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Life cycle assessment of single cell protein production–A review of current technologies and emerging challenges

Laura Fernández-López [a], Pablo González-García [a], Ana Fernández-Ríos [a], Rubén Aldaco [a], Jara Laso [a], Eva Martínez-Ibáñez [a], David Gutiérrez-Fernández [a], Marta M. Pérez-Martínez [b], Virginia Marchisio [b], Mónica Figueroa [b], David Baptista de Sousa [c], Diego Méndez [c], María Margallo [a,*]

[a] *Department of Chemical and Biomolecular Engineering, University of Cantabria, Av. Los Castros s/n, Santander, Cantabria, 39005, Spain*
[b] *EnergyLab Technology Center, Fonte das Abelleiras s/n, Vigo, Galicia, 36310, Spain*
[c] *ANFACO-CECOPESCA, Department of Sustainability and Circular Economy, Campus University 16, Vigo, Galicia, 36310, Spain*

A R T I C L E I N F O

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A B S T R A C T

Population growth trend will have a significant impact on the availability of food resources, leading to a surge in the development of various protein concentrates, including Single Cell Protein (SCP), which is derived from the biomass of unicellular organisms. The objective of this review is to analyze the application of Life Cycle Assessment (LCA) on SCP production, assessing the influence of the technologies on environmental outcomes and the challenges linked to LCA methodological choices. The articles included in the review were classified according to their LCA goal, distinguishing between those focused on the production of SCP for consumption, for feed valorization, for wastewater treatment and for conventional foods substitution in meals. Generally, most systems comprised three stages: feedstock production and pre-treatment, fermentation, and post-treatment, and in some cases, integration of SCP into the final product. The analysis revealed that the type of substrate has a great influence on the environmental profile of the product, as well as its pre-treatment. Electricity was also identified as the main hotspot in virtually all systems, being the most studied parameter in sensitivity analyses. Regarding the definition of LCA parameters, a lack of consensus on the description of system boundaries in the use of organic waste as substrate for SCP production is notable, leading to confusion about the actual associated impacts. Likewise, the study of the environmental performance of SCP based on its amino acid content and nutritional quality is one of the main challenges that would contribute to better evaluating its environmental behavior compared to other types of protein of vegetal and animal origin.

Introduction

Single Cell Proteins (SCPs) – also known as microbial or unicellular proteins – refers to protein derived from cells of microorganisms such as yeast, algae, fungi, and bacteria, which are grown on various carbon sources for synthesis (Najafpour, 2007). They present a promising substitute for animal- and plant-derived ingredients for feed and human nutrition, and have constituted a major focus of research for a long time (Zamani et al., 2020). This attention has primarily been and continues to be motivated by two key concerns. Firstly, the growth of the world population, which is projected to reach 9.7 billion people in 2050 (FAO, 2019) and which has coerced global proclivity towards producing more protein-rich foods to satisfy the future demand of consumers (Aidoo et al., 2023). Secondly, the critical environmental performance of food systems that produce around 50 % of food under conditions transgressing some planetary boundaries (Gerten et al., 2020). In fact, the main source of protein, vitamin B12 and essential amino acids is meat, which is identified as one of the most critical product respecting sustainability (Dagevos and Voordouw, 2013). Although according to some authors, the environmental footprint of livestock production is frequently overestimated due to overconsumption in middle- to high-income countries and a narrow interpretation of sustainability focused on climate change (Adesogan et al., 2020), its extraordinarily high water and energy consumption, land use or nitrous oxide and

* Corresponding author.
E-mail address: maria.margallo@unican.es (M. Margallo).

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ammonia emissions are undeniable (Detzel et al., 2021). Therefore, the higher content of animal protein in diets, the greatest burdens on the environment. In response to these challenges, novel foods are supposed to have the potential to reduce environmental impacts of diets while meeting essential nutritional needs (Mazac et al., 2022). SCP fulfils several of the aspects that characterize these products (European Union, 2015), standing out for their limited human consumption and their technological production and processing, whose perception towards consumers may still create a lack of acceptance (Siegrist and Hartmann, 2020). Moreover, it may overlap with future food, which can be produced in considerable volumes as a result of technological developments offering the potential to scale production levels up out of concern of the environment (Parodi et al., 2018). Consequently, the progressing prominence of microbial SCP in enhancing sustainable protein production and consumption (Aidoo et al., 2023) could lead to a future eco-friendlier food sector that will provide resilient diets and more consistent in the supply of essential nutrition in the face of acute biotic and abiotic stressors (Tzachor et al., 2021).

However, claims about the environmental sustainability of SCPs can only be based on and supported by methodologies that allow for objective study and measurements of their environmental impacts. Life cycle assessment (LCA) is the most widespread tool, which takes a holistic approach for evaluating the ecological footprint of products, adopting a life cycle thinking to address current challenges and needs related to the sustainability of production and consumption patterns (Notarnicola et al., 2017). In this field, SCPs have become an important focus of research in recent years, as their production has been developing, testing and adopting different conditions, techniques and resources that have the potential to achieve awesome environmental profiles. For instance, SCP can be produced by a wide range of microorganisms and species, including algae (e.g. *spirulina maxima*), bacteria (e.g. *Methylophilus* spp.), or fungal sources (e.g. *K.fragilis*) (Sharif et al., 2021), and which provide various advantages and challenges based on the protein concentration, essential amino acids balance (Ritala et al., 2017), growth velocity or compatibility with the feedstocks (Nyyssölä et al., 2022). The latter may constitute an important aspect in the sustainability of SCP, which can take advantage of circular economy principles by using renewable feedstocks like industrial or agricultural residues, as well as wastewater (Koukoumaki et al., 2023). This clash with the use of fit-for-purpose substrates, such as dextrose, corn starch or soybean meal, which despite exhibiting better controllability and more conversion options, have higher costs and less circular economy appeal (Jones et al., 2020). In addition, fermentation conditions embracing pH, temperature, aeration rate and nutritional requirements, such as carbon and nitrogen sources, not only strongly affect the yield and productivity of SCP production, but highly influence its environmental outcomes (Reihani and Khosravi-Darani, 2019).

So far, numerous articles have addressed the study of SCP production pathways but, to the best of our knowledge, none perform a comprehensive review of LCA studies. Therefore, the objective of the present review is to conduct a critical revision of LCA studies addressing SCP production. With the results, two directions are taken to carry out a comprehensive review. Firstly, the production systems are assessed from a technical and environmental perspective, which enables us to identify key features, which SCP technologies have been studied under this approach and what influence they have on the overall environmental performance. Secondly, the LCA characteristics of the articles reviewed are studied, which allows the identification of weaknesses and strengths in methodological choices and the proposal of improvements and a framework suitable for upcoming investigation. This research will set a benchmark for researchers, showing the environmental viability of the technologies and what options remain to be studied in order to continue progressing in this field.

Materials and methods

Literature search strategy

This review studies articles related to the application of LCA in SCP production, including the identification of the main technical characteristics of the systems, the key features of the LCA methodology and the interpretation of the environmental impacts. The search was conducted in the Scopus (Scopus, 2024) and Web of Science (WoS) (Web of Science, 2024) databases since they are the most comprehensive and reliable bibliographic data sources covering different scientific fields (Pranckutė, 2021). The terms "life cycle assessment" or "LCA" were searched in combination with "single cell protein" or "microbial protein" or "SCP". These terms must appear in the title, abstract or keywords of the scientific contributions. The search was not filtered by location or year, and the inclusion criteria were full-English articles. Furthermore, all subject areas not related to this topic were excluded, such as medicine, immunology or mathematics.

A total of 51 articles were found in the Scopus database, whilst the search in WoS conducted to 56 results (Fig. 1). From the former, 14 scientific articles were included after an exhaustive revision, whereas the remaining were discarded as they were reviews, did not develop a comprehensive LCA or the acronym SCP were referring to other terms, such as "sustainable consumption and production". From the latter, two new articles were found, summing up a total of 16 LCA papers obtained through the bibliographic search by terms. Additionally, two more articles were added for the review, which were found by searching for information in Google Scholar (Google Scholar, 2024), although they did not appear in the previous search with the keywords. However, their novel perspective on the application of LCA of microbial proteins in relation to other studies was the relevant aspect to consider their inclusion.

