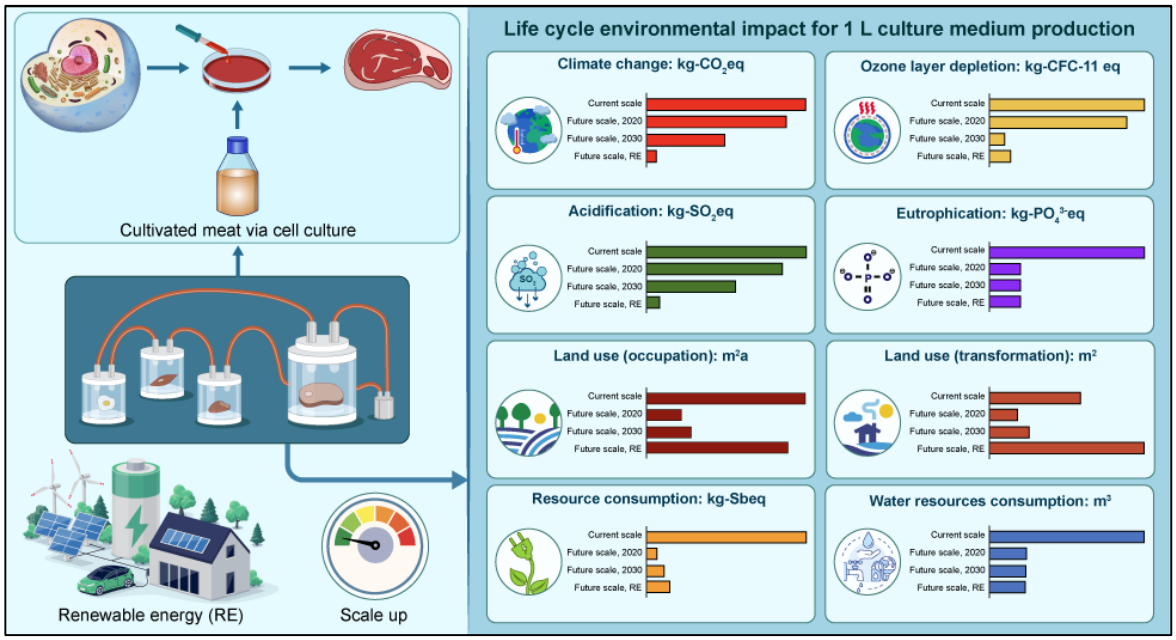
ABSTRACT. Cultivated meat is an alternative meat produced via cell culture. The culture medium accounts for most of the environmental impact of cultivated meat production. This study

quantitatively evaluated and analyzed the environmental impact of serum-free food-grade and complex culture medium production for cultivated meat by performing a life cycle assessment (LCA) based on activity data at the laboratory scale. In addition, specific measures were proposed to reduce the environmental impact further. LCAs were performed at current and future production scales. This study also evaluated the impact of multiple electricity sources on the environmental impact of culture-medium production. Expendables at the current scale, as well as electricity and animal-derived materials at both scales, are hotspots in the environmental impact of this culture-medium production. Among these components, the production of serum substitutes accounts for most of the environmental impact. As the scale shifts, the environmental impacts are expected to decrease by 11.3%–93.2% in all impact categories. As the composition of electricity sources changed, the impact on certain categories decreased. However, as the share of renewable energy increased, the impact on land use also increased significantly. This study promotes the practical application of new culture media for low-cost and low-environment-impact cultivated meat.

Graphical abstract



SYNOPSIS. This study evaluates the environmental impact of culture-medium production for cultivated meat and reports that electricity, animal-derived materials, and expendables are hotspots of this impact.

1. INTRODUCTION

Current meat production from livestock farming is one of the major sources of anthropogenic environmental impacts.[1] The consumption of animal-based foods has a larger environmental impact than other foods regarding greenhouse gas emissions, land use, water use, eutrophication, and biodiversity.[2] Additionally, global meat production is expected to increase by 76% between the beginning of this century and 2050.[3] Therefore, the environmental impact of livestock production will further increase.

Cultivated meat has attracted considerable attention as a new method of meat production. Cultivated meat aims to replicate conventionally produced meat via (stem) cell and tissue culture.[4] It is considered to have a low environmental impact and is expected to be an alternative meat that contributes to food security in the future.[5,6] Previous studies on the life cycle assessment (LCA) of cultivated meat have shown that environmental impacts of culture medium account for 48% to 89% of the total environmental impacts of cultivated meat production depending on the impact category,[7] and thus, the culture medium is a hotspot for the environmental impacts of cultivated meat production.[7,8] Therefore, research focusing on the environmental impact of culture medium is essential for reducing the environmental impact of cultivated meat production. However, peer-reviewed studies on detailed environmental impact assessments of culture media for cultivated meat production are lacking. Additionally, previous studies on the environmental impact assessment of cultivated meat production have focused on prospective future production systems when the production technology is developed on an industrial scale, and no studies have been conducted on current production systems.[9,10] Consequently, there is a problem that only optimistic and prospective evaluation results precede, and the actual environmental impact is not understood.

A culture medium is a liquid that contains nutrients required for cell culture, such as carbohydrates, proteins, growth factors, minerals, and vitamins.[5] Currently, culture medium containing Fetal Bovine Serum (FBS) is widely used. However, FBS is problematic because of the ethical perspective of being produced from bovine fetuses, the possibility of containing toxic substances such as endotoxins, and price instability.[11-13] Therefore, a shift to a serum-free culture medium is required for cultivated meat production.[14] Additionally, many culture media use chemical reagents; however, all culture medium components must be food-grade to develop and

sell edible cultivated meat products.[15] Culture media are classified into chemically defined media in which the components and concentrations are defined, and complex media in which the components and concentrations are not defined (typically from agricultural products, but this study's culture medium is from animal cell culture supernatant).[15] In general, using complex medium ingredients effectively reduces the price of the culture medium.[15] Therefore, focusing on serum-free food-grade and complex culture media is essential for the future development of cultivated meat.

