STUDY

Review and gap-analysis of LCA-studies of cultured meat

for The Good Food Institute

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Submitted by

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Abbreviations and acronyms

CED	Cumulative Energy Demand
CHO	Chinese hamster ovary (CHO)
DM	Dry matter
ELCD	European Life Cycle Database
FU	Functional Unit
GWP	Global Warming Potential
HVAC	Heating, Ventilation, Air-conditioning
ILCD	International Reference Life Cycle Data System
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
NREU	Non-renewable energy use
PLA	Polylactic acid
WULCA	Water use in Life Cycle Assessment

1 Goal and background of the study

An alternative for conventional livestock production is the so-called in-vitro cultivation of edible meat. This "artificial meat", also referred to as "cell-based meat," "cultured meat," or "clean meat", bears the potential to alleviate environmental, food security, human health, and animal welfare concerns associated with industrialized livestock herding and slaughtering for conventional meat production (Post 2012, Heinrich-Böll-Stiftung 2018).

The Good Food Institute (GFI) is a U.S.-based nonprofit organization that promotes plant-based meat, dairy and eggs as well as clean meat. The organization launched in February 2016 with the vision of creating a healthy, humane, and sustainable food supply. GFI targets scientists, policy makers and entrepreneurs to promote plant-based products and cellular agriculture.

GFI's campaigning work needs to be based on sound evidence regarding the environmental benefits of the promoted meat alternatives. Life Cycle Assessments (LCA) are the most appropriate tool for such an evaluation. There is already a multitude of LCAs regarding cultured meat. This review and gap-analysis of life cycle assessment (LCA) studies of cultured meat production systems aims to provide recommendations for a prospective LCA study of cultured meat in the near future.

The study first provides an overview of methodological specifics of the analyzed studies. Furthermore, the underlying data as well as assumptions made in the studies are analyzed and gaps are identified. The environmental impacts and the hotspots of the individual processes are reviewed. The report concludes with a discussion of the earlier described methodologies, data and findings, which leads to recommendations (e.g. goal and scope, functional unit, system boundaries) for a future LCA study of cultured meat.

2 LCA-studies on cultured meat production

To date, only a very limited number of environmental impact studies on cultured meat production are available. The analysis will be based on the publications shown in **Table 1**, which are to our knowledge the only publicly available LCA-studies of cultured meat so far.

The most recent LCA study available, carried out by Mattick et al. in 2015, used serum free media with soy hydrolysate as feedstock. The metabolic requirements of Chinese hamster ovary (CHO) cells were used as no data of skeletal stem muscle cells proliferation and differentiation were available to the authors. The anticipatory LCA-study assumed an annual production of 66,000 kg meat with a production size of 90 m^3 in the United States of America.

The first ever LCA study to be published on artificial meat was conducted by Tuomisto and Mattos in 2011. They projected an industrial-scale production site of cultured meat in different locations (Spain, Thailand, California). The projected scale was assumed with 30 m^3 production volume and 1,000 kg meat output per batch, with cyanobacteria hydrolysate as the main feedstock and stem cells from animal embryos.

In 2014, Tuomisto et al. amended the previous study by considering alternative production scenarios in Spain, in which the cyanobacteria-based feedstock was substituted with wheat and corn as energy and nutrient source. Moreover, instead of stirred-tank bioreactors, hollow-fiber reactors were assumed. The analysis stated the results of worst-case and best-case scenarios, depending on parameters like initial cell density and theoretical cell yield.

Smetana et. al. (2015) compared six meat analogues among others including chicken, in-vitro meat and insects. The study included the production of in-vitro meat, which relied on the data of Tuomisto and Mattos

Table 1: Reviewed LCA-studies

Author	Year	Title
Mattick C.S., Landis A.E., Allenby B.R., Genovese N.J.	2015	Anticipatory Life Cycle Analysis of In Vitro Biomass Cultivation for Cultured Meat Production in the United States
Tuomisto H.L., Teixeira de Mattos M.J.	2011	Environmental impacts of cultured meat production
Tuomisto H.L., Ellis M.J., Haastrup P.	2014	Environmental impacts of cultured meat: alternative production scenarios
Smetana S., Mathys A., Knoch A., Heinz V.	2015	Meat alternatives: life cycle assessment of most known meat substitutes

(2011) and Tuomisto et. al. (2012). It has to be mentioned that the cyanobacteria cultivation was modified with data from Smetana and Sandmann (2017), which according to the authors seemed to be more reliable and accurate. The goal was an evaluation of ready-to-eat meal at the consumer level and therefore amended the previously mentioned studies by further downstream processes like meat processing, distribution of product and final preparation at the consumer's home.

3 Method

This chapter describes the methodology and data used of the publications reviewed. It includes the respective scope such as functional unit, system boundary, geographical and temporal representativeness, multi-functionality, and impact categories as described in the International Organization for Standardization (ISO) standards 14040/14044. All studies attempted to follow the ISO standards of LCA, only Mattick did not state the compliance as according to the authors the interpretation of the guidelines could probably differ from those of others.

3.1 Functional unit and reference products

The functional unit provides a reference to which the inputs and outputs are related (Klöpffer and Grahl 2009). It defines qualitative and quantitative aspects of the good or service under study along with the questions: "what", "how much", "how well", and "for how long". The functional units used in the studies are stated in **Table 2**. It has to be noted that the protein and dry matter vary throughout the studies.

The reviewed LCA-studies compared lab-grown meat to numerous reference products e.g. beef, chicken, sheep, lamb, pork, poultry, fish, insect, soy meal, mycoprotein, milk, eggs and pulses.

3.2 Multifunctionality

If a product system or process has more than one output (i.e. multifunctionality), a procedure to distribute the impacts over all outputs need to be applied. For multifunctional products and multiproduct processes, the step-wise procedures (1) avoidance of allocation by sub-division, (2) system expansion, (3) physical and (4) economic allocation shall be applied according to ISO 14040/44. It is a well-known problem that the choice of allocation method may influence the results significantly.

