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# Recent findings on environmental sustainability and conversion efficiency of waste-to-protein pathways

**Review Article** 

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# Recent findings on environmental sustainability and conversion efficiency of waste-to-protein pathways



Armin Siegrist, Ashley Green, Moritz Gold and Alexander Mathys

#### Abstract

Research on the environmental sustainability and nutrient conversion efficiency of bioconversion technologies applied in waste-to-protein pathways is relevant from an early development stage on to identify optimal applications. This review summarizes the recent advances and remaining issues in this emerging research field. While black soldier fly larvae (BSFL) have been intensively studied, various other technologies such as other insect species, bacteria, fungi, microalgae, and worms, are currently underrepresented. Regarding environmental sustainability, which is mainly studied through life cycle assessment, the choice of functional unit is highly relevant for overall outcomes and comparability. Additionally, decisions on the burden of input materials and process substitution strongly influence the overall results. Substrates composed of different residual biomass streams strongly influence the process efficiency of BSFL, which is commonly expressed in feed conversion and protein efficiency rates. In contrast, residual biomass type, protein content, and amino acid profile are of minor importance for the protein composition of BSFL. Overall, the large variability of residual biomass types and bioconversion technologies necessitates better methodological alignment to produce comparable results across studies that collectively support decision-making.

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#### Keywords

Waste-to-nutrition, Alternative protein, Bioconversion, Circular economy, Black soldier fly larvae.

# Introduction

Residual biomass (RB; i.e., waste and side streams) utilization through waste-to-nutrition pathways is

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the nutritional circularity of food systems and decoupling their productivity from resource consumption. A recent review article by Javourez et al. [1] presented a comprehensive overview of the available conversion technologies. Within the field of bioconversion technologies, which utilize the metabolic capacities of organisms, research has increasingly focused on bacteria [2], fungi [3], insects, and microalgae [4] hereafter referred to as technologies. The ability of these organisms to transform residual nutrients into high-value proteins is a promising alternative to conventional agribased products. However, regulatory hurdles and product safety concerns related to hazardous or antinutritional compounds remain [5,6]. Furthermore, it is crucial to analyze the environmental sustainability of these processes early to avoid undesired trade-offs and burden-shifting when aiming to replace conventional protein sources [7]. This is a particularly challenging task considering the novelty of these technologies with limited production-scale data. Therefore, this article aims to document recent advances in the life cycle assessment (LCA) of bioconversion technologies used for alternative protein production, summarize the reported impacts of global warming, water, land, and energy use, and highlight remaining issues. As environmental sustainability and nutritional performance are strongly interlinked [8], this review further documents recent findings on protein quantity, amino acid (AA) profiles, and protein conversion in documented wasteto-protein pathways. A particular goal was to compare the effects of feed-grade RB streams, which have generally more value and competing utilization pathways, with non-feed-grade RB streams, which are commonly associated with safety concerns and regulated more stringently.

widely recognized as a promising approach for increasing

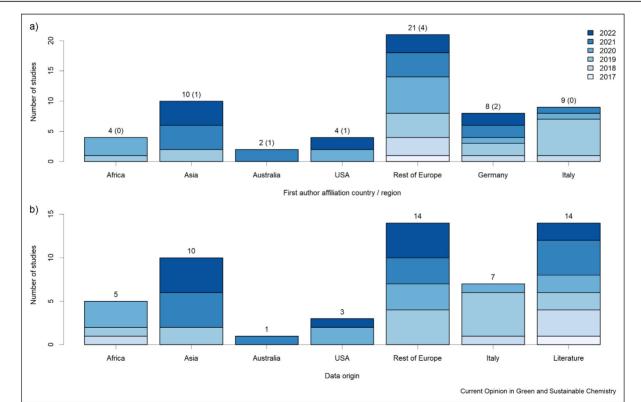
# Literature review

The Web of Science and Scopus databases were searched for keywords related to sustainability, feed conversion, RB, food or feed applications, and protein. Articles published in 2018 or later containing at least one keyword from each category were selected (n = 1441) and screened for quantitative information of interest (n = 114). Finally, the quality of the articles was assessed based on several exclusion criteria (n = 56). For a detailed workflow, please refer to Figure S.1. Although the selected articles cover research institutions from most continents, research activities on bioconversion