Based on the above background, this study conducted an LCA based on a company's actual culture medium production data to evaluate the current environmental impact of serum-free food-grade and complex culture medium for cultivated meat production. This study also conducted the LCA contingent on this culture medium production being more established and on a larger scale in the future and on using different electric power supply configurations. Furthermore, effective strategies for reducing the environmental impact of culture medium production were examined based on the LCA results. The significance of this study is threefold. First, this study quantitatively evaluated the culture medium's current and future environmental impacts, a key technology for low-cost and low-environmental impact on cultivated meat production. Second, this study identified hotspots in the environmental impact of culture medium production. Third, this study proposed specific measures to further reduce the environmental impact. These results support the practical application of new culture media for low-cost and low-environment-impact cultivated meat.

2. MATERIALS AND METHODS

This study used LCA for environmental impact assessment. LCA is a method for quantitatively assessing the environmental impact over the life cycle of a product or service and is standardized by the International Organization for Standardization (ISO).[16,17]

2.1 Composition of Target Culture Medium

The target of the study was the food-grade basal medium, designed based on Dulbecco's Modified Eagle's Medium (DMEM), with all components of DMEM replaced with food/food additives.[18] The serum substitute components are produced using a method in which multiple organ cells are co-cultured using the above basal medium, and the interaction of each organ cell secretes components that serve as a serum substitute to achieve a serum-free culture medium. The target culture medium consisted of food-grade basal medium and complex serum substitutes.

2.2 System Boundaries and Functional Unit

The system boundary of this study was cradle-to-gate, from raw material production to product manufacturing. Figure 1 shows a schematic of the system boundaries used in this study. Because the environmental impact of equipment manufacturing is small compared to equipment operation when the lifetime is considered, manufacturing the main body of the device and the plant was not included in this study's evaluation scope.[8] Also, the functional unit of this study was 1 L of culture medium. The basal medium was prepared by dissolving the raw materials in sterile water and filter sterilization. Serum substitute components were produced by co-culturing multiple organ cells in the above basal medium. Specifically, as shown in Figure 2, one bioreactor contained one type of organ cell, and multiple bioreactors were connected. All the bioreactors were filled with a basal medium and co-cultured by circulating the culture medium. The serum substitute components were prepared by collecting the culture supernatants. Cells

were extracted from eggs. Bioreactors and devices for exchanging the culture medium in the cell culture process used silicone tubes and other experimental expenses for conducting lines. The assembly process to install them, autoclaved sterilization, and gas sterilization were performed for each production lot. Animal-derived purified material and yeast extract were used as culture aids in the cell culture process to prepare serum substitutes. The disposal of waste generated in the production process and the cleaning process of each device after use were also included in the evaluation scope of this study.

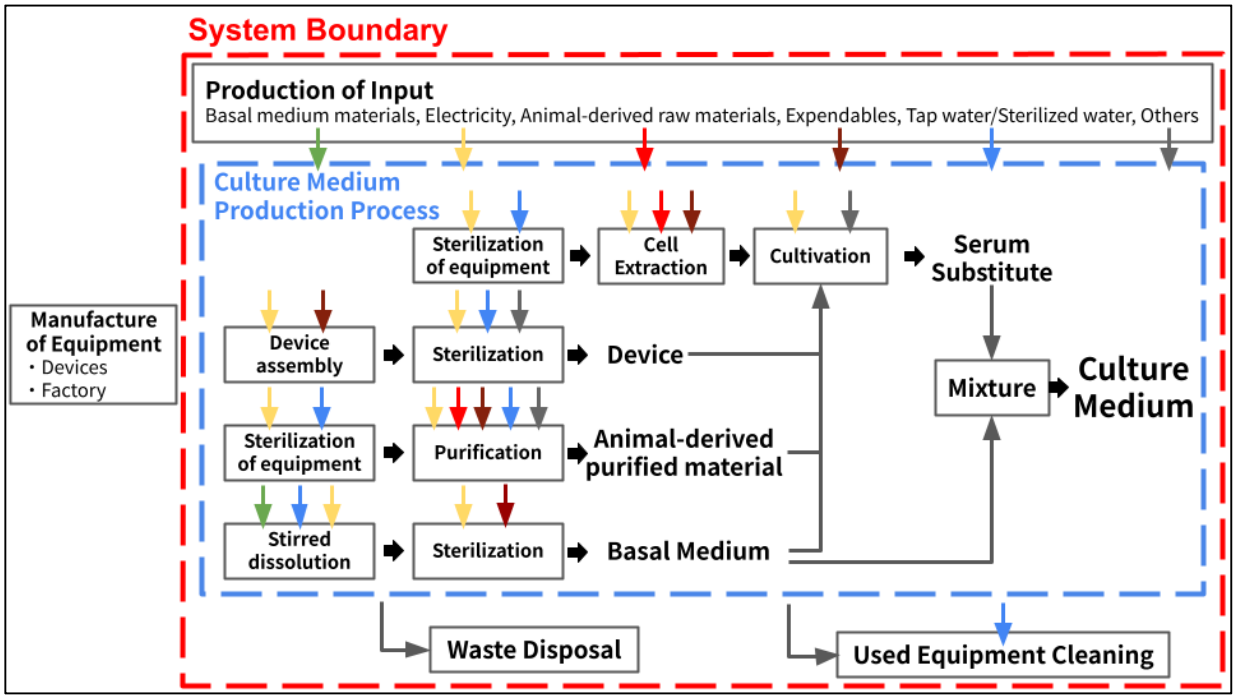


Figure 1. System boundary schematic.