Tuomisto (2011) and Smetana applied only weight-based allocations (e.g. biomass suitable for hydrolysis) throughout the study. The mass allocations for the cyanobacteria made in Tuomisto (2011) apply for the amended study of Tuomisto (2014) as well. Further, the allocation of feedstocks of by-products of wheat and corn feedstock production (Williams, Audsley et al. 2006) were allocated by economic value.

Mattick's environmental impacts for in-vitro biomass inputs and their coproducts are allocated on a gross chemical (calorific) energy basis (e.g. corn wet milling).

For the benchmarking of Tuomisto's and Mattick's results, the environmental results of beef, pork, lamb and poultry from different literature were used. The results were reported in live weights or carcass weights, for which different conversion factors (edible meat of live weight, beef 37% - 43%, pork 56%, lamb 34% and poultry 56%) were used. In order to obtain a comparable functional unit of edible biomass, an economic allocation was applied such that impacts were assigned to meat and its coproducts on the basis of relative market value (88.5% to 92.4% to edible parts).

Table 2: Functional units of reviewed studies

Study	Functional Unit
Mattick	1 kg of Chinese hamster ovary (CHO) cell biomass with 17% dry matter and 7% protein content
Tuomisto (2011)	1 kg cultured meat with 30% dry matter and 19% protein content (minced beef type)
Tuomisto (2014)	1 kg cultured meat with 30% dry matter and 19% protein content (minced beef type) 1 kg protein
Smetana	The satisfaction of a consumer with 1 kg protein-enriched product ready for the consumption Calorific energy content (3.75 MJ) of ready for consumption product Digestible protein content in final product

3.3 System boundaries

The system boundary sets which processes are part of the product system and are included in the assessment. The boundaries of the reviewed studies are shown in **Figure 1**. While Mattick and Tuomisto chose a cradle to factory-gate approach, Smetana analyzed the environmental impacts from cradle to consumer table.

The cleaning of the bioreactor and facility energy requirements were only considered by Mattick, while on the other side the reactor production was only considered by Tuomisto. Both included different nutrient media productions, the sterilization and hydrolyzation as well as the cell cultivation (by Mattick split into proliferation and differentiation). Furthermore, both excluded the growth factor production and stem or CHO cell collection as well as the wastewater treatment, the spent media recycling and the treatment of other waste products.

3.4 Life Cycle Impact Assessment (LCIA)

LCIA provides the foundation for analyzing the potential contributions of resource extractions and emissions in a life cycle inventory (LCI) to a number of potential environmental impacts. LCI results are, according to ISO 14044, classified into impact categories, each represented by a category indicator. The relative contribution of each input and output within the product system is assigned to impact categories and converted into indicators that represent the corresponding potential impacts on the environment. The result obtained in the classification phase are multiplied by the characterization factors of each substance within each impact category (Menoufi 2011). There are several different LCIA-methods (e.g. ReCiPe, CML, TRACI, IMPACT) available, which differ in quantity of impact categories and height of characterization factors.

Smetana used ReCiPe v.1.08 (Heijungs, Huijbregts et al. 2009) and IMPACT 2002+ while Mattick used CML 2001 and the Cumulative Energy Demand method. Tuomisto used, among others, the IPCC method and a water footprint method from Kounina et. al. (2012). Tuomisto did not mention which exact method was used to assess the primary energy demand. Land use is so far only restricted to direct land use, as for indirect land use change the cause-effect relationship is difficult to identify as well as to quantify, although this phenomenon has indeed significant effects especially on the GWP. Still, due to the methodological difficulties, indirect land use change is not considered in any of the investigated studies. For land use, Tuomisto assessed the land requirement in hectare. Mattick quantified the land occupation according to the ecological footprint method by Frischknecht and Jungbluth (2007) associated with human activities while excluding time-integrated land in m^2a. Smetana assessed land occupation in m^2a according to Heijungs et al. (2009). Water use was assessed in Tuomisto (2011) according to Milá I Canals et al. (2009) and Tuomisto (2014) according to Kounania et al. (2012) which uses the blue water footprint and country-specific water scarcity characterization factors.

All studies emphasize the relevance of global warming potential, energy, water and land use. Mattick also analyzed the edible energy produced and the eutrophication potential. Smetana considered many more impacts such as depletion of resources, ecotoxicity and acidification, considering the comparison of other meat products in the analyzed studies.

4 Data acquisition and Life Cycle Inventory

In the inventory phase of an LCA, a system model is constructed that models the emissions and resource consumption for each stage of the life cycle of the analyzed product. (DIN ISO 14040, 2006, DIN ISO 14044, 2006) This chapter focuses on the clean meat production processes modelled in the different LCA-studies.

The lab-grown meat of Smetana is based on data of Tuomisto (2011) and Tuomisto et. al. (2012), the inventory data will not be discussed in this chapter. All analyzed studies are based on hypothetical production processes and simulation models as currently no large-scale production facility of clean meat exists. Hence, all studies heavily rely on assumptions, literature and calculations based on mathematical formulas. The key assumptions of the cell cultivation of the three studies and additional data considered are stated in **Table 3**.

Mattick:

The cultured meat is assumed to be produced with a serum free media supplemented with soy hydrolysate. The metabolic requirements of muscle cell cultivation