technologies in waste-to-protein pathways seem to be heavily concentrated in Germany, Italy, and Europe in general (Figure 1a). Nevertheless, the geographic distribution of experimental sites and studied facilities (Figure 1b) indicates a wide coverage of climatic conditions, which can be relevant for the performance of bioconversion technologies. Only Razzaq et al. [9] investigated incorporating RB-sourced fungal proteins in noodles. In contrast, all other studies have focused on feed applications. Over half of the selected LCA studies analyzed insect production, while over 75% of publications reported on insect proteins. In both cases, the dominant insect species was *Hermetia illucens*, commonly called black soldier fly (BSF), owing to the high substrate plasticity of BSF larvae (BSFL).

# Environmental life cycle assessment

Based on the environmental impact data reported for various bioconversion pathways (Table 1), technologies and individual studies are difficult to compare because of the use of different functional units (FUs). Depending on the primary focus of the study on either protein provision or RB treatment, the authors adopted different perspectives when describing the same process. Consequently, they either reported their results against an FU based on RB input, biomass output, or protein output. For better harmonization, it is therefore strongly recommended to find a consensus on the most suitable FU for bioconversion technologies or at least provide the necessary data to convert one FU to another, for example, the moisture and protein content of the final product. Another issue is the lack of data and varying impact assessment methods for certain impact categories, e.g., for worm-based applications [10,11] or water and energy use in general (Table 1). This further reduces comparability and leads to one-dimensional analyses based on global warming potential (GWP), which may overlook relevant trade-offs. For example, emissions from energy generation are often a dominant contributor to the overall GWP impacts of bioconversion technologies [2,3,12,13]. This is in contrast to that of animal-based proteins, where GWP impacts are mainly driven by land use change, manure management, and, in the case of ruminants, enteric fermentation [14]. Therefore, increasing the variety of studied impact categories based on standardized methods is critical for achieving more comprehensive assessments.



Geographic distribution of the selected research articles by (a) the affiliation of the first author and (b) the location of the experimental site or studied facilities. If a first author in (a) had affiliations in multiple regions, the study was counted for all of them. The number above each bar indicates the total number of articles and the number in brackets refers to the number of review articles and book chapters. The literature category in (b) includes review articles, book chapters, and original articles that summarized protein contents or AA profiles from previous studies in a review section or relied exclusively on literature data for their LCA.

Figure 1

Summary of GWP, land, water, and energy use related impacts reported by recent life cycle assessment (LCA) studies investigating bacteria-, fungi-, insect-, microalgae-, or worm-based waste-to-protein pathways. Bacteria include purple non-sulfur bacteria; fungi include *Neurospora intermedia* and *Fusarium venenatum*; insects include *Hermetia illucens*, *Musca domestica*, *Protaetia brevitarsis seulensis*, and *Tenebrio molitor*; microalgae include *Galdieria sulphuraria* and *Chlorella vulgaris*, and worm species include *Eisenia fetida*. For specific values of a species or study, please refer to the Supplementary Data.