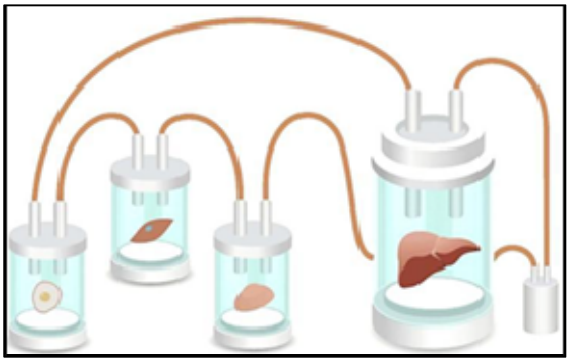


Figure 2. Cell culture for the preparation of serum substitutes.

2.3 Scenario Analysis

This study conducted LCA for the case in which culture medium production is more established and on a larger scale in the future. Table 1 summarizes the changes from present to future scales. The production volume per lot was assumed to be approximately 700 times higher. The current scale used silicone tubes and other expendables conducting lines, etc. In the future, it is assumed that all piping from the main body of the device to the conductors will be stainless-steel piping for multiple uses. Assumably, this will considerably reduce expendables. The cleaning method will assumably change from hand washing to Cleaning in Place (CIP), and the sterilization method will change from autoclaved and gas sterilization to Sterilization in Place (SIP) using steam. In the cell culture process for preparing serum substitutes, the temperature and air were regulated by placing all bioreactors in a single incubator at the current scale. However, it is assumed that the temperature and air will be controlled for each bioreactor in the future. It is also assumed that the source of cell extraction changes from eggs to cell lines.

Table 1. Comparison of present and future scales.

	Current Scale	Future Scale

Production volume per 1 lot (ratio)	1	700
Device	Tubes and other expendables required	Multi-use
Heater/Aeration	Managed in one incubator	Managed by individual reactors
Cell Source	Egg	Cell line
Cleaning	Hand-washing	Cleaning in Place(CIP)
Method of Sterilizing Device	Steam pressure sterilization /Gas sterilization	Sterilization in Place(SIP)

This study used data on Japan's average electricity source composition in 2020 ("Current Energy Mix") as the energy source used at the current scale. In addition to the "Current Energy Mix," future-scale LCA was conducted using Japan's expected electricity source composition in 2030 ("Energy Mix in 2030") announced by the Japanese Agency for Natural Resources and Energy, and electricity source composition for solar and wind power generation only ("Renewable Energy Mix").[19] Table 2 shows the specific electricity source composition for "Current Energy Mix," "Energy Mix in 2030," and "Renewable Energy Mix."

Therefore, in this study, as shown in Table 3, LCA was performed for three scenarios in addition to the current scale, which is the actual and current culture-medium production process. All three scenarios targeted the future scale and used the following data as energy sources: "Current Energy Mix" for [Future Scale, 2020], "Energy Mix in 2030" for [Future Scale, 2030], and "Renewable Energy (RE) Mix" for [Future Scale, RE].

Table 2. Electricity source composition of each energy mix.

	Current Energy Mix	**Energy Mix in 2030**	**Renewable Energy Mix**
Solar	1.9%	16.0%	50.0%
Wind	0.9%	5.0%	50.0%
Petroleum etc.	1.7%	2.0%	-

Coal	32.5%	19.0%	-
LNG	41.9%	20.0%	-
Other gases	3.1%	-	-
Nuclear	4.4%	20.0%	-
Geothermal	0.2%	1.0%	-
Hydropower	10.0%	11.0%	-
Biomass	2.3%	5.0%	-
Waste	0.4%	-	-
Hydrogen, Ammonia	-	1.0%	-
Others	0.7%	-	-

Table 3. Scenario Description.

Scenario	**Scale**	**Energy Source**
Current Scale	Current	Current Energy Mix
Future Scale,2020	Future	Current Energy Mix
Future Scale,2030	Future	Energy mix in 2030
Future Scale, RE	Future	Renewable Energy (RE) Mix

2.4 Data Collection.

IntegriCulture Inc. (Japan) provided data for the culture medium used in this study. Data were collected from the company's standard operating procedure for culture medium production and the entire production process from March 2023 to June 2023. Literature was used to obtain information that these methods could not collect.[18]

Information on future manufacturing processes was collected from the company for future LCA, and inventory data was created based on this information. The device's electricity consumption in the future scale was predicted using the method by Kawajiri et al..[20] In addition, data on electricity and water consumption in the SIP/CIP were obtained from the literature

information.[8] The details of the future-scale LCA methodology in this study are described in S.1 of the Supporting Information (SI).

2.5 Life Cycle Inventory (LCI).

Table 4 shows inventory data for the evaluation scope. Table 4 summarizes the classifications and materials, amounts used per liter of culture medium production, data sources, and purposes for each present and future scale. Each input and output was classified by electricity, expendables, waste, amino acids, glucose, vitamins + inorganic components + trace elements, animal-derived raw materials, tap water + sterilized water, and others. The results and other information are presented based on this study's classification.

Table 4. Inventory data for 1 L of the culture medium production.