Analysis of study findings

Each study was appraised independently to compile all the necessary information for critical analysis. This process was carried out in two steps. Firstly, attention was directed towards the SCP production processes themselves. The type of substrate, pre-treatment, fermentation conditions, separation processes and intended application of the product, as well as any additional stage to produce raw materials, were identified and studied. This will enable us to recognize which systems and components have been subjected to an exhaustive environmental evaluation and which options remain unexplored for further assessment. Moreover, knowledge of these aspects will make it possible to associate greater or lesser environmental impacts with the raw materials or technologies used in each system, helping to draw conclusions about the suitability of employing certain techniques. Secondly, LCA methodological features were assessed by the description of the goal and scope, including the definition of the functional units (FUs), allocation and system boundaries, the compilation of the life cycle inventory (LCI), both background and foreground data, the evaluation of the software, methods, and impact categories, the application of uncertainty or sensitivity analyses, and the main conclusions extracted from the papers.

Articles were classified in four categories according to the goal of the systems. The first group included research addressing the LCA of single SCP production systems, which assess the performance of the life cycle stages and identify the main hotspots (class 1). The second category contained papers focused on SCP production for organic waste or wastewater treatment (class 2), while a third group was created to comprise microbial protein production for animal feed valorization (class 3). A last category encompassed articles that address the environmental performance of the introduction of microbial protein into meals, coinciding with the two additional articles found in Google Scholar (class 4).

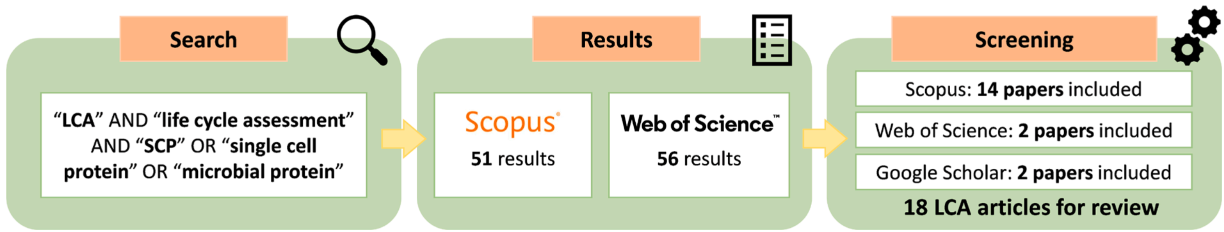

Fig. 1. Search and review criteria scheme.

Results and discussion

Findings of the literature review

From the publications included in the review, information about the geographical distribution of the articles, as well as the time evolution was retrieved to observe trends and draw conclusions. LCA studies addressing SCP production amount to 16, whose publication is comprised between 2018 and 2024 (Fig. 2), indicating the emerging environmental challenges of this sector. The trend of publications along the years is fluctuating but seems to be rising, with the highest number of articles in 2020 (five publications), evidencing the growing interest in alternative protein products. However, although it is an acceptable number of articles that can provide interesting insights, there is still a wide range of study approaches to unmask its potential and improve the environmental profile of SCP up to its optimization. This becomes evident when comparing environmental studies related to SCP with, for example, those in the meat sector, which total more than 600 publications (Scopus, 2024). It is also worth mentioning that one-third of the articles were published in the multidisciplinary journal of Science of the Total Environment, and followed by Journal of Cleaner Production and Environmental Science and Technology. Some papers focused on SCP production for waste valorization were published in more specific journals, such as Water Research or Waste Management.

The geographical distribution is interesting, since many data will depend on location and shows trends in technology. Fig. 3 depicts the number of articles combining LCA and SCP production, distinguished in colors according to their classification in this review. Europe, and particularly Northern countries, are clearly at the forefront of investigations, with 11 of 18 publications conducted by first authors from these regions. Finland has an important research activity, mainly focused on the production and environmental assessment of microbial protein intended for human consumption, and followed by United Kingdom, which in addition investigates its valorization in animal feed.

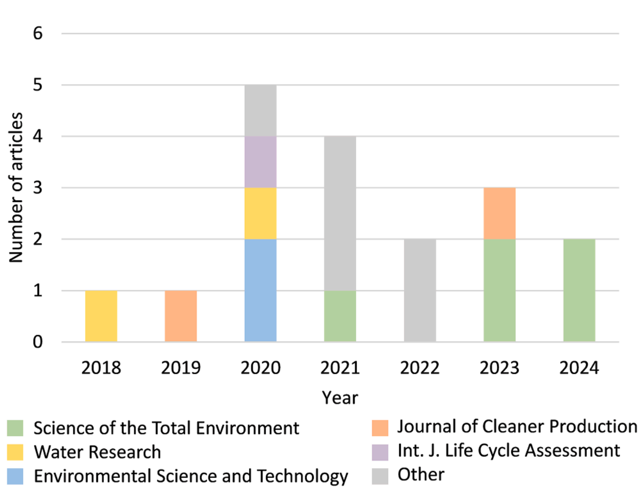

Fig. 2. Distribution of publications addressing LCA of SCP production across journals and years.

Greater awareness of environmental and food systems implications, as well as greater initiative to solve current problems, may be the main drivers of this trend in Europe. On the contrary, the limited contribution of Asian and North American countries is mainly focused on the assessment of SCP as a technique for organic residues or wastewater treatment. The fact that China and United States, which have conducted most researches, are the two most polluting countries in the world may be a feasible explanation of this tendency.

Technological aspects of SCP production

Currently, there are different processes for obtaining SCP, depending on the substrates available or the target of the product, since the SCP must meet certain requirements for the human or animal consumption. In general, the production system of SCP includes: i. pre-treatment of the substrates, ii. fermentation, iii. separation of the SCP from the main stream and additional post-treatments, and, when applicable, iv. the adequacy of the SCP for product incorporation into food or dietary supplements. This scheme can vary by introducing additional steps to obtain biogas (bioH_2 and bioCH_4) or O_2 and H_2 by electrolysis. The entire process is shown in Fig. 4, which provides a summary of the main stages of microbial protein production. Specific characteristics of the reviewed papers are explained in detail in Sections 3.2.1-3.2.5.

Substrates

Substrates must contain an adequate source of carbon and nitrogen along with other nutrients, such as phosphorus, to guarantee the proper growth of microorganisms (Chama, 2019). Table 1 shows the substrates used in each paper reviewed. Although fit-for-purpose feedstock like natural gas, dextrose or soybean meal still attract attention, in recent years, the use of waste or industrial organic by-products has been popularized since it reaches the most profitable economic levels (Spalvins et al., 2018). This is particularly evident in the LCA studies, which almost entirely consider waste as feedstock for microbial protein production. Organic waste valorization into SCP is one of the most widespread, being assessed in six papers, and including residues from citrus (Chen et al., 2021), rice cultivation (Upcraft et al., 2021), or varied organic waste from restaurants (Khoshnevisan et al., 2020). Wastewater is also an important source of study, especially in the papers from class 2, whose objective is precisely to find a treatment mechanism that could apply circular economy principles. It is common that effluents come from food industries, such as potato processing (Spiller et al., 2020), or fruit juices production (Chen et al., 2020), but municipal wastewater can be utilized too (Marami et al., 2022).