Category	FU <sup>a,b</sup>	GWP <sup>b</sup> (kg <sub>CO2-eq</sub> )	LU <sup>b</sup> (m <sup>2</sup> * a)	WU <sup>b</sup> (m <sup>3</sup> )	ED <sup>b</sup> (MJ)	Data
Microalgae	1 kg dry protein	8.70-12.49	0.25-0.32	NA	202.8-248.5	[15]
Fungi	1 kg dry protein	23.7	4.4	2.2	NA	[3]
Insect	1 kg dry protein	2.4-18.0	-1.3-9.8	-0.07-0.39	NA	[16-18]
Microalgae	1 kg dried BM <sup>b</sup>	0.3-19.7	0.03-0.74	0.2-6.4	13.20-18.04	[13,19,20]
Insect	1 kg dried BM <sup>b</sup>	-6.4-12.0	-16.8-61.0	2.8-11.0	-108.0-84.2	[12,21-25]
Insect (c <sup>c</sup> )	1 kg dried BM <sup>b</sup>	-2.9-8.4	-3.6-22.5	-14.0-103.9	19.5-141.4	[24,26]
Worm	1 kg dried BM <sup>b</sup>	2.2-6.3	NA	NA	NA	[10,11]
Fungi	1 kg fresh BM <sup>b</sup>	0.1-0.2	0.05-0.09	0.02	NA	[27]
Insect	1 kg fresh BM <sup>b</sup>	0-1	0	NA	1-10	[6]
Bacteria	1 ton fresh RB <sup>b</sup>	220.3-322.2	-62810196	-8.041.65	NA	[2]
Insect	1 ton fresh RB <sup>b</sup>	35	NA	NA	NA	[28]

<sup>a</sup> Where possible results based on fresh matter or RB were converted and reported based on 1 kg of dried BM.

<sup>b</sup> FU, functional unit; GWP, global warming potential; LU, land use; WU, water use; ED, energy demand; BM, biomass; RB, residual BM.

<sup>c</sup> c = consequential approach (all other studies followed an attributional approach).

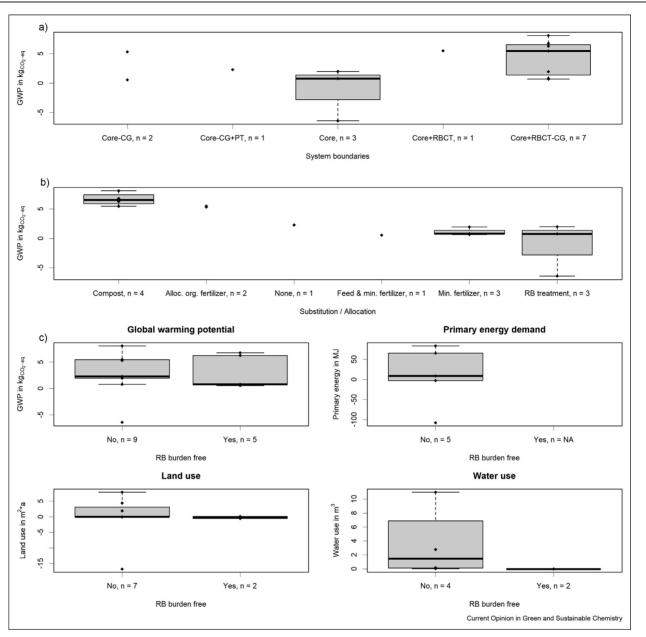
The range of the reported impacts for one technology can be vast. While this is often attributed to differences in FUs across studies, variations in outcomes are also driven by other underlying assumptions, such as the choice of system boundaries, substitution (system expansion), allocation, and burdens of RB. According to studies that reported impacts at the group level, transport processes are of minor importance (<20%) in the considered impact categories compared to the core processes (defined as processes that occur within factory gates) of RB preparation, bioconversion, and post-processing [10,11,13,15-17,22,23]. The operation phase caused the most impacts, but where reported, capital goods contributed up to 15% of the overall impacts [22]. Regarding substitution or allocation effects, the surveyed papers reported significant GWP impact reductions of 30-70% from replacing mineral fertilizers with processed insect residues [12,23]. In comparison, substituting compost by residues or allocating a share of the impacts to insect residues used as organic fertilizer vielded significantly smaller reductions of 10-20% [18,22,24]. As evidenced, the potential impact reduction of using bioconversion instead of conventional RB treatment (e.g., composting) can be very high but depends strongly on the RB treatment technology [13,15,17,21]. The allocation of burdens to the RB substrate can also be a relevant contributor to the overall impacts [3], especially if animal-based residuals are involved. For example, Roffeis et al. found that almost 70% of the total impacts originate from RB (e.g., manure) [22].