Classification	Material	Current Scale			Future Scale			Purposes
		Amounts used/1L culture medium		Data Source	amounts used/1L culture medium		Data Source	
Electricity		2.98E+00	kWh	A	4.67E+00	kWh	B[8],C,D[20]	Regulation of cell culture environment, Sterilization, Assembly of devices, Clean bench, Centrifuges, Agitation, SIP, CIP
Expendable	Rubber glove	1.68E+00	pair	A	9.34E-02	pair	C	Wearing for hygiene control
	Hydrophilic PES	6.45E+00	g	A	3.22E+00	g	C	Laboratory expendables Equipment expendables Sterilization filters
	S3 Resin	9.77E-01	g	A	1.01E-01	g	C	
	Agar	2.52E+00	g	A	2.51E-01	g	C	
	Silicone rubber	1.35E+01	g	A	-	-	-	
	Thermoplastic elastomer	2.23E+01	g	A	-	-	-	
	Polystyrene	5.11E+01	g	A	-	-	-	
	Polypropylene	1.66E+01	g	A	-	-	-	
	Paper	1.79E+01	g	A	-	-	-	
Waste	Industrial waste, Waste plastics	1.41E+02	g	A	3.85E+00	g	C	
	Industrial waste, Plants and animals residue	7.72E+01	g	A	2.51E-01	g	C	
	Industrial waste, Waste animal oil and waste	2.47E+01	g	A	-	-	-	
	Industrial waste, Waste alkali	5.78E+01	g	A	-	-	-	
	Industrial waste, Waste paper	1.79E+01	g	A	-	-	-	
	Other wastes	4.05E+00	g	A	-	-	-	
Amino acids		1.58E+03	mg	A,B[18]	1.57E+03	mg	B[18],C	Basal medium materials ※Details on the breakdown in S.2 of SI
Glucose		5.04E+03	mg	A,B[18]	5.03E+03	mg	B[18],C	
Vitamin, Inorganic components, Trace elements	Inorganic compornents	1.05E+04	mg	A,B[18]	1.04E+04	mg	B[18],C	
	Trace elements	7.06E-02	mg	A,B[18]	7.04E-02	mg	B[18],C	
	Vitamin	3.60E+01	mg	A,B[18]	3.59E+01	mg	B[18],C	
Animal-derived raw materials	Egg	5.43E+01	g	A	-	-		Cell source
	Animal-derived purified product's material	3.15E+01	g	A	1.26E+01	g	C	Cell culture support
Tap water, Sterilized water	Tap water	8.33E+00	L	A	1.59E+00	L	B[8],C	Equipment and instrument cleaning, Basal medium, Animal-derived purified products
	Sterilized water	1.29E+00	L	A,B[18]	1.13E+00	L	B[18],C	
Others	Sewage treatment	8.55E+00	L	A	1.63E+00	L	C	
	Ethanol	1.50E+01	g	A	-	-	-	Disinfection
	Chlorine bleache	7.13E-01	g	A	-	-	-	
	Ethylene oxide	1.58E+01	g	A	-	-	-	Gas sterilization
	Scaffold	1.29E+00	g	A	4.74E-01	g	C	Cell culture
	Yeast extract	1.81E-01	g	A	1.58E-01	g	C	Cell culture support
	Alkaline cleaning solution	-	-	-	5.16E-02	g	B[8],C	CIP

Regarding data sources, data obtained from the actual manufacturing process is shown as A, data based on literature information as B, data provided by the company regarding future scales as C, and data obtained by prediction using the method described by Kawajiri et al..[20] Details on the breakdown of basal medium materials are in S.2 of SI.

We used the LCI database IDEA Ver. 3.3 (IDEA) from the LCI database.[21] IDEA is the most comprehensive inventory database available in Japan. IDEA shows the environmental burden intensity for each product, which traces back to the upstream processes, specifically, "from cradle to gate." In this study, the LCI quantified the environmental load substances generated in this culture medium production by multiplying the amount of each material used and the waste generated, as presented in Table 4, by the corresponding environmental burden intensity in IDEA. "Current Energy Mix" used in this study's [Current Scale] and [Future Scale, 2020] was based on the "electricity, Japan, FY2020" environmental burden intensity in IDEA. IDEA provides information on the environmental burden intensity for generating 1 kWh of electricity using each electricity generation source. By multiplying these by the energy source composition proportion in Table 2, we calculated the environmental burden intensity for "Energy Mix in 2030" and "Renewable Energy (RE) Mix." Alternative methods for addressing the lack of environmental burden intensity in IDEA were explained in S.3 of the SI.

2.6 Life Cycle Impact Assessment (LCIA).

In this study, the Life-cycle Impact assessment Method based on Endpoint modeling 2 (LIME2), an LCIA methodology that reflects the environmental conditions of Japan, was used in the LCIA.[22] The environmental impact is quantified by multiplying the amount of each environmental burden calculated in the LCI by the LCIA coefficient and classifying the amount of each environmental burden with the impact category. This study performed LCIA for the following eight impact categories considered particularly important for culture medium production: climate change, ozone layer depletion, acidification, eutrophication, land use (occupation), land use (transformation), resource consumption, and water resources consumption. The results for all impact categories evaluated in LIME2 are shown in S. 4 of the

SI.

3. RESULTS AND DISCUSSION

Figure 3 shows the environmental impact of each material at current and future scales. Table 5 shows the environmental impacts of producing 1L of culture medium for this study.