Figure 1: System boundaries of analyzed studies

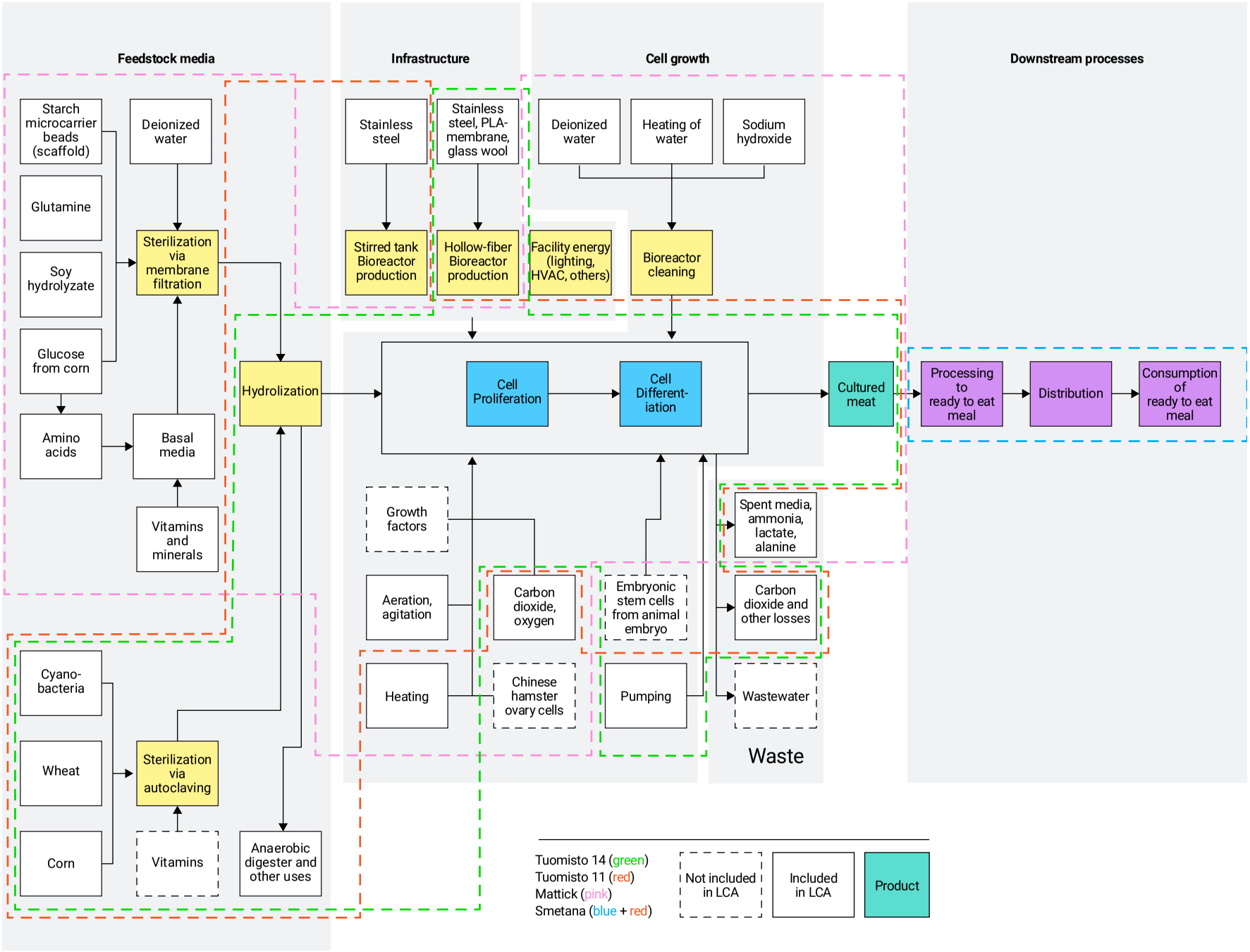


Table 3: Key assumptions and considered data of LCA-studies (adapted and amended from Mattick et. al. (2015))

	Mattick	Tuomisto (2011)	Tuomisto (2014)
Feedstock origin and amount	Glucose, glutamine, soy hydrolysate and basal media (total: 1.86 kg / kg meat)	Cyanobacteria hydrolysate (0.72 kg / kg meat)	Cyanobacteria hydrolysate (0.72 kg / kg meat), wheat, corn (2 kg / kg meat)
Cell origin and metabolic requirements	Chinese hamster ovary (CHO)	Stem cells from animal embryo	Stem cells from animal embryo
Initial cell density in bioreactor	$2x10^5$ cells/mL	not stated	$1x10^6$ cells and $2x10^4$ cells
Maximum cell density	$4x10^6$ cells/mL to account for growth inhibition due to metabolic by-products	$1x10^7$ cells/mL	$1x10^8$ cells/mL and $2x10^8$ cells/mL
Mass of one cell	$3.5x10^{-12}$ kg (17% DM, 7% protein (42% on a DM basis))	$3.33x10^{-12}$ kg (30% DM, 19% protein)	
Batch duration	11 days (Proliferation: 5 days, differentiation: 3 days, cleaning: 3 days)	60 days (cell cultivation)	90 days (cell cultivation)
Scaffold material	Corn starch microcarrier beads	Excluded	Excluded
Bioreactor design	6x15.000 L stirred-tank reactors, filling capacity: 100%	30x1.000 L stirred-tank reactors, weight 93 kg, filling capacity 80%, 20 years lifetime	hollow-fibre bioreactor, membrane from PLA, 5mm thick stainless steel and 25mm thick glass wool, 20 years lifetime
Agitation/mixing	449 W (29.9 W/m^3), 1.5 m/s	16 W/m^3, 100 rpm	pumping calculated with low efficiency of 0.5, 16 W/m^3, 100 rpm
Aeration/sparging	Atmospheric air at 2 kg O_2/kWh plus 4% CO_2	0.05 vvm	0.05 vvm
Sterilization of culture medium	Microfiltration membranes	Autoclaving	Autoclaving
Deionization of water	Included	Excluded	Excluded
Culture temperature	37°C	37°C	37°C
Energy for heating water	23°C to 37°C	Excluded (could be considered part of the sterilization energy)	Excluded (could be considered part of the sterilization energy)
Energy to maintain cell culture temperature	Included	Excluded	Included

are based on Chinese hamster ovary cell cultivation. The material and energy flows of the proliferation and differentiation phase are based on a simulation model with many assumptions. The nutrient media is changed between these phases. In total, a batch duration of 11 days is presumed (proliferation 5 days (based on hybridoma cells), differentiation 3 days and cleaning 3 days). It was assumed that the proliferation is terminated before ammonia concentration reaches 2 mM, as higher concentration would inhibit further cell growth. Corn starch based microcarrier beads were assumed as scaffold material in the bioreactor. Due to the fact that no large-scale cultured meat production facility exists so far, the facility size of a brewery was assumed. The mix of fuels for meat production was assumed to be the same as for the brewery industry. The required energy of the building for lightning, HVAC and other purposes was assumed to be equivalent to a warehouse. The cleaning of the bioreactor is assumed in a three-step procedure from literature (In the beginning and end the reactor is rinsed with deionized water, sodium hydroxide solution and heating up to 77.5°C). Further, Mattick presumed that all dry ingredients travel 500 km by diesel truck.