In this study, the influence of these parameters is explicated using BSFL because it is the most studied technology. We focused on the BSFL studies measured with attributional assessments and an FU of 1 kg of dried biomass (Figure 2). Despite the small sample of seven studies covering 14 RB streams, the analysis yielded several relevant observations. First, the inclusion or omission of capital goods, final product transport, and RB collection and transport are not visible in the overall GWP impact (Figure 2a, Figure S.2). In contrast, the effects of substitution or allocation are strongly visible in the overall GWP impacts (Figure 2b). Compost and organic fertilizer substitution were expressed in higher overall emissions (>5 kg CO2-eq/kg dry larvae biomass) compared to studies that assumed a substitution of mineral fertilizer or RB treatment (<2 kg CO2-eq/kg dry larvae biomass). Surprisingly, the study by Modahl et al. [16] reported comparatively low GWP impacts (3.8 kg CO2-eq/kg dry larvae biomass) without considering substitution effects. Finally, the analyzed data indicates that the allocation of burdens to RB inputs is generally reflected by higher overall impacts across various impact categories (based on median values; Figure 2c). Overall, the findings highlight the relevance of methodological choices in LCA and the need for alignment to increase comparability.

# Protein quantity

This paragraph summarizes the findings on the total dry matter (DM) protein content of RB and post-conversion products in waste-to-protein pathways. Prandi et al. [29] measured the protein composition of almost 40 different RB streams (specifically, food waste). Together with values from other recent publications [12,30–37], they offer an excellent overview of DM protein content in potential substrates (Figure 3a). We define non-feedgrade RB based on European Union (EU) legislation definitions [6]. On average, this biomass contains 15%

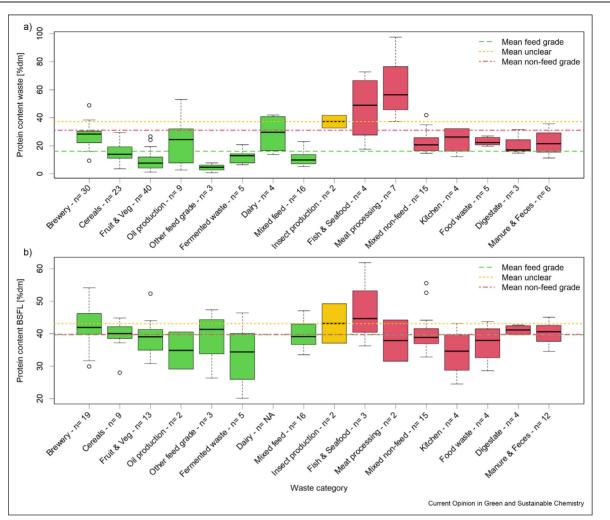




Environmental impacts reported by different attributional life cycle assessment (LCA) studies analyzing black soldier fly larvae (BSFL) production systems based on a functional unit (FU) of 1 kg dried biomass of larvae (meal). **a**) Global warming potential (GWP) impacts under consideration of different system boundaries (Core = residual biomass (RB) preparation & bioconversion, post-processing, and capital goods (CG), PT = product transport to the point of use, RBCT = RB collection & transport). Please refer to Figure S.2 for an illustration of the different system boundaries. **b**) GWP impacts based on different substitution or allocation approaches (Alloc. = economic allocation). **c**) GWP, energy, land, and water use impacts of studies considering RB to be burden-free or not. Further studies were needed to analyze the impacts of energy, land, and water use in a) & b). Please note that influencing factors could not be isolated, e.g., comparing substitution and allocation approaches included studies with different system boundaries. Data sources: [12,16,18,21–24]. For underlying data, please refer to Supplementary Material.