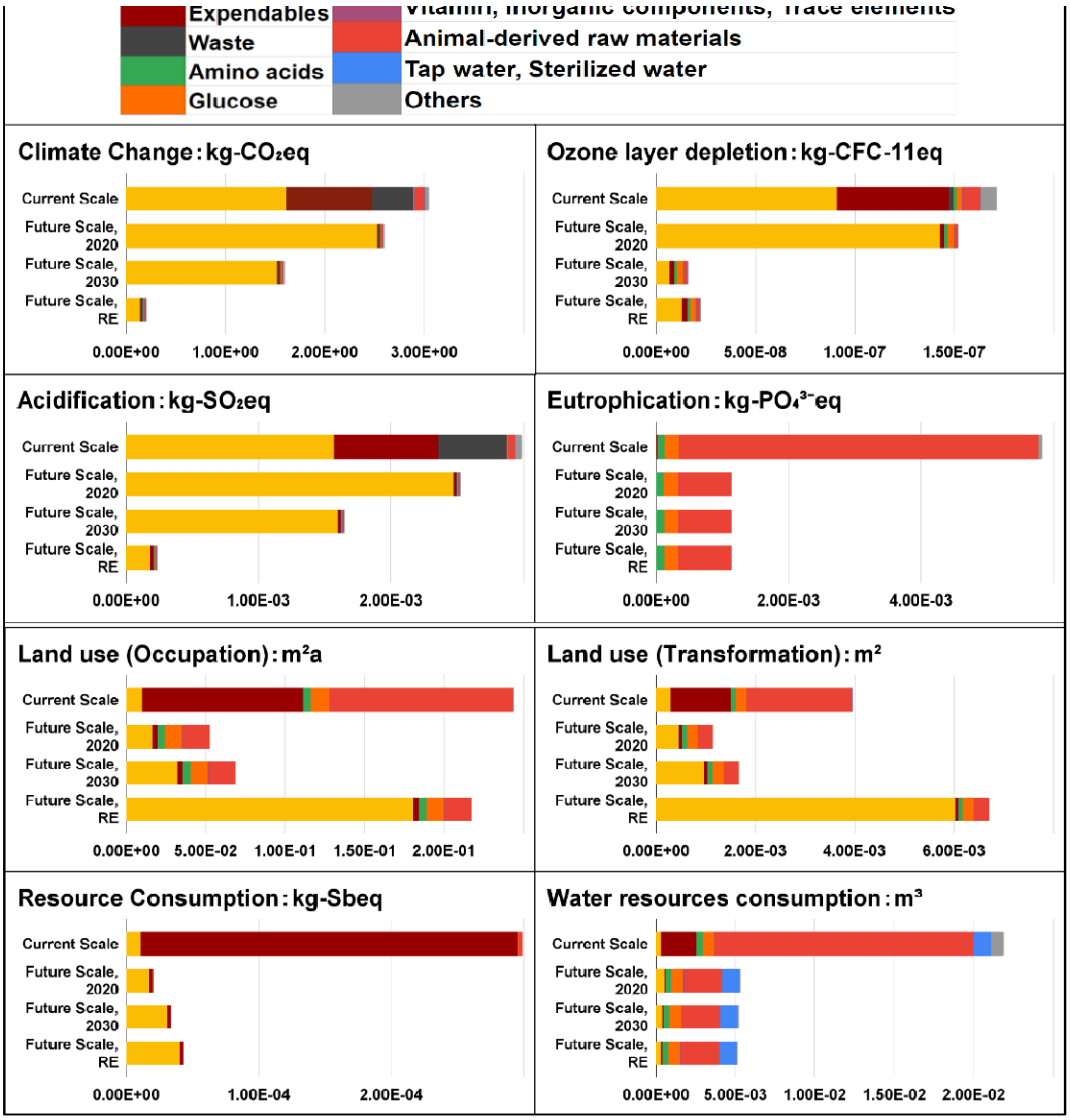


Figure 3. Environmental impact of each material for 1 L culture medium production.

Table 5. Environmental impact of 1 L culture medium production.

Impact Category	Unit	Current Scale	Future Scale, 2020	Future Scale, 2030	Future Scale, RE
Climate change	kg-CO_2eq	3.05E+00	2.60E+00	1.59E+00	2.11E-01
Ozone layer depletion	kg-CFC-11eq	1.72E-07	1.52E-07	1.62E-08	2.26E-08
Acidification	kg-SO_2eq	2.99E-03	2.52E-03	1.65E-03	2.37E-04
Eutrophication	kg-PO_4^{3-}eq	5.84E-03	1.15E-03	1.15E-03	1.15E-03
Land use (Occupation)	m^2a	2.44E-01	5.28E-02	6.90E-02	2.17E-01
Land use (Transformation)	m^2	3.95E-03	1.16E-03	1.67E-03	6.71E-03
Resource consumption	kg-Sbeq	2.99E-04	2.03E-05	3.42E-05	4.39E-05
Water Resources Consumption	m^3	2.18E-02	5.35E-03	5.23E-03	5.16E-03

3.1 Current Scale.

Electricity accounts for more than 50% of climate change, ozone layer depletion, and acidification. Animal-derived raw materials accounted for 47.5% to 92.8% of eutrophication, land use (Occupation and Transformation), and water resources consumption. Expendables accounted for a large proportion (10.1%–94.8%) in the seven categories except eutrophication. Therefore, electricity, animal-derived raw materials, and expendables are hotspots in the environmental impact of the current culture-medium production.

Regarding the breakdown of electricity consumption, 53% was consumed by adjusting the culture environment, such as temperature and air control, in the cell culture process to prepare serum substitutes, and 27% was consumed by sterilizing instruments and equipment. Thus, reducing electricity consumption through these processes is important. Cell culture is common in cultivated meat production, and considerable studies are being conducted to improve cell culture efficiency.[23-25] As the cell culture efficiency increases, the electricity consumption of this process is expected to decrease. In addition, because this process is performed on a laboratory scale, the

equipment and experimental apparatus are divided into smaller pieces, just as a bioreactor or other device comprises several parts. They must be sterilized individually, which tends to increase the number of sterilization procedures required. As technology becomes more established, these parts will be integrated into the device, and the number of sterilizations will be reduced. In sterilization, the larger the weight of the sterilization target, the lower the electricity consumption per unit weight.[26] Therefore, as the number of sterilization cycles decreases owing to more established technology, the electricity required for sterilization is also expected to decrease.

Animal welfare is generally recognized as one of the advantages of cultivated meat. It is necessary to avoid using animal-derived raw materials in the production of culture medium from the perspective of environmental impact shown in this study and animal welfare and consumer acceptance.[26] Therefore, there is a need to transition to using cell lines as a cell source or collecting biopsy cells from low-environmental-impact livestock without slaughter.[28,29]

Expendables accounted for a large proportion of the seven impact categories except for eutrophication, and the use of expendables increased the amount of waste. Therefore, it is essential to reduce the use of expendable products. Expendables include equipment such as conductors for connecting multiple bioreactors, experimental instruments such as pipettes, and rubber gloves for hygiene control. Equipment expendables are expected to be reduced by integrating all equipment parts and making them multiusable. In addition, expendables should be reduced through mechanization of the process, and the amount of expendables used per 1L of culture medium production should be reduced by improving the productivity of one lot.

3.2 Future Scale.

[Future Scale, 2020] decreased environmental impacts from 11.3% to 93.2% in all impact categories compared with [Current Scale]. Therefore, the transition from the current to future scale effectively reduces the environmental impact of culture-medium production.