Pre-treatments

The substrates used to obtain SCP are frequently subjected to some treatments to facilitate their use in later stages. This is especially necessary in cases where residues and effluents are used. LCA studies on microbial production have assessed a wide range of pre-treatments, including physical and biological techniques. Most implemented methods comprised enzymatic hydrolysis, which is frequently used for food waste substrates (Kobayashi et al., 2022), and anaerobic digestion, which aims to generate biogas from wastewater to be used as culture

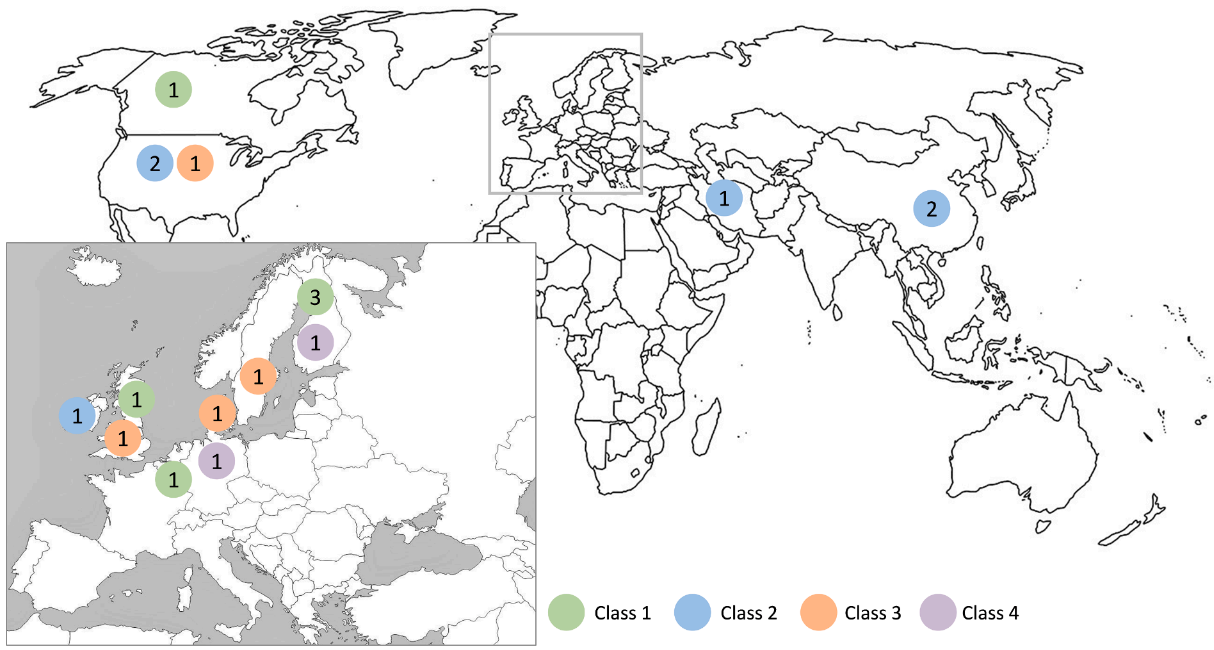

Fig. 3. Geographical distribution of articles addressing LCA of SCP production. Articles belonging class 1 are represented in green, class 2 in blue, class 3 in orange and class 4 in purple.

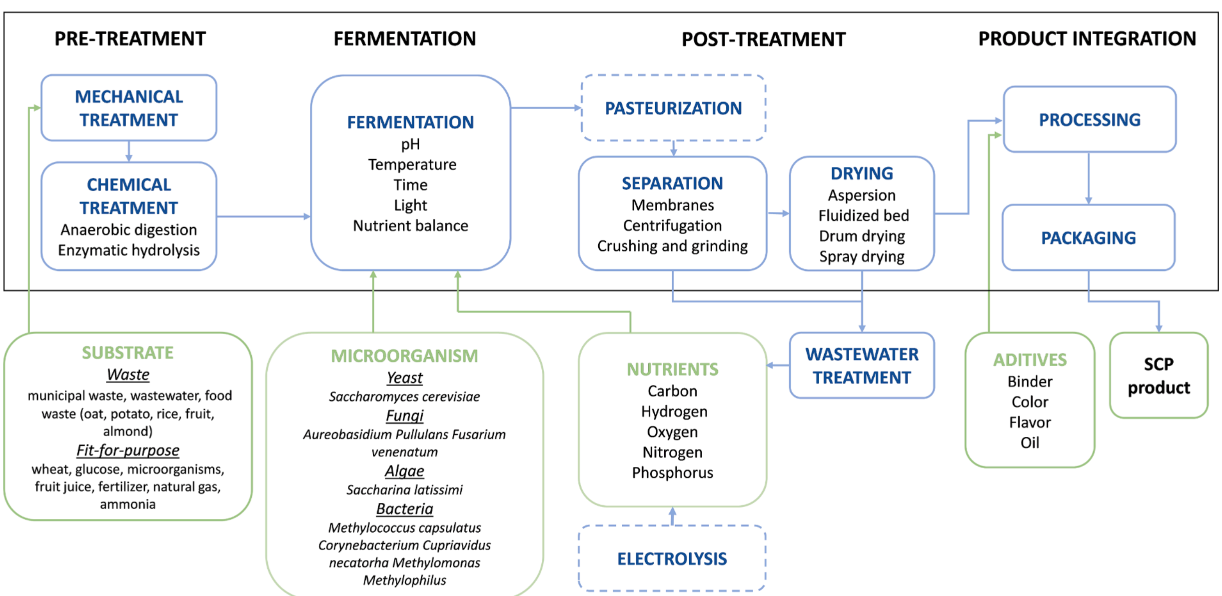

Fig. 4. Flow diagram of SCP production with different features and technologies. Green boxes represent resources and blue boxes processes. Dashed lines represent optional processes in the production of SCP.

medium (Wang et al., 2023). The latter was also assessed in combination with organic waste, which constitute one of the most impactful units on climate change of the whole SCP system (Khoshnevisan et al., 2020). Simpler pre-treatments, such as pellets formation with the substrate, or homogenization and mixing to achieve a uniform medium were analyzed by Chen et al. (2021) and Aidoo et al. (2024) respectively, which demonstrated their little contribution to the overall environmental impacts. In contrast, the use of an industrial shredder to reduce particles size suppose a relevant hotspot in microbial protein production with purple non-sulfur bacteria (LaTurner et al., 2020).

Fermentation

In fermentation microorganisms grow and generate biomass by using substrate as carbon source. The cultivation process begins with microbial screening, which involves obtaining microorganisms from soil, air and water. In most LCA articles, this stage was either outside the system boundaries or was not mentioned in the scope definition. Järviö et al. (2021) considered an additional stage of microorganism propagation previous to fermentation, but it did not specify its influence on environmental results. Likewise, Chen et al. (2021) included yeast cultivation within the system boundaries and reported petty contribution of the materials required for its growth.

Regarding the type of microorganisms, bacteria, algae, fungi and yeast have been used in the different reviewed studies. Bacteria were used in 13 out of 18 papers, being the most studied microorganism. LaTurner et al. (2020) used purple non-sulfur bacteria that may result

Table 1
Key attributes of the reviewed articles processes: substrate, pre-treatment, fermentation, microorganism and post-treatment.