more protein than the feed-grade RB. In particular, animal-based residuals from insect production, and fish and meat processing showed very high protein values (median: 37-56% protein). Among feed-grade substrates, the protein contents of side-streams from beer, dairy, and oil production stand out (median: 20-31%

protein). With regard to post-conversion products, a wide range of DM protein contents has recently been reported for microalgae (13–53%) [4,38,39], fungi (17–70%) [9,40], and insects (20–64%) [4,23,35,37,41–55]. Looking at median values, microalgae (36%) and insects (40%) show significantly higher protein levels compared





Dry matter (DM) protein contents of (a) residual biomass, i.e., waste & side streams potentially used as rearing substrates, and (b) black soldier fly larvae. They are grouped by the category of biomass. The groups are colored based on their legal status as feed substrates in the European Union according to Bosch et al. [6]. Insects are not clearly classified as feed- or non-feed-grade because their status depends on their rearing substrate. The reported values include crude and true protein. Please refer to the Supplementary Material for absolute values and a description of specific residual biomass types. Data sources: [4,12,23,29–37,42–54].

to fungi (11%). The only identified value for bacterial protein (*Lactobacillus kefir*) was 54% DM [40]. Most studies did not report the protein contents of both the rearing substrates and post-conversion products, except for insect larvae, where a positive sample correlation (r = 0.41) was found (Figure S.3). Interestingly, the mean DM protein content of BSFL did not differ between feed- and non-feed-grade rearing substrates, even though the reported protein content ranged from 20% to 60% (Figure 3b), which is often a challenge in animal feed applications.

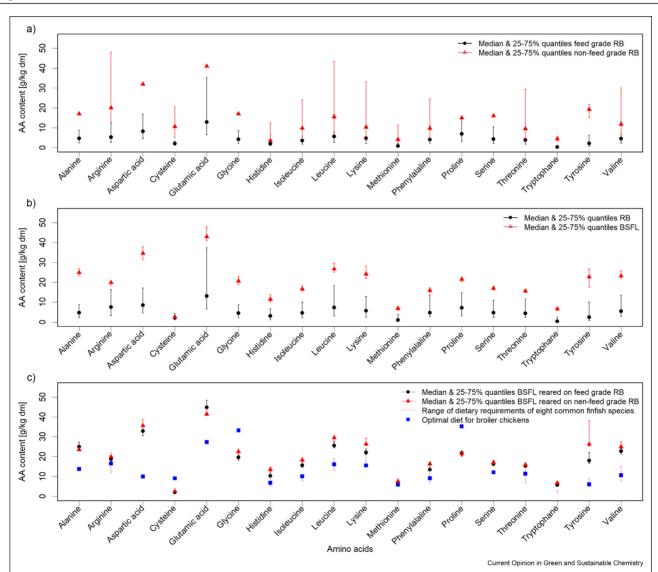
A recently debated topic is the conversion of measured nitrogen (e.g., by the Kjeldahl or Dumas method) to protein. Many studies still rely on the standard nitrogento-protein conversion factor of 6.25 [36,46,51,52], which overestimates the true protein content of most RB and bioconversion products because it includes non-protein nitrogen, such as chitin from insects. Therefore, an increasing number of researchers have applied factors derived from the measured total AA content [29,47– 49]. In the case of BSFL, 4.76 is typically used, which is almost 25% below the standard factor [47–49]. Unfortunately, the applied factor is often not specified. It is essential to provide the applied conversion factor and the measurement method when reporting the protein content to improve cross-study comparisons.