Tuomisto (2011):

Tuomisto (2011) assumes cyanobacteria hydrolysate as energy and nutrient source with a protein content of 64% dry matter for growth and proliferation of the muscle cells. 720 g of biomass from cyanobacteria is assumed to produce 1 kg of meat. The cyanobacteria biomass flows are based on experience from lab scale production at the University of Amsterdam. The production in an open pond, harvesting, facility, maintenance, transport and sterilization (auto-claving) are based on estimated, calculated and experimental data for example for diesel and electricity requirements. It is assumed that the initial water input for the cyanobacteria system is seawater. The stem cells from animal embryos are grown in a cylinder stirred-tank bioreactor with the hydrolysate, growth factors and vitamins. The biomass flows are based on laboratory scale production data with an assumed hydrolysis yield of 50% of cyanobacteria biomass whereas only 20% is used for anaerobic digester and is therefore allocated to the cultured meat production. The assumed culture meat yield is 50% of the hydrolysate and the other 50% are lost in form of CO_2 air emissions and other losses. The power input for agitation and aeration was estimated based on literature or simulation models. The volume of the culture is assumed to be 30 m^3, by assuming maximum muscle cell density of $1x10^7$ cells/mL and weight of a cell $1x10^{-12}$ kg/cell. Therefore, each reactor with a volume of 1 m^3 produces 10 kg dry matter (DM) of cultured meat during 60 days in 37°C. Water needed for muscle cell cultivation is 30 m^3, and the DM content of the end product (cultured meat) is 30%. It is assumed that 80% of the water used for the cell culturing process is recycled without any treatment.

Tuomisto (2014):

Tuomisto (2014) is based on the assumptions made in Tuomisto (2011) with further small adaptations. Beside hydrolysate from cyanobacteria, wheat and corn were assumed as feedstock. For the production of 1 kg cultured meat 2 kg of wheat and corn feedstock were assumed. Instead of a stirred-tank bioreactor the process includes a hollow fiber reactor which helps to replicate the capillary system. The cell density was assumed with $2x10^8$ cells/mL in a best-case scenario and with $1x10^8$ cells/mL in the worst-case. Overall, a 90-day production period at 37°C was assumed. The pumping was expected to have a low efficiency of 0.5, electric heating was used for the reactor and the nutrient media was assumed to be changed every three days.

Professional LCAs shall use professional software to create a model of the production process with the collected inventory data. Further software can be used to assess multiple scenarios, sensitivity and allow the use of professional LCI-database for robust and reliable results. The used LCA-software and LCI-databases vary through all analyzed studies. Smetana and Mattick used SimaPro (version 8 and version 7.3.3) linked to databases like ecoinvent (version 3 and version 2.0), US LCI, ELCD (version 2.0) and DK food LCA database. Tuomisto instead used Microsoft Excel with the ELCD database to assess the environmental impacts.

Sensitivity and scenario analysis

According to ISO 14044, sensitivity analysis is a systematic procedure for estimating the effects of the choices made regarding methods and data on the outcome of a study. The analysis evaluates the robustness of the study results and conclusions by varying data and assumptions made in the study. Sensitivities can be assessed with the Monte-Carlo simulation. It is a numerical method to solve mathematical problems by replacing point estimates with random variables. The method enables simulation of any process whose development is influenced by random factors (Liu 2006).

Tuomisto (2011) and Mattick performed a Monte Carlo Analysis to assess model sensitivity in identified areas of uncertainty. Mattick included the sensitivity of facility size, facility energy consumption, cell growth rate, cell density, mass increase during differentiation, and crop yield for corn and soy beans. Tuomisto (2011) included the parameters cyanobacteria protein content, hydrolysis yield, culture meat yield during muscle cell cultivation, construction and maintenance of cyanobacteria, production ponds, harvesting and cultivation of bacteria, fertilizer, transportation distances, electricity consumption of sterilization, steel, aeration, rotation, allocation of cyanobacteria and fresh water use. Furthermore, the geographical location of the production process (California, Spain and Thailand) was assessed in scenarios.

Tuomisto (2014) chose to analyze the impact of different feedstocks. Besides cyanobacteria hydrolysate, corn and wheat were chosen in a scenario analysis. For the muscle cell cultivation, a best and worst case for initial and maximum cell density were assessed as well.

Smetana verified the variability of results by choosing two alternative functional units (3.75 MJ energetic value of final product and 0.3 kg of digested proteins). He also chose an alternative LCIA-method (IMPACT 2002+), but the results of this alternative differ little from the previously chosen method.

5 Review of potential environmental impacts

This chapter provides the findings of potential environmental impacts of the cultured meat production of the reviewed studies. Tuomisto and Mattick show a similar trend for the impacts of the cultured meat production systems, with comparable energy use and lower global warming potential, lower land use and lower water use for cultured meat compared to beef. Smetana shows much higher environmental impacts for the ready to eat meal made from cultured meat compared to conventional meat and other meat substitute meals. According to the author the higher impacts can be explained due to different data for the cyanobacteria cultivation and higher related impacts as well as the additional steps of processing, distribution and consumption of the meal.

Global Warming and Energy Demand

Figure 2 and **Figure 3** show the GWP and the CED of cultured meat production from Mattick and Tuomisto (2014). The triple amount of GHG and energy use impacts in Mattick can be explained by the altered composition of the growth medium (mainly basal media and glutamine production), as well as the cleaning of the reactor, which was considered in Mattick's study. Tuomisto (2014) stated the major energy inputs in the cultivation of cultured meat consist of the heating energy required to heat the nutrition media and maintain the bioreactor temperature at 37 C°. Therefore, the preliminary hypothetical calculation of the bioreactor energy use had the highest contribution to the primary energy and GHG emissions of cultured meat production. The energy requirement may be reduced by modifying the process and reactor, for example, by using heat exchangers. More research is required for developing suitable bioreactors for large scale cultured meat production.