Study	Substrate	Substrate pre-treatment	Additional compounds for fermentation (nutrients, pH regulators…)	Type of fermentator	Microorganism	Post-treatment of SCP
Class 1 – SCP production						
Aidoo et al. (2024)	Crude pea starch	Water addition to raise moisture up to 70 % and mixing	$(NH_4)_2SO_4$, $KHPO_4$, H_2SO_4 and $MgSO_2$	Solid-state aerated reactor (30 °C, 20 h)	Yeast (*Saccharomyces cerevisiae)*	Decanting centrifugation, drying, cooling and storage
Kobayashi et al. (2023)	Oat side stream	Drying, enzymatic hydrolysis and sterilization of supernanant	KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and $(NH_4)_2HPO_4$	Stirred aerated bioreactor with cooling (20–30 °C, 20 h).	Yeast (unspecified)	Centrifugation, washing and drying
Järviö et al. (2021)	None	None	NH_3, inorganic salts, H_3PO_4, NaOH, CO_2, O_2 and H_2 (produced by own electrolysis)	Continuous stirred tank reactor	Hydrogen-oxidizing bacteria	Pasteurization, centrifugation, and drying
Upcraft et al. (2021)	Rice straw	Mixture of rice straw and ionic liquids, followed by evaporation, filtration and washing. Enzymatic hydrolysis of pulp, filtration and evaporation to obtain a final liquid hydrolysate	O_2, NH_3, glucose, and H_3PO_4	Continuous airlift fermenter	Fungis (*F. Venetatum)*	Centrifugation
Sillman et al. (2020)	None	None	NH_3, P, S, CO_2. KH_2PO_4 and $MgSO_4$ as culture medium	Bioreactor with in-situ electrolysis	Bacteria (*C. Necator)*	Centrifugation and evaporation
Spiller et al. (2020)	Wastewater from potato industry	None	SFT, $(NH_4)_2SO_4$	Two aerated bioreactors	Aerobic heterotrophic bacteria	Two settlers, centrifugation, and drying
		Anaerobic digestion	NH_3, CH_4, $FeCl_3$	Open raceway pond and aerated bioreactor	Microalgae and aerobic heterotrophic bacteria	Two settlers, centrifugation, and drying
		Hydrolytic, acidogenic and acetogenic fermentation	None	Outdoor tubular photobioreactor and aerated bioreactor	Purple non-sulfur bacteria	Ultrafiltration, centrifugation, and drying
Class 2 – organic residues and wastewater treatment						
Wang et al. (2023)	Manure, food waste, and wastewater	Anaerobic digestion to produce biogas	NH_4OH, KH_2PO_4, and trace metal salts	Aerated bioreactor	Methanotrophic bacteria (unspecified)	Dewatering, drying and sterilization
Marami et al. (2022)	Wastewater and biopulp	Anaerobic digestion and biological biogas upgrading for methane production from wastewater	NH_4—N and P (produced in a bio-electrochemical system from rejected water of the digestor)	Stirred aerated tank reactor	Methanotrophic bacteria (unspecified)	None
Chen et al. (2021)	Citrus waste	Pelleting	Soybean meal	Unspecified	Yeast (unspecified)	None
Chen et al. (2020)	Wastewater from peach, apple and kiwi juices production	None	NH_4Cl, KH_2PO_4, $MgCl_2$	Unspecified	Unspecified	Recovery (unspecified technique) and drying
Khoshnevisan et al. (2020)	Biopulp from organic municipal solid waste, supermarket waste, and residues from restaurants	Anaerobic digestion and subsequent centrifugation, pasteurization and dilution of supernanant	Ammonium mineral salts, N, trace elements, CH_4	200 ml bottles in a shaker incubator	Methanotrophic bacteria (unspecified)	None
LaTurner et al. (2020)	Food waste	Industrial shredding, acid-phase digestion and dilution	NH_3, P	Anaerobic photobioreactor	Purple non-sulfur bacteria	Hollow fiber membrane filtration, centrifugation and drying
Class 3 – animal feed valorization						
Bergman et al. (2024)	Spent sulfite liquor	None	H_3PO_4, NH_3, and KOH	Aerated bioreactor	Fungis (*P. Variotti)*	Filtration, washing, drying, and gridring
Owsianiak et al. (2022)	Starch-rich wastewater	None	Unspecified	Aerated bioreactor	Aerobic heterotrophic bacteria (unspecified)	Centrifugation, drying, and production of fishmeal with SCP
Couture et al. (2019)	None	None	CH_4 used as culture medium, additional nutrients (unspecified)	Unspecified	Heterotrophic bacteria (unspecified)	Harvesting, condensing, and drying
	Wheat byproduct from biorefinery	Enzymatic pretreatment	Unspecified	Unspecified	Yeast (unspecified)	Harvesting, condensing, and drying
Tallentire et al. (2018)	Wheat grain	Grinding and liquefaction	$(NH_4)_2HPO_4$, H_2SO_4	Anaerobic fermenter	Yeast (unspecified)	Distillation and separation

(*continued on next page*)

Table 1 (*continued*)

Study	Substrate	Substrate pre-treatment	Additional compounds for fermentation (nutrients, pH regulators…)	Type of fermentator	Microorganism	Post-treatment of SCP
	None	None	CH_4 as medium, O_2, NH_3, H_2O, $MgSO_4$, $FeSO_4$, $CuSO_4$, K_2SO_4,	U-loop fermenter	Bacteria (*Methylococcus capsulatus* and *Alcaligenes acidovorans*)	Separation, homogenization, and drying
Class 4 – SCP introduction in meals						
Mazac et al. (2023)*	Unspecified	Unspecified	Unspecified	Unspecified	Hydrogen-oxidizing bacteria (unspecified)	Unspecified
	Unspecified	Unspecified	Unspecified	Unspecified	Fungi (unspecified)	Unspecified
Smetana et al. (2021)	Wheat grain	Milling, concentrating, glucose extraction	Unspecified	Unspecified	Fungi (unspecified)	Texturizing and cutting of mycoprotein, mixing and cooking of burgers

* SCP production process is not shown in the article since life cycle inventory is compiled from literature.

environmentally and economically viable to process food waste and produce a protein supplement. Spiller et al. (2020) also utilized this microorganism, and compared its performance with that of heterotrophic bacteria and microalgae for wastewater treatment. Results evidenced clearly better environmental outcomes in resources and ecosystems related indicators when microalgae were used in combination with heterotrophic bacteria, while purple non-sulfur bacteria led to lower impacts in human health categories. Hydrogen-oxidizing bacteria (HOB) for feed or food purposes was considered by Järviö et al. (2020) to evaluate the performance of a system in which crops are not needed as a source of carbon. As a result, saving of land resources and a reduced impact on eutrophication were achieved. Methanotrophic bacteria (MOB) was studied in several papers, and it is used to achieve growth from methane. The weakness of these systems lies mainly in the anaerobic digestion to which the substrate must be subjected to produce the biogas from which methane is obtained (Marami et al., 2022). On the other hand, five articles considered yeast as microorganism. Compared with the use of bacteria, yeast is likely to achieve a significant improved performance for the same production system, especially in terms of greenhouse gas emissions and water consumption (Couture et al., 2019). Finally, fungi SCPs were analyzed in four researches. Bergman et al. (2024) proved that SCP from *P. variotti* achieves lower burdens in climate change and biodiversity impacts than soy protein concentrate, but higher in energy demand and freshwater eutrophication. In addition, this microorganism was considered in the two papers addressing the production of microbial protein for introduction in meals, since it is the only microbial source of a high proportion of protein biomass that has market approval.

In general, SCP can be produced in different fermentation systems, including solid, semi-solid, surface and submerged fermentations, allowing the latter to achieve the highest yield (Suman et al., 2015). Most LCA articles did not specify what type of fermentation is carried out, but usually points out some features such as aeration, agitation, temperature or operation time. Stirred aerated bioreactors were the most frequently used and evaluated. The role of this fermenter for SCP production using wastewater as substrate was quite significant in relation to the environmental impacts (Owsianiak et al., 2022), with contributions between 15 % and 30 % to the total (Marami et al., 2022). This percentage could increase to almost 50 % and 60 % for the global warming indicator if a reactor with in-situ electrolysis (Sillman et al., 2020) or cooling (Kobayashi et al., 2023), respectively, is utilized, mainly due to the use of electricity. Elements that need to be added to reactor to maintain proper growth such as carbon, hydrogen, oxygen, nitrogen and phosphorus, were also key for the environmental results. Preparation of the culture medium with compounds including KH_2PO_4, $(NH_4)_2HPO_4$ and $MgSO_4{\cdot}7H_2O$ could suppose around 20 % of the climate change impact, 50 % of marine eutrophication and acidification, or 30 % of water consumption (Kobayashi et al., 2023). Depending on the chemicals, these contributions could increase up to 67 % in marine ecotoxicity or 71 % in freshwater ecotoxicity (Aidoo et al., 2024).

Post-treatment

Post-treatments frequently include separation of SCP, concentration and drying, as well as additional stages for sterilization or pasteurization of the product to avoid contamination by pathogenic bacteria and to reduce enzymatic activity (Järviö et al., 2020). Depending on the system, post-treatments could not constitute an important driver on environmental degradation. For instance, centrifugation and evaporation contributed approximately 15 % to the total carbon emissions in bacteria protein production (Sillman et al., 2020). On the contrary, the centrifugation and drying performed by Owsianiak et al. (2022) entailed a quite significant impact. Itemizing post-treatments, most papers considered separation of microbial protein from the culture medium by centrifugation. The centrifugal force can accelerate the sedimentation process of particles that tend to do so spontaneously (density higher than that of the liquid), or in those that tend to float (density lower than that of the liquid) (Kalstein, 2023). Some authors substitute centrifugation with a filtration stage (Bergman et al., 2024) or includes ultra-filtration (Spiller et al., 2020) or hollow fiber membrane filtration (LaTurner et al., 2020) prior to centrifugation, which evidently adds burdens to the process. The drying stage was quite common in most LCA studies, whose impacts varied depending on the type of dryer, including spray dryers (Spiller et al., 2020), fluidized bed (Kobayashi et al., 2022) or drum drying (Järvïo et al., 2021).