[Future Scale, 2020] results showed that electricity accounts for a large share of 86.1–97.8% of the impacts of climate change, ozone layer depletion, acidification, and resource consumption. It is also assumed that with the transition from the current to the future scale, expendables and waste will decrease because of the multi-use of the entire equipment, mechanization of processes, and increased production of culture medium per lot. Animal-derived raw materials will decrease because of a change in the cell extraction source from eggs to cell lines. Consequently, the proportion of amino acids and glucose, the raw materials of the basal medium, is expected to increase in eutrophication, land use (Occupation and Transformation), and water resources consumption. However, animal-derived purified materials for assistance in the cell culture process will be used on a future scale, and animal-derived raw materials will account for a large proportion of eutrophication, land use (Occupation and Transformation), and water resources consumption.

This study used grains as the extraction source for basal medium ingredients, such as amino acids and glucose. However, a shift to algae and other extraction sources with lower environmental impact is necessary to further reduce the environmental impact of culture medium production in the future.[30] Also, as mentioned above, the use of animal-derived raw materials should be avoided. In the cell culture process for preparing serum substitutes, further research and development are needed in a direction that does not require animal-derived raw materials.

An increase in electricity consumption per 1L of culture medium production is assumed from the current to the future scale. Electricity is expected to account for a large share of the impact categories. Therefore, scenarios [Future Scale, 2030] and [Future Scale, RE] were considered with different electricity sources at future scales. For the main changes in the electricity source composition, in [Future Scale, 2030], it is assumed that the share of solar and nuclear power will increase and that the share of coal and LNG will decrease compared to [Future Scale, 2020]. The electricity composition of [Future Scale, RE] is assumed to consist only of solar and wind power, eliminating coal, LNG, nuclear power, hydroelectric power, and other sources accounting for a large share of the electricity composition in the other two scenarios. The share of renewable energy in electricity composition is assumed to increase in ascending order: [Future Scale, 2020], [Future Scale, 2030], and [Future Scale, RE]. As a result of these changes, the impact of ozone layer depletion is considerably reduced in [Future Scale, 2030] compared to [Future Scale, 2020]. However, the impacts of ozone layer depletion and resource consumption increased from [Future Scale, 2030] to [Future Scale, RE]. Compared with the environmental impacts of their respective current processes, they showed a slight increase. Impacts on climate change and acidification decreased considerably as the share of renewable energy in electricity composition increased. Eutrophication and water resources consumption were not significantly affected by changes in electricity composition. However, land use, both occupation and transformation, notably increased as the share of renewable energy increased.

The transition to renewable energy is essential for reducing the environmental impact of this culture medium, sustainable development, and solving various current environmental problems.[31] However, the results of this study indicated that an increase in the percentage of renewable energy in the electricity source mix used in the culture medium production considerably

increases the impact on land use. The impact of renewable energy on land use has been discussed in many previous studies.[32-34] Increased land use may lead to greenhouse gas emissions due to the loss of primary plant production and loss of biodiversity due to habitat loss, change, and fragmentation.[35] It may also undermine the advantage of cultivated meat, which requires less land use for meat production compared to livestock production, as shown in previous studies.[6–8] One way to address such issues related to renewable energy and land use is to use wastelands, building roofs, urban areas, and water surfaces as sites for power generation facilities, or share the land with other land uses such as agricultural land and pasture land.[34-36] Among these measures, those that are not difficult to adapt to culture medium production may include installing electricity generation facilities on the rooftops of production plants and sharing land by installing them within production sites. However, there is potential for debate regarding land use for renewable energy, as some argue that renewable energy uses less land than other forms of energy because the efficiency of power generation varies depending on local environmental characteristics, such as wind and solar radiation, and because additional fuel extraction and transportation are not required.[37,38] The priority is reducing electricity consumption to reduce the environmental impact of culture medium production. The results of this study indicated that the cell culture process for the preparation of serum substitutes consumes the most electricity at current and future scales. As described above, as the efficiency of cell culture improves, electricity consumption in this process and the overall production of the culture medium is also expected to be reduced. An increase in electricity consumption was assumed from the current to the future scale. However, this is mainly due to the technological shift that is expected to occur when adjusting the culture environment: at the current scale, multiple bioreactors can be managed together in a single incubator due to their small scale, and in the future, each bioreactor

will have to be managed individually as they become larger in volume. The impact of this change is significant. Therefore, it is possible that the benefits of scale can be realized if the scale is further increased from that assumed in this study. Thus, it is important to recognize that while using renewable energy in production is essential for producing this culture medium, it also involves issues such as land use. The first goal is to reduce electricity consumption. Comprehensively considering various environmental impacts and methods of installing power generation facilities is also necessary.

3.3 Basal Medium Components and Serum Substitute Components.

The results of this study are discussed with a focus on the basal medium and serum substitute components. Figure 4 shows the contribution of each substance to the respective impact categories by basal medium and serum substitute components at current and future scales.