Water and land use

As mentioned in chapter 3.4, different LCIA-methods were used to calculate the water and land use. Hence no direct comparison of the results is possible. Tuomisto (2014) showed that the muscle cell cultivation accounted for 82% of the indirect water use and the highest water input was needed for replacement of evaporation loss in cyanobacteria cultivation and for muscle cell cultivation (blue water). Mattick and Smetana did not assess any water use. Due to the agricultural processes the feedstock production (e.g. basal media, soy hydrolysate) is the main contributor to land use in both results of Tuomisto and Mattick. The land use/occupation in m^2/1 kg ready to eat meal of Smetana's analysis was 2 to 5 times lower than competitor products.

6 Discussion

Goal and scope

ISO 14040/44 states that the goal and scope shall be clearly defined and shall be consistent with the intended application. The intended application, the reasons for carrying out the study, the intended audience and whether the results are intended to be used in comparative assertions intended to be disclosed to the public shall be stated. The scope of an LCA shall clearly describe the product system studied, i.e. the functions of the product system, the functional unit, system boundary, allocations, LCIA methodology, assumption, limitations, data requirements, data quality and others. In general, goal and scope sets the basis for an LCA study and is therefore essential.

All analyzed LCA-studies provided information on what portion of global burdens can be associated with the clean meat production and hence fall under the LCA-mode attributional. Moreover, the reviewed studies estimate product systems that do not exist in large scale and are forward-looking and prospective approaches.

For GFI, an attributional LCA estimating the present and future life-cycle environmental impacts using scenarios is recommended. Comparison of final results with competitor products is further recommended. The study should assess the present and the future process and should clearly describe the scale up to a commercial process, so that readers can understand the main assumptions and bottlenecks. The goal and scope of the study may be revised due to unforeseen limitations, constraints or results.

Functional Unit

A comparison only considering the mass of the meat (specially for minced meat) is not sufficient and may lead to false conclusions. For example, a much higher water content in the meat could lead to much lower environmental impacts, but the analyzed product would be of lower quality. The nutritional value of the clean meat could be customised to the needed food application in the future. This advantage over conventional meat is not clearly indicated by the analyzed LCA-studies. So far, all studies used a weight-based functional unit with indication of dry matter and/or protein content (see **Table 2**).

Figure 2: Global Warming Potential of cultured meat

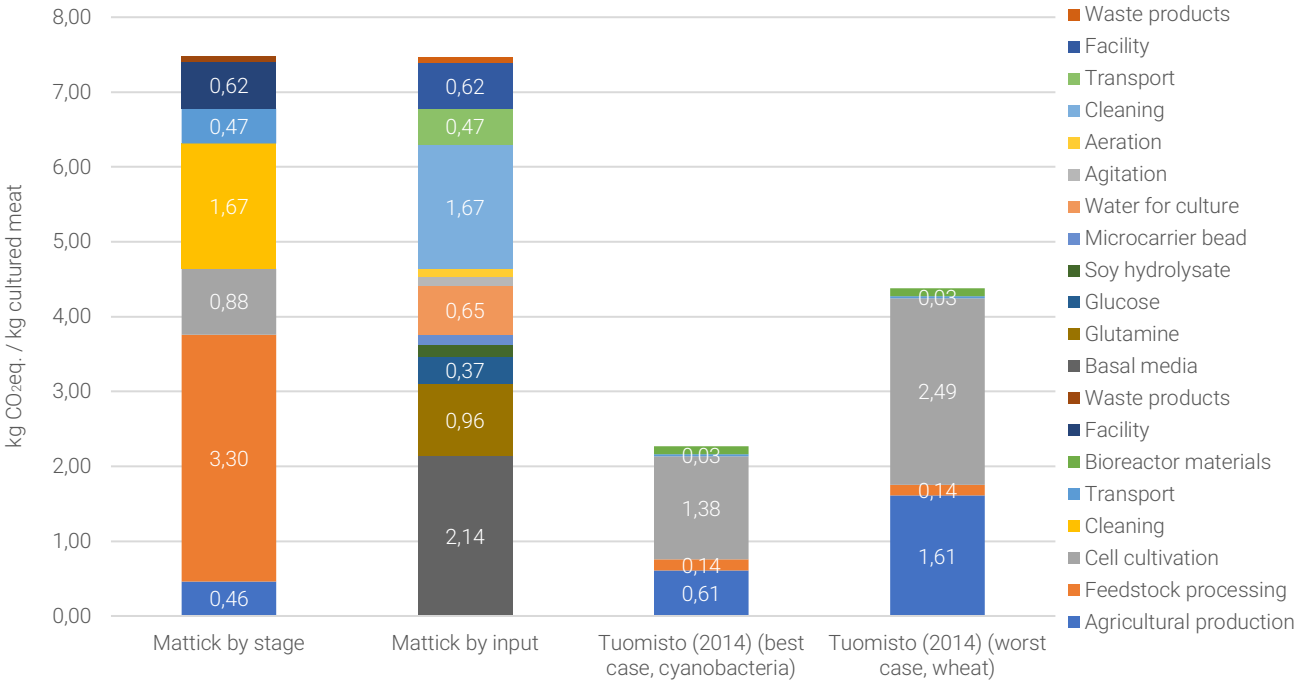
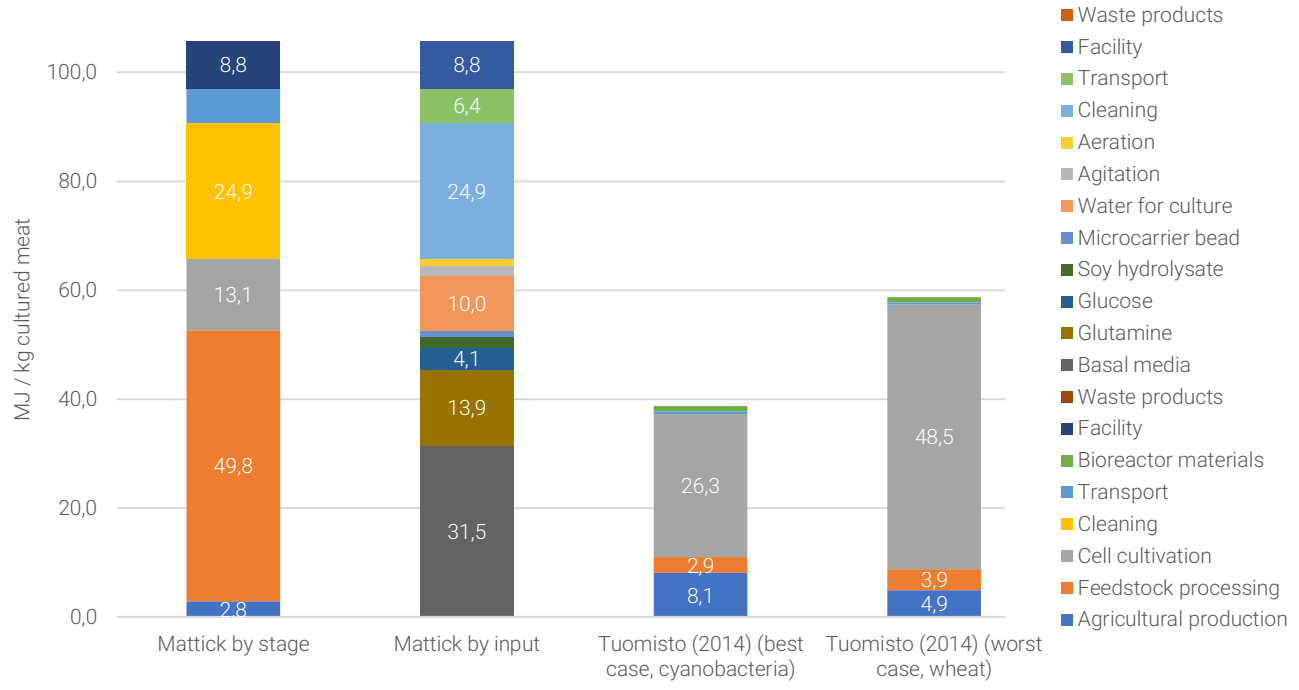