SCP processing

SCP usually appear in powder form, like flours or commercial protein supplements and then, it is added to the final product. This step was not described in most of the papers. Owsianiak et al. (2022) considered the introduction of the microbial protein powder in fishmeal, in order to produce a more protein-rich feed. However, it did not consider any additional stages after drying. On the other hand, Smetana et al. (2021) performed the texturizing, mixing and cutting of SCP to produce hamburgers, and considered the final cooking of the product. With this purpose, gluten and wheat flour were added to the mycoprotein to give meat a texturization. Next, some type of oil, such as sunflower or palm oil, was added to give it the shape of a raw mycoprotein hamburger and after cooking and assembly, the final shape of the hamburger is obtained. This hamburger showed incredibly low burdens if compared with conventional beef ones, and they were mainly associated with damage in ecosystems quality and human health (Smetana et al., 2021).

Environmental aspects

The objective of this section is to review the application of the life cycle assessment tool in microbial protein production to identify the main challenges and problems of LCA studies. Moreover, this analysis will allow the better understanding of the environmental performance and hotspots of this system. Tables 2–4 sum up all the main elements of the reviewed studies. This section is divided into the four steps of the LCA methodology: i. goal and scope, ii. life cycle inventory, iii. life cycle impact assessment, and iv. interpretation.

Definition of the goal and scope

The ISO 14040 standard establishes the definition of the objective and scope as the first step to develop the LCA of a product, process or service. The objective states the intended application, the reasons for conducting the study, and the intended audience, and people to whom the study results are intended to be communicated. The scope includes the definition of the function and functional unit (FU), the description of the product system and its boundaries, the allocations and all the assumptions that have been considered (ISO, 2006a, 2006b).

Functional unit

The FU is the measure of the function of the studied system and gives a reference to the related inputs and outputs (ISO, 2006a, 2006b). Its adequate selection is an issue of major importance since different FU could lead to a significant variability in the interpretation of the results and conclusions.

If the function of the system is the production of any food or feed, studies usually employ output or product-related FUs. The most common FUs comprise mass- and nutrient-based references, and, to a lesser extent, resource-related FU, e.g. 1 Ha of cultivated land. This trend is similar in LCA studies of SCP (Table 2). The production of different types of protein were evaluated by Spiller et al. (2020), Upcraft et al. (2021), Järviö et al. (2020), Kobayashi et al. (2022), Sillman et al. (2021) and Aidoo et al. (2024), employing 1 kg or ton of protein as FU. When the protein is introduced in the meal or food, the function is not the production of the protein and the FU consider this new product. For instance, Mazac et al. (2023) compared the environmental and nutritional impacts of meals including novel/future foods with those of vegan and omnivore meals using as FU one meal. Smetana et al. (2021) compared several burgers composed of beef and substitute materials using two FUs related to the product, one raw burger patties of 113 g and one ready to eat cooked burger made of burger patty, bread buns and burger sauce to represent relevance to the sensory testing and potential changes reflected due to burger cooking. FU (2) also represents an extended system (cradle-to-consumer) compared to FU (1) referencing cradle-to-gate boundary.

When the function of the protein is animal feed, the FU could reflect the mass of protein required to feed or to produce the final product, like one ton per year of shrimps (Owsianiak et al., 2022), or one kg rainbow trout (Bergman et al., 2024). In other cases, the reference was related to the amount of protein or the live weight of the animal. Couture et al. (2019) that compared salmon feeds based on protein from soy, methanotrophic bacteria, and yeast ingredients employed 660 g of protein equivalent to 1 kg of soy protein concentrate as FU, and Tallentire et al. (2018) used one bird grown to a live weight of 2.2 kg to assess the environmental implications of replacing soybeans with novel ingredients in chicken feed formulations.

Despite some studies highlighted that a nutritional FU based on the protein quality would be more appropriate, this type of reference value was not already applied. Considering the quality of protein, i.e. the bioavailability and digestibility of its essential amino acids, would conduct to better understanding of their nutritional implications and how they could meet possible nutritional deficiencies. Furthermore, this characteristic would enable to compare the environmental performance of SCPs with other protein-rich products, as well as different production techniques and biomass that lead to the SCP with the best amino acids

Table 2
Main aspects of the reviewed LCA studies: function, FU, system boundaries and allocations.

	Function	FU	Approach	Allocation
Waste/wastewater treatment				
Chen et al. (2020)	Treat a wastewater	1 m^3 wastewater	Cradle to gate	Economic and mass
Khoshnevisan et al. (2020)	Treat biopul waste	1 ton biopulp	Cradle to gate	System expansion
LaTurner et al. (2020)	Treat food waste	Amount food waste generated per day by a 50,000 inhabitants city	Cradle to gate	System expansion
Chen et al. (2021)	Treat citrous residues	1 kg of fresh citrous residue	Cradle to gate	Mass a and system expansion
Marami et al. (2022)	Treat a wastewater Produce an animal supplement	27.3 million m^3 sewage/year 1 ton of pure protein	Cradle to gate	System expansion
Wang et al. (2023)	Produce bioethanol	1 MJ bioethanol	Cradle to gate	System expansion
Animal feed valorization				
Owsianiak et al. (2022)	Feed shrimps	Kg of SCP to produce 1 ton per year of shrimps	Cradle to gate	System expansion
Bergman et al. (2024)	Feed rainbow trout	kg feed required to produce 1 kg rainbow trout	Cradle to gate	Economic and mass
Tallentire et al. (2018): de WoS	Feed chickens	one bird grown to a live weight of 2.2 kg	Cradle to gate	Economic
Couture et al. (2019)	Feed salmons	660 g of protein equivalent to 1 kg of soy protein concentrate	Cradle to gate	Economic and mass
Introduction SCP in meals				
Mazac et al. (2023)	Elaborate meals with novel/future foods	One meal	Cradle to consumer	No
Smetana et al. (2021)	Elaborate crude burguers Elaborate a burguer meal	1) One raw burger 2) One cooked hamburger, bread rolls and sauce	Cradle to gate Cradle to consumer	No
SCP production				
Spiller et al. (2020)	Produce SCP from a wastewater	1 ton of crude protein	Cradle to gate	No
Upcraft et al. (2021)	Produce mycoprotein from agricultural residus	1 kg of protein	Cradle to gate	Economic
Järviö et al. (2020)	Produce microbial protein	1 kg of microbial protein product prior to packing	Cradle to gate	System expansion
Kobayashi et al. (2022)	Produce SCP from oat side-stream	1 kg SCP product	Cradle to gate	Economic and mass
Sillman et al. (2021)	Produce protein-rich biomass	1 kg protein	Cradle to gate	No
Aidoo et al. (2024)	Produce SCP	kg of dried SCP with a 10% moisture content	Gate to gate	No

Table 3
Sources of primary data and databases.

	Primary data					Secondary data (database)				
	Lab scale	Pilot plant scale	Industrial scale	Literature	Others	Ecoinvent	Agri-footprint	Agribalyse	Sphera	Other
Kobayashi et al. (2022)	x			x		x	x	x		
Mazac et al. (2023)				x		x		x		
Upcraft et al. (2021)		x				x				
Järviö et al. (2020)		x				x				
Owsianak et al. (2022)				x		x				
Aidoo et al. (2024)				x		x				
Couture et al. (2019)			x	x		x			x	
Tallentire et al. (2018)			x	x		x	x			
Sillman et al. (2021)				x					x	
Chen et al. (2020)		x		x		x	x			
Smetana et al. (2021)				x		x	x			
LaTurner et al. (2020)				x		x				
Bergman et al. (2024)			x	x		x				
Wang et al. (2023)					x	x				x
Chen et al. (2021)	x	x		x		x				
Marami et al. (2022)		x		x		x				
Spiller et al. (2020)				x	x	x				
Khoshnevisan et al. (2020)	x					x				

profile. In this line, the door is open to advance the development of complex nutritional FU, and the application of the DIAAS (Digestible Indispensable Amino Acid Score) protein quality scoring system to provide valuable outcomes and new perspectives (McAuliffe et al., 2023).