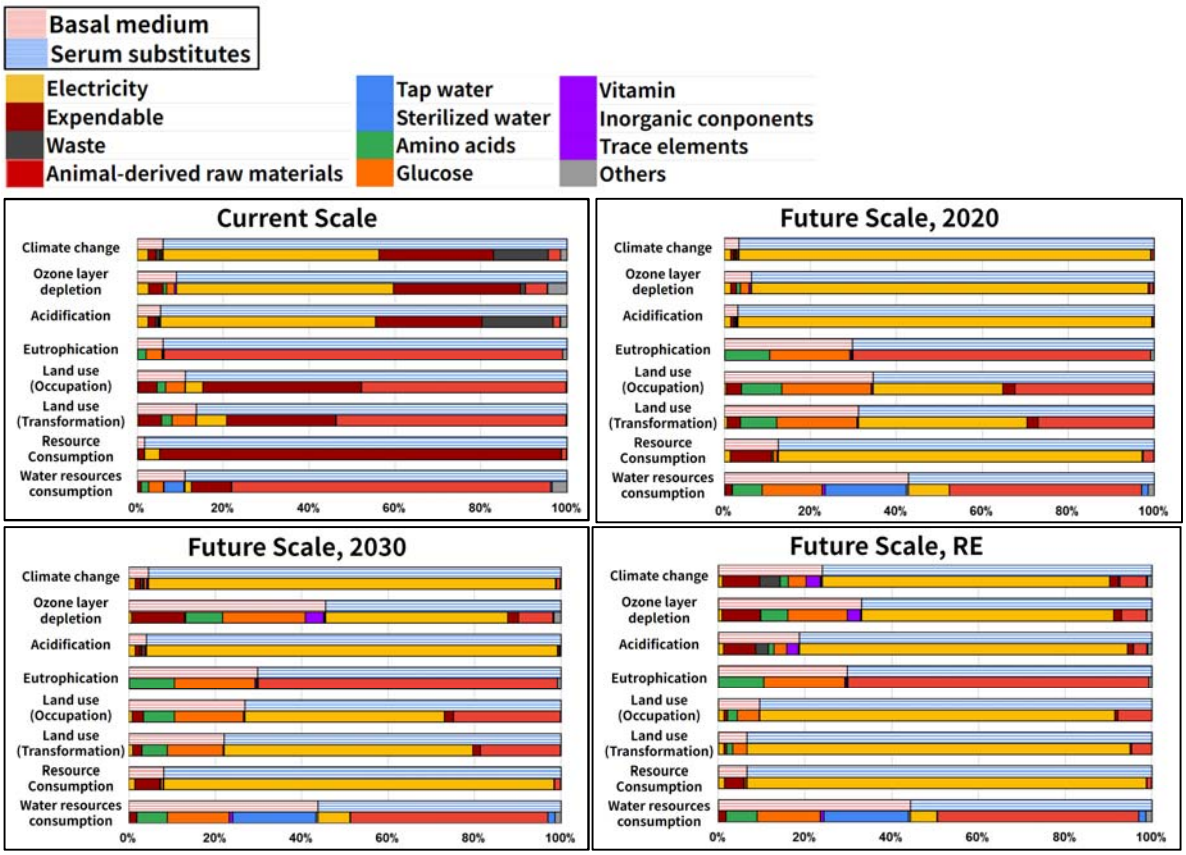


Figure 3. Contribution of each substance to respective impact categories by basal medium and serum substitute components at current and future scales.

At both scales, in all scenarios and impact categories, the proportion of serum substitutes was greater than that of the basal medium components. At the [Current Scale], the basal medium component accounted for 2%–14% of each impact category. The share of basal medium components increased with the transition to the future scale, accounting for 30%–43% of eutrophication, land use (Occupation and Transformation), and water resources consumption in [Future Scale, 2020]. In the [Future Scale, 2030], ozone layer depletion, eutrophication, land use (Occupation and Transformation), and water resources consumption accounted for 22%-45%,

and in the [Future Scale, RE], climate change, ozone layer depletion, acidification, eutrophication, and water resources consumption accounted for 19%-44%.

Tuomisto et al.[7] showed the contributions of amino acids, glucose, FBS, water, vitamins + minerals, and growth factors to the environmental impacts of DMEM+FBS and Essential8 production. This study compared the results of Tuomisto et al.[7] with those of this study for climate change, eutrophication, acidification, water resources consumption, and land use (Tuomisto et al.[7] do not distinguish between occupation and transformation). However, the results of the environmental impact of culture medium production by Tuomisto et al.[7] were evaluated using only the raw materials data listed above. They did not consider expendables, electricity, and waste from mixing and sterilization. In addition, FBS was substituted with the LCI data for urea, and growth factors were substituted with the LCI data for amino acids. Therefore, it should be noted that this is not a complete comparison.

For the basal medium, when electricity, expendables, and waste were not considered, the tendency of amino acids and glucose to account for a large proportion of all impact categories of eutrophication, climate change, acidification, water resources consumption, and land use were similar in Tuomisto et al.[7] and this study. The same tendency was observed in water resources consumption, with water in addition to amino acids and glucose accounting for large proportions. Therefore, as mentioned above, changing the source of extraction of basal medium raw materials to one with a lower environmental impact is necessary.

Comparing the proportion of serum substitutes in this study's results with the FBS of DMEM+FBS and growth factors of Essential8 in Tuomisto et al.[7], serum substitutes accounted for a considerably larger proportion in any scenario and impact category in this study; this is due

to the use of electricity and animal-derived raw materials in the cell culture process to produce serum substitute components and the addition of many expendables to these materials at the current scale. This study focused on co-culturing multiple cells as a substitute for serum components in cell culture. Still, other methods are commonly used as substitutes, such as combining various growth factors and recombinant proteins such as albumin, insulin, and transferrin.[39] Trinidad et al.[40] conducted a detailed environmental impact study of the growth factor. They indicated that the growth factor accounts for 0.4%–23% of the total global warming potential (GWP) of cultivated meat production and that the influence of growth factor production on the environmental impact of cultivated meat production may not be negligible. These proportions represent a percentage of the total GWP of cultivated meat production, which would account for a larger impact if it were a proportion of the culture medium production. Sinke et al.[8] evaluated the environmental effects of albumin, transferrin, and insulin-like growth factor (IGF) production using private company data. Sinke et al.[8] showed that albumin accounts for a large proportion of the environmental impact of culture medium production because the concentration in the medium is the highest when used. Therefore, based on the results of Trinidad et al.[40] and Sinke et al.[8] and those of this study, the serum substitute component is expected to account for a large proportion of the environmental impact of culture-medium production. Thus, the reduction in the environmental impact of serum substitute production in this culture medium is directly related to the decrease in the environmental impact of culture medium production, which is essential. As described above, measures to reduce or eliminate the use of expendables, animal-derived raw materials, and electricity, as well as to improve the efficiency and scale-up of cell culture, would greatly contribute to reducing the environmental impact of serum substitutes in this study and the culture medium overall.