Figure 3: Cumulative Energy Demand of cultured meat



For example, Tuomisto compares cultured meat to beef production in Ireland (Casey and Holden 2006) and Sweden (Kumm 2002), which do not consider nutritional value (dry matter, protein, energy and water content). Furthermore, the conventional meat composition (fatty acid composition, fat, cholesterol, vitamins, minerals, lipids content) is influenced by species, sex, age, physical exercise and nutrition. The fatty acid composition for example has effect on meat quality, fat tissue firmness, color, shelf life and flavor (Cobos and Díaz 2014). Therefore, for a comparison with competitor products, the nutritional and quality aspect have to be considered in the functional unit.

Potential reference products for a comparative LCA-study

The LCA studies compared lab-grown meat to numerous reference products e.g. beef, chicken, sheep, lamb, pork, poultry, fish, insect, soy meal, mycoprotein, milk, eggs and pulses. As the name lab-grown meat already states, the main purpose of meat cultivation is the replacement of conventional meat. For that reason, in-vitro meat tries to mimic the texture and taste of traditional meat products. The skeletal muscle cells utilized for cell cultivation are taken from meat-supplying animals. Therefore, on one hand the comparison with beef, chicken, lamb and other meat seems to be most suitable. On the other hand, considering only the nutritional value (e.g. protein content) of all kinds of products, whose main purpose is the supply of protein would be suitable for comparison. As the human body cannot produce all amino acids themselves, essential amino acids have to be consumed with food. Consequently, the value of the proteins (essential and non-essential amino acids) would be interesting to be covered in a new study. Of course, the choice of reference products strongly depends on the goal of the study as well as the function and purpose of the product.

Multifunctionality

The allocation methods varied throughout the studies. Side-streams and by-products (e.g. feed grade) that accrue during the cultured meat production (e.g. ammonia, lactate, CO_2 and alanine) could potentially lower the environmental burdens of the meat. By allocating some of the environmental impacts to these streams and products, e.g. by physical or economical value, the burdens of the production process could be lowered. Therefore, it is recommended to consider and allocate these streams and products, which can have a high influence on the outcomes of the LCA study.

For the benchmarking products, Mattick and Tuomisto used similar conversion factors and economic allocation. In a future study it has to be taken care that the allocation of environmental impacts of counterparts are stated. Any discrepancies should be clearly described and justified. A sensitivity analysis of the different suitable allocation methods as provided by Mattick would be helpful to fully understand the impact of the choices made.

System boundaries

As shown in **Figure 1**, the processes, design and materials considered vary throughout the LCA studies. To analyze the impacts related to the cultured meat production, a cradle to factory gate approach would be suitable but shall be consistent with the goal and scope of the study. The following attributional processes should be part of the product system and are classified as upstream processes:

- Feedstocks/Nutrient media cultivation and processing
- Production of semi-products used in the core process (e.g. deionised water, scaffold materials)
- Production of other ingredients (e.g. growth factors, minerals, vitamins)
- Production of auxiliary products used such as acids for cleaning
- Impacts due to the production of electricity and fuels used in the upstream module
- Manufacturing of primary, secondary and tertiary packaging
- External transportation to the core processes
- Upstream processes not listed may also be included

The following attributional processes should be part of the product system and classified as core processes (cell cultivation, gate to gate):

- Internal transportation
- Feedstocks/Nutrient media hydrolyzation and sterilization
- Cell cultivation (e.g. split up into proliferation and differentiation, but as detailed as possible)
- Harvesting of meat from scaffold material or other harvesting activities
- Bioreactor and equipment cleaning
- Waste and wastewater treatment (waste generated during cell cultivation and other core activities)

- Recycling and reuse of media and other materials (e.g. wastewater, scaffold)
- Manufacturing processes not listed may also be included

The production of the raw materials used for production of the product shall be included. A minimum of 99% of the total weight of the declared product and its packaging shall be included. Animals required for stem cell and cell extraction, growth factors and vitamins could be neglected if lower than 1% of the total weight, but as the impacts of these products are unknown, neglecting is not recommended. That said, it may be very difficult to obtain reliable data.