Finally, when the goal of the study is to evaluate the treatment of a waste or effluent, the FU is usually related to the input flow, not to the final product. For instance, Chen et al. (2020) and Marami et al. (2022) employed the input volume of wastewater that feed a wastewater plant, whereas Khoshnevisan et al. (2020), LaTurner et al. (2020) and Chen et al. (2020) considered the mass of biopulp, citrous and food waste to treat. However, when the final product of that treatment is valorized or compared with other products, the function of the system change. Marami et al. (2022) used two FUs, 27.3 million m^3 of inlet raw sewage stream per year to assess the environmental performance of the WWTP, and one metric ton of finished pure protein to compare the environmental impacts of 2nd generation microbial protein with other proteinaceous feed sources. Other waste valorization study was conducted by Wang et al. (2023) that evaluated the environmental impacts of integrated corn stover-to-ethanol biorefineries that incorporate both codigestion of organic waste and different strategies for utilizing biogas, including the conversion to single-cell protein. In this case as the main function is the obtention of bioethanol the functional unit of the system was defined as 1 MJ bioethanol.

System Boundaries

The scope of the analysis will depend on the LCA approach. Therefore, "cradle to grave" and "cradle to cradle" involve all stages of the life cycle. Other less complete approaches limit the beginning or/and the end of the cycle. "Cradle to gate" and "cradle to consumer" exclude some downstream processes after the gate (such as consumption and end of life), limiting the study from the beginning of the cycle to a specific "gate"; "gate to grave" from a midpoint to the end of life stage, or "gate to gate" for intermediate or specific parts of the life cycle (Ruiz-Salmon, et al. 2021). The main differences between "cradle to gate" and "cradle to consumer" is that the later encompasses from the beginning of the cycle to households, including the integration of SCP into commercial products. Moreover, in the reviewed papers "cradle to gate" reaches up to the gate of the factory and it is used more for experimental SCP studies, being the final product the protein powder, whereas "cradle to consumer" is used for industrial or pilot plant cases studies.

We found that 83 % of the articles employed a "cradle to gate" approach due to the importance of upstream processes and the experimental nature of the studies, since SCPs constitute a market segment in full development that needs to be optimized. The integration of SCP powder in final products constitutes the "cradle to consumer" approach that was used in 11 % of the papers, to evaluate meals with novel/future foods (Smetana et al., 2021) and burgers (Mazac et al., 2023).

In this review, waste and wastewater studies applied a "cradle to gate" approach, but it is important to differentiate between the meaning of cradle and grave in a product and waste LCA. Whilst waste and product LCAs normally shares the same grave, LCA practitioners tend to view the "cradle" for waste as the moment that an item becomes, or is perceived as, valueless and is thrown out, sent for recycling or for biological treatment (Clearly, 2009). Thus, the cradle of household waste is usually the dustbin and starts with null impact (Margallo, 2014). Only one paper applied a "gate to gate" approach excluding raw materials extraction and preparation, and downstream processes. Aidoo et al. (2024) analyzed a solid-state fermentation system that utilizes crude pea starch as substrate for SCP production, considering the raw crude pea starch as a waste slurry from the pea protein extraction industry. This type of system boundaries are used when the process itself has the highest impact, being raw materials waste or having a low contribution, and the consumption and end of life stages are negligible.

It is remarkable that end of life stage was not addressed in all of the reviewed papers. This omission is likely due to the experimental or pilot nature of the technology, as well as due to the general negligible influence of this life cycle stage to the overall impacts.

Allocation

Multifunctional processes are very common in industrial and waste management sector. Few processes produce a single output, have only one input or are based on a linear relationship between raw material inputs and outputs. In these cases, it is important to address allocation procedures for systems involving multiple products and for recycling systems (ISO, 2006a, 2006b). To handle this problem, the ISO 14040 standard establishes as a first solution to expand the system boundaries or divide the process into subprocesses. When this solution is not possible, the allocation should be assigned based on physical causation, or based on other criterion, such as economic-value, mass, or energy (Margallo et al., 2014).

In the reviewed papers, authors have applied system expansion and mass and economic allocations to solve that multifunctionality. System expansion was applied in six papers and in most of the waste and wastewater treatment studies since several products and byproducts are generated, such as fertilizer, gasoline, heat, power or animal feed. Seven papers do not apply system expansion, employing energy and/or mass allocation or analyzing the differences between both perspectives. Besides, in other studies allocation strategies were not mentioned in the methodology, leading to confusion as to whether assignations were not considered or whether this information was omitted in the manuscript even though it is included in the LCA modelling.

Table 4
Software, LCIA method, and impact category used in reviewed studies.

				Midpoint categories																		Endpoint categories			
				GWP	LU	WC/WU	Acidification		Eutrophication		Ecotoxicity				ODP	HOFP	PMFP	RO	IRP	Energy/material		Damage			
	Software	Method					AA	TAP	MEP	FEP	FETP	METP	TETP	HTP (c/nc)						CED/NRE	SOP/ME/ADPf	HH	EQ	RA	CC
Kobayashi et al. (2022)	Simapro	Midpoint	Recipe (H)	x	x	x		x	x	x															
Mazac et al. (2023)	Simapro		Recipe (H) and AWARE	x	x	x		x	x	x															
Upcraft et al. (2021)	Simapro		Recipe	x	x	x		x		x															
Järviö et al. (2020)	Simapro		Recipe (H) and AWARE	x	x	x		x	x	x				x											
Owsianak et al. (2022)	Simapro		Recipe (H)	x																					
Tallentire et al. (2018)	Simapro		Recipe (H)	x	x																				
Aidoo	OpenLCA		Recipe (H)	x	x	x		x	x	x	x	x	x	x	x	x			x		x				
Couture et al. (2019)	GaBi		Recipe (H)	x	x	x		x	x	x															
Sillman et al. (2021)	GaBi		CML	x	x	x				x[2]															
Chen et al. (2020)	Unspecified		IPPC	x																					
Smetana et al. (2021)	Simapro		IMPACT	x	x		x	x		x[2]	x[3]		x	x	x			x	x	x	x				
LaTurner et al. (2020)	Simapro		TRACI	x	x	x	x[1]			x[2]	x[3]			x	x	x	x								
Bergman et al. (2024)	Simapro		IPPC, Recipe (H), ICLD, CML-LCA, CED	x	x		x[1]				x[3]									x					
Wang et al. (2023)	–		IPPC	x																					
Chen et al. (2021)	Unspecified		Unspecified	x			x[1]			x[2]	x[3]										x				
Marami et al. (2022)	Unspecified	Endpoint	Recipe (H)																			x	x	x	
Spiller et al. (2020)	Simapro		Recipe (H)																			x	x	x	
Khoshnevisan et al. (2020)	Simapro		IMPACT 2002+																			x	x	x	x
				16	11	8	1 11	7	6 16	6	1 11	1	2	4	**3**	**2**	**1**	**1**	**2**	**2**	**3**	**2**	**2**	**2**	**1**

GWP: Global warming potential; EP: eutrophication potential; LU: land use; WU/WC: water use/consumption; AA: aquatic acidification; AA: terrestrial acidification potential; MEP: marine eutrophication potential; FEP: freshwater eutrophication potential; FETP: freshwater ecotoxicity potential; METP: marine ecotoxicity potential; TETP: terrestrial ecotoxicity potential; HTTP: ecotoxicity potential; ODP: ozone depletion potential; HOFP: ozone formation potential; PMFP: fine particular matter formation, RO: respiratory organics; IRP: potential CED: cumulative energy demand; NRE: non-renewable energy use; SOP: ozone formation potential; ME: mineral extraction; ADPf: abiotic depletion fossil; HH: damage to human health; ED: damage to ecosystem quality; RA: damage to resources availability; CC: damage to climate change. HH: human health; EQ: ecosystem quality; RE: resources; ODP: ozone depletion potential; UE: energy use.