3.4 Sensitivity Analysis.

Laboratory equipment changes frequently because cell cultures are extremely sensitive to contamination with bacteria, microorganisms, and other contaminants. Consequently, the number of expendables used, such as experimental instruments and equipment, varies widely. Therefore, a sensitivity analysis was conducted in this study by examining the impact of a 10% change in the amount of expendables used on the results of this study.

Figure 5 shows the percentage change in the environmental impact of culture-medium production in each scenario for a 10% change in expendable usage. The sensitivity analysis showed that the [Current Scale] was most affected by the change in expendable usage in all impact categories under all scenarios. For resource consumption, the impact was a 9.5% change, followed by climate change, acidification, ozone layer depletion, and land use (transformation and occupation). However, eutrophication and water resources consumption were less affected by changes in expendable usage in all scenarios. In the [Future Scale, 2020], resource consumption; in the [Future Scale, 2030], ozone layer depletion; and the [Future Scale, RE], climate change, ozone layer depletion, and acidification, a change in the environmental impact of more than 1% was observed for a 10% change in expendable usage. However, no change was observed in the magnitude relationship between scenarios owing to a 10% change in expendable usage across all impact categories. Therefore, the results of this study were relatively stable, with a 10% change in expendable usage.

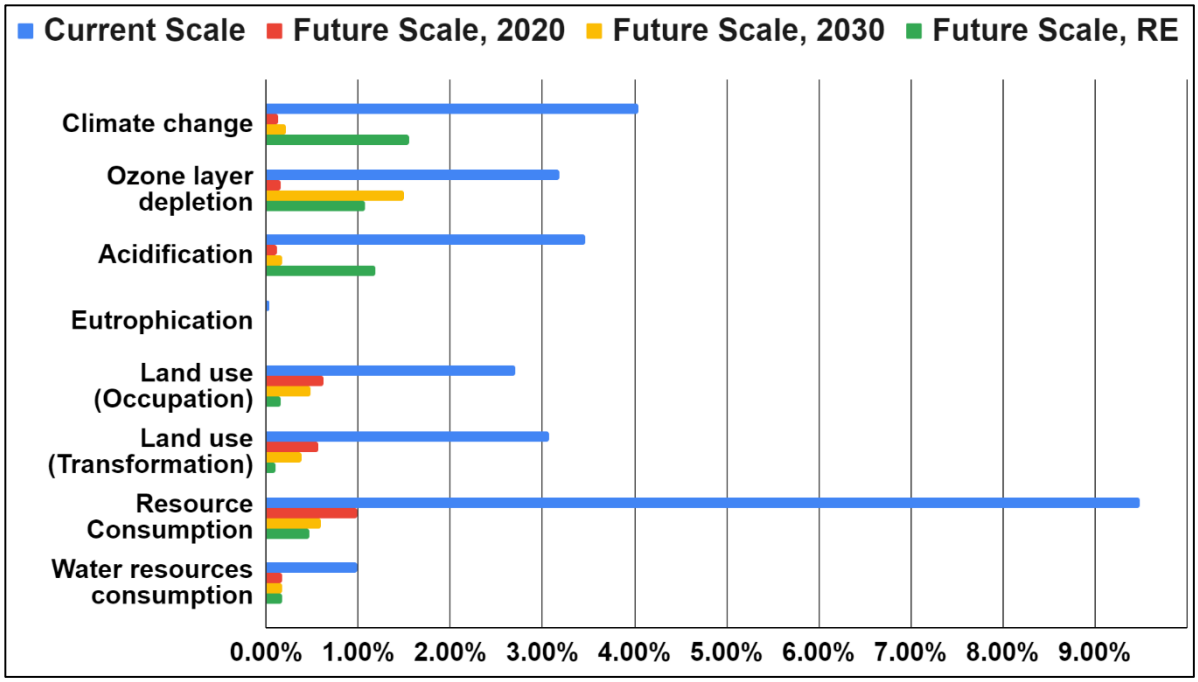


Figure 4. Sensitivity analysis results: The percentage change in the environmental impact of culture medium production for a 10% change in expendable usage.

3.5 Limitations and Future Prospects of This Study.

Various culture media and production methods are available, and research and development are currently in progress. Although this study evaluated serum-free food-grade and complex culture media, the environmental impact is expected to vary depending on the type of culture medium and production method. Therefore, although there are currently no detailed LCA studies for culture media other than this one, it is essential to conduct LCA studies for different types and production methods of culture media in the future and compare the results with those of this study, as this will contribute to entirely reducing the environmental impact of culture media. In addition, the data used in this study were collected from only one company because there is currently only one example of a serum-free food-grade and complex culture medium produced using cell interaction. Thus, the results of this study may differ from those of LCA for other

cases using the same culture medium type. Therefore, LCA studies should be conducted if other cases related to the same culture medium type arise. Moreover, although this study conducted an LCA only on the culture medium production process, the ultimate purpose of the culture medium under study was to cultivate meat. An LCA covering the entire cultivated meat production process will be necessary in the future, which will require the findings of culture medium production obtained in the present study.

ASSOCIATED CONTENT

Supporting Information. S.1. Prediction of electricity consumption of bioreactors used at future scales; S.2. Details of the basal culture medium raw materials; S.3. Alternative methods for determining environmental burden intensity; S.4. Life-Cycle Impact Assessment (LCIA).

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ABBREVIATIONS

LCA, life cycle assessment; FBS, Fetal Bovine Serum; ISO, International Organization for Standardization; DMEM, Dulbecco's Modified Eagle's Medium; CIP, Cleaning in Place; SIP, Sterilization in Place; RE, Renewable Energy; SI, Supporting Information; IDEA, LCI database IDEA Ver. 3.3; LIME2, Life-cycle Impact assessment Method based on Endpoint modeling 2; GWP, global warming potential.

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