Additional to the above-mentioned processes aligned with the ISO 14040, it is recommended to include the manufacture, maintenance and decommissioning of capital equipment (e.g. bioreactor production, etc.). Additional operations, such as lighting and heating should also be taken into consideration (e.g. the energy and heating demand of the facility, air- and cleanroom-conditioning) to provide a full picture of the process impacts. The impact of the location can also be considered.

Life Cycle Impact Assessment

Due to its relevance, the global warming potential is often a central element of LCAs and can be evaluated even alone in a carbon footprint. Due to its process relevance, the cumulative energy demand, water use, land use and eutrophication are major points to be considered in artificial meat cultivation.

In North America, the TRACI 2.1 midpoint-oriented-method is well established. TRACI stands for "Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts" and was developed by the U.S. Environmental Protection Agency. In Europe, the ILCD/JRC is currently revising their method in accordance with the "Product Environmental Footprint" initiative. However, other methods such as CML and ReCiPe are well-established and credible. Impacts that are not among the ILCD recommended list are energy use (CED or NREU) and water footprint assessment (e.g. by AWARE method). As mentioned above, in our opinion both could add valuable information to the analysis.

Depending on the goal of the LCA it might be helpful to apply a method that is (1) scientifically acknowledged and (optional) (2) commonly used in the USA e.g. to apply for funding. The final choice depends mainly on the goal of the study as well as the intended audience. All described methods are implemented in standard LCA software.

Life Cycle Inventory

Mattick, in contrary to Tuomisto, assumed CHO cells' metabolic requirements as these cells are heavily optimized and the most common for mass production of e.g. therapeutic proteins. Tuomisto used stem cells which are very close to the cells used in clean meat production, but which lack any kind of industrial data. The cell origin, cell type and the resulting metabolic requirements are important to calculate most of the material and energy flows of the muscle cell cultivation. The media input to meat varied throughout the studies as shown in **Table 3**. Therefore, data of specific lab experiments with most suitable cells like stem cells from animals should be used for the assessment and simulation of the cultivation process e.g. to achieve correct numbers of media inputs. Along with that, the cell growth (initial and maximum density) is crucial to calculate the yield of the process. Tuomisto (2014) assumed a doubling of cells every 48 hours while in Mattick's calculation the doubling in proliferation phase was every 27 hours. Here, also primary and measured data from specific experiments would be recommended. Tuomisto did not include the biogenic CO_2 emissions during muscle cell fermentation, because the CO_2 will be emitted back to the air when the meat is consumed. The ILCD guidelines recommend to include, list and report the GHG emissions and removals arising from fossil carbon sources and biogenic carbon sources/sinks separately in the inventory and results for transparency (Sustainability 2010, Manfredi, Allacker et al. 2012).

The choice of reactor design and energy requirements will stay uncertain as long as no specific bioreactor for meat cultivation is on the market. The agitation, aeration, heating of water for culture and pumping for heat exchanger are based on scientific calculations partly based on equipment properties and efficiency ratios. Hence, these flows lack certainty. The facility energy demand (lighting, heating, ventilation and air conditioning) was assumed from a warehouse, but an estimation closer to a biotech facility would be more suitable as the future clean meat production probably also requires higher hygiene standards (e.g. cleanroom conditions).

If a hypothetical production has to be assessed the data should include prospective energy efficiencies, state of the art technologies from most similar processes from industry e.g. biotech-industry and measured data. Best case would be primary and measured data directly from the specific process steps.

For a future LCA study it is advised to depend the considered production process as well as the underlying

data on the actual cultured meat manufacturing process. That means the study should rather be based on an available lab scale process and measured primary data than on assumptions and literature of similar processes and equipment. Other data, like material or energy flows (e.g. wheat and corn production), that enter the production system should rather come from selected generic databases than from literature. In addition, the cleaning of the reactors was assumed from literature dating back to 1994. Hence, the reference year of the data and literature used in the study should be as current as possible.

Note: *It must be noted that LCA studies carried out during an experimental and modelling stage of development have mostly higher environmental impacts than industrial scale processes. This is based on high uncertainties present in the data inventory and an experimental production route subject to optimization. The level of the potential environmental impacts are to be considered anticipatory and expected to change/lower along the development path associated to increasing knowledge and decreasing grade of uncertainty according to the technology development related to LCA. (Villares, Işıldar et al. 2017)*

Scenario and sensitivity analysis

Specially for hypothetical processes with numerous assumptions and a high degree of choice e.g. the location, energy source, a sensitivity and scenario analysis are highly recommended. Parameters and choices with high uncertainty and high impact on the results should be considered as well as supposed low impacting parameters. A sensitivity analysis like Monte Carlo can provide deeper insights on the reliability and robustness of the study. A scenario analysis can raise awareness of e.g. most suitable production locations for an upcoming facility or the influence of the energy source used in the process.

Tuomisto's scenario analysis showed that the switch from cyanobacteria hydrolysate to wheat or corn results in around 140% higher feedstock impact for GHG and 400% higher land use. The results further showed that energy use and GHG emissions were most sensitive to the changes in energy requirements for muscle cell cultivation (aeration and rotation). Also, the change to 100% allocation of cyanobacteria had a substantial impact on the results.

In Mattick's sensitivity simulation model, specifically the cell density, mass increase during differentiation, facility size and cell growth rate underlie the highest sensitivities. Compared to the baseline impacts the results of the sensitivity analysis vary between -50% to +250%. The choice of allocation (mass, economic or gross chemical energy) of inputs and coproducts barely influenced the overall results.

Since impacts of the LCA studies were quite variable due to choices and assumptions made, the following process steps are recommended to include in a sensitivity and scenario analysis.

- Different electricity and heat source (renewable, mix, maybe energy production from waste biomass from process)
- Different input quantities and sources of media and feedstock (cyanobacteria & soy hydrolysate, corn, wheat, other suitable feedstocks)
- Recycling of media/feedstock components
- Include growth factors production
- Include scaffold application and materials used
- Include cell collection and different cell densities and growth rates (initial and maximum)
- Different batch time / growth time in bioreactor
- Bioreactor and process design (Stirred-tank, hollow-fiber and perfusion reactor)
- Different scales
- Allocation of inputs and by-products
- Different functional units (e.g. 1 kg meat and 1 kg protein)
- Different cell types (e.g. fat, muscle)

It has to be noted that in a future assessment different hotspots may occur, due to further development in the field of cultured meat production and equipment used, which are not listed. Of course, these hotspots should be considered as well.