Economic allocation was used by Upcraft et al. (2021) in the cultivation of raw materials (rice grain and straw) and during the stages of lignocellulosic mycoproteins production (paste of mycoproteins and furfural). Tallentire et al. (2018) used the coproduct economic allocation methodology to calculate the environmental burdens of producing novel ingredients to replace soybeans.

Four cases combined or analyzed economic and mass allocations and one system expansion with mass allocation. Chen et al. (2020) used both allocations in the different wastewater treatment plants to establish the amount of nutrients that are used from the fruit juice stream and recovery waters. Kobayashi et al. (2022) applied economic allocation for secondary oat stream impacts, and mass allocation for the assignment of the liquid (supernatant) and solid outputs from enzymatic hydrolysis since their economic values were unknown. Couture et al. (2019) and Bergman et al. (2024) analyzed the differences between mass and economic allocation. The former allocated economically the inputs ingredients coproduced and, in a sensitivity analysis, compared the results from economic versus mass allocations. The latter allocated the environmental impacts of spent sulfite liquor, pulp and lignosulfonate based on the monetary value and, in a sensitivity analysis, applied a mass allocation. Finally, Chen et al. (2021) adopted a system expansion to handle multi-functional processes, assuming that the outputs replaced four types of corresponding products: animal feed, electricity, fertilizer and biorefinery products. This approach was combined with a mass allocation with ratio of 1:1 citrus residues and processed fruit.

Life cycle inventory

The life cycle inventory involves data collection and calculation procedures to quantify system inputs and outputs (ISO, 2006a, 2006b). LCI includes usually primary or foreground data and secondary or background data provided in most of the cases by LCA databases. Table 3 collects the main primary and secondary data. Regarding primary data, 44.4 % of the papers compiled the LCI from own experiments carried out at lab or pilot scale plant. Even, Chen et al. (2021) combined data from lab and pilot plant scale. This is due to the low maturity of the technology and the need to conduct experiments under different conditions and resources to optimize its production. One drawback of these studies is that the extrapolation of the results to industrial scale is limited. In the case of SCP studies, this preliminary assessment may highlight the critical sources of environmental impact in the process life cycle, and the areas where improvements should be made when implementing these techniques to a larger scale (Muñoz, 2006).

On the other hand, 16.7 % of the studies used data based on their own results at industrial scale or provided from companies. Tallentire et al. (2018) collected data from industrial suppliers to evaluate the use of other proteins for animal feed, Couture et al. (2019) conduced the LCA at industrial scale replacing soy ingredients with novel SCP, and Bergman et al. (2024) used primary data from a pulp mill biorefinery. Finally, 72.2 % of the papers included bibliographic data based on scientific papers, either to supplement primary data or as a primary source of data.

The quality and geographical, technological and temporal representativeness of primary data has a strong influence on LCA results. In fact, Takano et al. (2014) identified these aspects together with the allocation rules, the system boundaries, the modelling approaches and choice of secondary database for life cycle inventories as some of the major causes for different LCA results. For secondary data, Ecoinvent (Moreno Ruiz et al., 2019) and GaBi professional (now Sphera) (Sphera, 2023) are the most widely used databases. Despite, both are process-based databases, they use different modelling approaches and notable differences have been reported in LCA results. So, the selection of an LCI database can become strategic depending on the motivation of the study (Pauer et al., 2017). In this review we found that Ecoinvent was used in 94 % of the papers (17 of 18 papers) for their analysis. This database is well-known due to its advantages such as the detailed information compared to unitary processes. Nonetheless, some papers combined Ecoinvent with other databases such as Gabi (Sphera, 2023), Agri-footprint (Blonk Consultants, 2019) and Agribalyse (Agribalyse, 2022).

Life cycle impact assessment

This section analyzed those aspects that have more influence on LCA results, the LCA software and the selection of the impact method and impact categories. LCA modelling usually requires the use of a software, being GaBi (Sphera, 2023) and Simapro (Prè Consultants, 2019) two of the most popular for LCA-practitioners. Some discussion and comparison have been published regarding the differences between SimaPro and GaBi. Lopes Silva et al. (2019) identified two main factors contributing to differences between software tools, the process of importing back-end data sets and the absence of standardized rules for implementing life cycle impact assessment (LCIA) methods. Speck et al. (2015) analyzed the LCA publications from 2010 to 2013 in the International Journal of Life Cycle Assessment and Journal of Industrial Ecology and they found that a total of 116 articles used SimaPro, 38 articles GaBi, and seven articles used other LCA software. In our review we found a similar trend (Table 4); Simapro was employed in 61 % of the articles, whereas two papers employed Gabi (2 papers) and one paper OpenLCA (GreenDelta, 2020). Some of the publications do not included information or do no use any LCA software.

Regarding the LCIA method, 83 % of the reviewed papers employed midpoint categories, positioning as the most attractive indicators to display LCA results. All these methods considered the intermediate effects of the impact categories, making this option the most suitable for environmental intervention (European Union, 2010). Recipe with Hierarchist perspective (H) (Huijbregts et al., 2016) was used in 64 % of the papers, either alone (6 papers) or in combination with AWARE (Boulay et al., 2018) (2 papers) to evaluate the water use or with other methods (1 paper). Bergman et al. (2024) combined the category of freshwater eutrophication of ReCiPe with IPPC method for climate change (Stocker et al., 2023), acidification of ILCD 2011 midpoint (Posch et al., 2008; Seppälä et al., 2006), land use of CML (CML, 2024), cumulative energy demand (CED) (Frischknecht and Jungbluth, 2003) and the category of biodiversity loss methods developed by Kuipers et al. (2021) and Hélias et al. (2023).

Other methods were applied in one paper each one: IMPACT+2002 (Jolliet et al., 2003), TRACI (EPA, 2023), and CML. These methods included generic categories of acidification, eutrophication and ecotoxicity, so there is not distinction between marine and freshwater eutrophication, aquatic and terrestrial acidification, and human, freshwater, terrestrial and marine ecotoxicity. For climate change, the IPCC method is gaining in popularity due to the European Commission recommendation that propose the Product Environmental Footprint (PEF) as a common way of measuring environmental performance. In fact, based on our search, IPCC method was employed in three SCP papers since 2020.

GWP (global warming potential) is the most important impact category, used in all studies with midpoint indicators. This is the most common category in environmental analysis, but special interest is shown in food-related items, which account for approximately 25 % of annual GHG emissions (Fernández-Ríos et al., 2021). Eutrophication (marine, freshwater and unspecified) was analyzed in 16 papers, whereas 11 publications included different acidification indicators and several types of ecotoxicity. Land use was measured in 68 % of the midpoint studies and water use or consumption in 50 % of the studies because these are some of the major concerns and deficits of the food sector. Other authors studied ionizing radiation, ozone depletion and formation, fine particulate matter formation, respiratory organic and resources and energy indicators.

Endpoint methods were employed in 27 % of the studies, being ReCiPe perspective (H) and IMPACT+2002 used in two and one papers, respectively. In these methods the categories of damage to human health, ecosystems and resource availability were analyzed. Several authors highlight the benefit of single score or endpoint indicators for communication, however, the large uncertainty associated with such metrics was evident in some studies (Laso et al., 2018).

Interpretation

The interpretation consists of the presentation of the results according to the objectives. The conclusions of the life cycle inventory and impacts are assessed to provide an easily understandable and complete evaluation and to present the results of an LCA in a coherent way (ISO, 2006a, 2006b). All the authors carried out an interpretation of the results, in which, for the most part, they identified the hotspots of the processes in order to focus on the different problems and areas in which the production process of SCP should improve. For the interpretation of results, SCP production for food purposes was compared in relation to GWP, LU (land use), WU (water use), TAP (terrestrial acidification), FEP (freshwater eutrophication) and MEP (marine eutrophication). In doing so, outcomes from Upcraft et al. (2021), Järvïo et al. (2020), Kobayashi et al. (2022) and Aidoo et al. (2024) were assessed, since they used the same software, impact method and comparable FUs. To establish a fair comparison between the different studies, a FU of 1 kg of protein was established, since it provides clearer data and avoid working with products with a% protein. For studies that did not use this FU, their results were transformed to 1 kg of protein to unify units.

Lowest greenhouse gas emissions were achieved for yeast SCP production from crude pea starch, estimated at 0.61 kg CO_2 eq./kg protein (Aidoo et al., 2024). Similar results were obtained by Järvïo et al. (2021) considering a production system with hydrogen-oxidizing bacteria in which hydropower energy is used (1.6 kg CO_2 eq./kg protein). However, if electricity from the Finnish grid mix is applied, burdens rose up to 12.89 kg CO_2 eq./kg protein, evidencing that electricity is one of the most critical resources in SCP production. The worst performance was shown for mycoprotein production using rice straw as substrate (Upcraft et al., 2021) and yeast-based microbial protein from oat residues (Kobayashi et al., 2023), of approximately 23 kg CO_2 eq. and 22 kg CO_2 eq./kg protein, respectively. Generally, culture medium production as well as fermentation were the major contributors to climate change as consequence of the electricity consumption for mixing, aeration or cooling. In comparison with other protein-rich foods, SCP seems to be competitive with beef and eggs. On the other hand, chicken (3.9 kg CO_2 eq.), tofu (18.5 kg CO_2 eq.), or pork (16.8 kg CO_2 eq.) report better results than most of microbial proteins (Ritchie et al., 2020), which makes it necessary to optimize configurations, technologies and materials use in order to replace conventional foods with this novel product.

In terms resources consumption, SCP production systems using agricultural products or waste as substrate entailed higher land occupation. For instance, land use of SCP production without feedstock was estimated at 1.18 m^2a crop eq. (Järvïo et al., 2021), while this impact grew up to 2 m^2a (Kobayashi et al., 2023) and 4.39 m^2a (Upcraft et al., 2021) when organic waste is utilized as carbon source. Accordingly, upstream processes involving substrate generation and culture medium production were the main drivers of impact. All in all, non-feedstock SCP systems appear to be favorable under this perspective. On the contrary, results varied considerably between systems in terms of water use, but were not as dependent on the substrate. This point did not have a great influence on the amount of water used, but it did affect which stage is most intensive on this resource. In cases where an agricultural residue is used, most of the water consumption goes into the cultivation of the crop, whereas if there is not feedstock, the synthetic culture medium is the main demander. Some values of the water footprint of the reviewed articles are 1.3 m^3 (Kobayashi et al., 2022) and 2.23 m^3 (Upcraft et al., 2021). Generally, these footprints can be considered quite high when compared to other products such as veal, which reports 0.25 m^3/kg protein (Agribalyse, 2022).

With regard to other indicators, impacts differed considerably between systems and technologies. For marine eutrophication, Kobayashi et al. (2023) reported $5.50 \cdot 10^{-3}$ kg N eq./kg protein and associated 98 % of the impact in the culture medium production stage to the enzymatic hydrolysis to which oat side stream is subjected before fermentation, and specifically to enzyme production. Lower burdens were obtained by Järvïo et al. (2021), of $4.60 \cdot 10^{-4}$ kg N eq./kg protein, which did not consider any feedstock and, therefore, had no pre-treatment. Instead, impacts were led by the production of raw materials for the culture medium, specifically carbon dioxide, and by the use of electricity in fermentation. Indeed, the marine eutrophication potential decreased to $1.93 \cdot 10^{-5}$ kg N eq./kg protein when renewable energy is applied. The values reported for this indicator can be found in comparable ranges with other products such as chicken, but lower than more contaminating meats such as beef (Aidoo et al., 2024). Similar trends were obtained for the freshwater eutrophication potential. Greater impacts were estimated by Upcraft et al. (2021), of $1.30 \cdot 10^{-2}$ kg P eq./kg protein, and followed by Kobayashi et al. (2022) ($8.00 \cdot 10^{-3}$ kg P eq.) and Järvïo et al. (2020) ($3.50 \cdot 10^{-3}$ kg P eq.). In all the researches, electricity for fermentation was identified as the main hotspot. This resource was also the main driver of acidification potential, as identified by Upcraft et al. (2021) and Järvïo et al. (2020), which calculated 0.16 kg SO_2 eq./kg protein and $3.20 \cdot 10^{-2}$ kg SO_2 eq./kg protein, respectively. For its part, Kobayashi et al. (2023) showed a similar contribution to the impact from the fermentation, medium preparation and feedstock production stages, reaching 0.12 kg SO_2 eq./kg protein.

More and more studies include in the interpretation section a sensitivity analysis to assess the influence of key parameters in environmental outcomes. Most of the reviewed papers conducted a sensitivity analysis related to energy sources, allocation rules, etc. The use of energy has influence on most of industrial processes as well as on fermentations and drying stages for SCP production. Therefore, Järviö et al. (2021), Sillman et al. (2020), and Khoshnevisan et al. (2020) analyzed the use of alternative energy sources or the influence of the location in the energy mix's impacts, and Spiller et al. (2020) evaluated the energy related parameters and ammonia emissions of the process. On the other hand, allocation methods constitute one of the major sources of uncertainty in LCA results. Couture et al. (2019) and Bergman et al. (2024) conducted a sensitivity analysis to compare mass and economic allocation, whereas Marami et al. (2022) and Tallerine et al. (2018) analyzed the influence of the monetary value on the economic allocation. Finally, other authors assessed the efficiency, operational and design conditions (Järviö et al., 2021; Upcraft et al., 2021; LaTurner et al., 2020), and the characteristic of inputs and outputs (Chen et al., 2020; Wang et al., 2023; Tallerine et al., 2018; Aidoo et al., 2024).

Future challenges

As SCP are getting noticeable in research field and the LCA analysis is an indispensable tool to address the environmental performance of products, the combination of them is essential to create an appropriate evaluation and to improve the environmental performance of the production processes. Animal feed purpose is a field known and safe as the majority of studies reported it, but it is not the same case for human food. The intersection of two aspects of interest, human food and waste substrate, needs to be investigated to optimize processes and provide a kind of protein with lower environmental impacts than traditional sources of protein, that implies a variety of consumer options and perhaps, a change in how meat industry processes their products, among others. However, the fact that some substrates are recycled process streams may generate controversy in the general public. Another aspect that may influence the consumers preferences are the sensorial characteristics of the products. Food neophobia and meat attachment as psychological constructions create obstacles in the interest of change and diet transition. Future investigations should consider these points in detailed and analyze how this attitude could be surpassed.

Conclusions

The objective of this work is conducting a thorough review to uncover the fundamental similarities among SCP production methods and establish a unifying framework for its understanding under an LCA approach.

In relation to the LCA methodology, the FU definition was identified as the most relevant, as well as the most concerning characteristic in LCA studies, since its selection could lead to misinterpretation of the results. A nutritional based FU might be an option that contribute to better comparison with other products to food or feed purpose, rather than weight base FU that are common in LCA of food products. In this regard, the application of a protein quality scoring system is strongly recommended, as it can best compare the intended function of protein-rich foods. Regarding the environmental performance of SCPs, the main hotspot was the high electricity consumption, being the main contribution stages fermentation and culture medium production. If these steps are optimized to achieve better efficiency, the footprints and the process impacts will decrease. Use alternative energy from renewable sources would be another option to take into consideration to reduce the negative effects of the processes. All in all, the valuable insights reported in this review provides a foundation for future research to build on in order to facilitate the development of more efficient and sustainable SCP production systems.

CRediT authorship contribution statement

Laura Fernández-López: Writing – original draft, Investigation. **Pablo González-García:** Writing – original draft, Investigation. **Ana Fernández-Ríos:** Writing – review & editing. **Rubén Aldaco:** Supervision. **Jara Laso:** Writing – review & editing. **Eva Martínez-Ibáñez:** Writing – review & editing. **David Gutiérrez-Fernández:** Writing – review & editing. **Marta M. Pérez-Martínez:** Writing – review & editing, Funding acquisition. **Virginia Marchisio:** Writing – review & editing. **Mónica Figueroa:** Writing – review & editing, Funding acquisition. **David Baptista de Sousa:** Writing – review & editing, Project administration. **Diego Méndez:** Writing – review & editing, Project administration. **María Margallo:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Maria Margallo reports financial support was provided by University of Cantabria. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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