7 Recommendations

The findings of the reviewed LCA studies and the recommendations for a future LCA study of a hypothetical production process of cultured meat relying on lab scale data of industry are briefly listed.

Goal and scope

The LCA approach shall be selected depending on the goal and scope of the study. One can distinguish two approaches: attributional and consequential. An

attributional approach is recommended in order to evaluate and/or to compare processes or products. Moreover, this approach allows to identify the most impacting process parameters and the technical optimization potential. In contrast, an evaluation of the (societal) consequences of the technology can be better performed in a consequential approach. Typical target audience here are policy makers.

In order to provide feedback for the industry, an attributional approach is the most suitable. Moreover, a so-called prospective LCA might be suitable. This includes scale-up data as well as potential changes of the circumstances.

Functional unit and reference products

The function of the product is the heart of each LCA study. Accordingly, one should select this with caution and align the choice with the intended goal of the study. The following options are suitable:

- protein [kg]: especially suitable to evaluate different protein sources including plant-based proteins.
- meat in weight [kg]: especially suitable for evaluations only considering meat, e.g. of different origin. To eliminate at least the water content, one may define the FU as dry matter. Furthermore, the nutritional value should be stated.
- energy [kcal]: common indicator in food.

It should always state if any bones or other not edible parts are considered and if the scaffold material is incorporated in the meat. The type of meat (minced meat, steak) should be specified as well.

It is worth to keep in mind that food is a natural product whose properties are subject to natural fluctuations. Moreover, the properties (e.g. protein content, energy content, micronutrients) differ from type to type. Therefore, a 1:1-comparison is hardly possible and might be better represented by an average value. A 1:1 comparison is not per se a no-go, but should be explicitly reported and kept in mind for the interpretation.

Multifunctionality

To address multifunctionality, all established methods, mass, economic or energy allocation, are suitable, however, physical allocation is recommended by the standards. If the nature of the by-products is very different from the main product, an economic allocation is globally applicable. As a kind of best-practice, most studies evaluate the impact of the allocation procedure in a sensitivity assessment.

System boundaries

As for now, most studies' goal is proof of concept of the technology and therefore process-centered, which means that a cradle-to-gate assessment is sufficient. As a non-commercial product, this part would be entirely based on assumptions. The assessment should include all relevant steps from tissue procurement and cell banking, proliferation, tissue perfusion and differentiation on a scaffold as well as the harvesting.

A cut-off can be applied. Cut-off means "a specification of the amount of material or energy flow or the level of environmental significance associated with unit processes or product system to be excluded from a study" in order to reduce complexity. (DIN ISO 14040, 2006)

While infrastructure plays a crucial role in lab scale processes, it is in most cases less relevant for upscaled industrial high-volume processes. Therefore, it should be considered, but it is not a must. This depends highly on the goal and scope. If the studies' goal is strongly emphasizing a product comparison, it would be helpful to include the infrastructure if the competitor product includes it as well. If the process and its optimization is central, it is less important. In low-TRL assessments, it might increase complexity and completeness, but will add uncertainty. The key questions for this choice are: Does it add value? Does it provide necessary and beneficial information?

Life Cycle Impact Assessment

It is recommended to at least assess the global warming potential, energy demand, land and water use. Beside the water use, a water scarcity assessment according to the AWARE method, which includes geographical and temporal parameters, could give deeper insights into the weaknesses of cultured meat production. The AWARE method is the WULCA consensus characterization model of water scarcity footprints conducted by LCA- and water-experts and represents the state of the art on how to assess potential impacts from water use in LCA. The energy use is recommended to be assessed with the Cumulative Energy Demand (CED) and non-renewable energy use (NREU). Finally, the eutrophication potential is recommended to be included in the analysis to show the significance of nutrient input into the ecosystem due to feedstock production, as mentioned by Mattick (2018).

Data acquisition and Life Cycle Inventory

It might be very difficult to get industrial data for clean meat. Data sources are extrapolated lab-scale data, simulation models or literature data from similar processes, e.g. from food or pharma. The latter can help to fill gaps and to better understand the potential of the technology. The data origin should be of the following range of priority: measured, calculated, expert estimates, estimated data. A smart combination of these sources should help to get the best data available.

For process-centered assessments, lab data enhanced with simulated process models might be suitable. However, for a product comparison the exclusive use of lab-scale data might lead to misinterpretation in the audience by ignoring the huge potential of learning curves. Still these approaches have its limitations and uncertainties remain high.

A reliable comparison of results of different sources should require the same data sources, same LCIA method and a professional LCA software. LCA databases which assure the data quality are strongly recommended. The exact version of software and database should be stated to boost the reliability and transparency of the study and are stated as responsibility of LCA practitioners by Baitz (2018).

An inventory data quality assessment according to Weidema et. al (1996) could further help to highlight weaknesses of data used (Reliability, Completeness, temporal and geographical correlation).

Scenario and sensitivity analysis

Especially in low-TRL processes a scenario analysis can provide valuable insights. Scenarios can reflect:

(1) directly process-related choices (e.g. choice of input materials like electricity source, heat source or scaffold material),

(2) alternative (sub-) processes and

(3) changes in the framework conditions (e.g. long-term changes in the energy mix).

Scenarios can include: electricity and heat source, scaling effects, nutrient media composition and feedstocks, growth factor production, scaffold application and material, cell collection, density and growth (initial and maximum), batch-time, bioreactor design, and by-products. It could be an asset to test different functional units as well.

The study can be complemented by a so-called prospective LCA in order to answer questions like “what will happen?”, “what can happen?” or “how can a specific target be reached?”. To do so, a hypothetical scale-up and optionally an outlook into a future technosphere can be included (e.g. changes in the energy mix and transportation, feedstock provisions). This is especially relevant in comparative studies as a comparison on lab-scale may cause premature and potentially wrong conclusions.

A sensitivity analysis e.g. via Monte Carlo Simulation should be conducted to address the uncertainty of the LCI-data as well as the impact of methodological choices.

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