#### Protein quality

Among the identified studies focusing on waste-toprotein pathways, AA profiles were almost exclusively found for BSFL [37,43,46,47,52,56] except for one report on the AA composition of mealworms [55] (see Supplementary Data). This indicates that research on the protein quality of other organisms has been strongly underrepresented in recent years. The AA composition of RB itself is highly variable (Figure 4a) owing to the diversity of the analyzed substrates [29,34,43,47,57] and potentially varying measurement conditions. In general, meat-, fish-, and manure-based substrates classified as non-feed-grade in the EU [6] show higher DM AA contents than feed-grade substrates, such as cereal,

Figure 4

brewery, fruit, and vegetable side-streams (Figure 4a). This agrees with the presented findings for overall protein content (Figure 3a). When comparing the AA profiles of BSFL to RB, all AAs in larvae, except for cysteine, were elevated (Figure 4b). These findings underscore the suitability of BSFL for bioconversion with the primary goal of converting RB into high-quality protein sources for food and feed. The reported AA profiles of BSFL fed on non-feed- and feed-grade substrates show a low discrepancy (0-3%) between medians) across studies, except for tyrosine, where non-



Amino acid (AA) profiles in g/kg dry matter of (a) feed- and non-feed-grade residual biomass (RB; i.e., waste & side streams potentially used as rearing substrates) (n = 1-41) (b) overall RB (i.e., feed- and non-feed-grade), and black soldier fly larvae (BSFL; n = 13-54), and (c) BSFL reared on feed- and non-feed-grade residual biomass (n = 4-19). In this context, residuals refer to waste or side-streams of biomass that can be used as rearing substrates for BSFL and not residues from the bioconversion, i.e., BSFL residues. The optimal dietary profile for broiler chicken and dietary requirements for various fish species were added as a reference in (c). Chicken diets were adapted from He et al. [58] under consideration of chicken AA digestibility reported in Schiavone et al. [59]. The dietary requirements of fish were obtained from Lall et al. [61]. Data sources: [29,34,37,43,46,47,52,55–57].

feed-grade substrates led to higher values (+7% of median) (Figure 4c). Compared with an optimal dietary profile for broiler chickens [58,59], BSFL (25% quantile) exceed the requirements for most AAs while being deficient in cysteine, glycine, and proline. This should be carefully monitored when formulating animal diets, as discussed by Heuel et al. [60] and He et al. [58]. Regarding fish diets, methionine and cysteine are critical in BSFL-based formulations [52].

# Feed conversion & protein efficiency

Data on feed conversion (FCRs) and protein efficiency rates (PERs) along waste-to-nutrition pathways are very sparse. This represents a key data gap because both rates can vary significantly among feed sources [35,51]. Therefore, they are important performance indicators of bioconversion processes. Furthermore, they are commonly used in feeding trials to study the effect of replacing conventional protein sources with postconversion products in animal diets (e.g., larvae meal replacing fishmeal). However, FCRs and PERs are always influenced by many factors and operational parameters that differ among studies. Generally, the FCR is calculated by dividing the weight of feed provided to an organism by its weight gain, while the PER is obtained by dividing the organism's weight gain during bioconversion by the amount of protein provided in the feed [33,53,55]. Thus, a low FCR and a high PER are desired. However, the calculation methods are associated with critical uncertainties; for example, some studies have reported ingested feed instead of the feed provided for FCR [30,35,41,51,52]. Another disparity arises from the use of dry- or fresh-matter-based weights. However, such specifications are often lacking. This makes meaningful cross-study comparisons difficult and requires future alignment.

Reported FCRs for BSFL and mealworm larvae reared on various RB substrates range from 1.1 to 14.5 (n = 25)and 4.4 to 5.5 (n = 2), respectively [33,35,41,51]. The available data showed no significant difference between feed- and non-feed-grade rearing substrates (see Supplementary Data). PERs are not found in any bioconversion technology. For fish species studied in feeding trials, reported FCRs and PERs for feed blends containing worm or insect meal were in the range of 1.03-2.83 and 0.33 to 2.45, respectively [52,53,55,62-64]. The deviation from FCRs and PERs found for control diets was generally below 20% [52,55,62-64]. However, one study found significant improvements of the FCR and PER by the supplementation of postconversion proteins (FCR, -38%; PER, +48%) [53] while another study reported opposite findings (FCR, +75%; PER, -41%) [62]. The available data shows no clear correlation between the inclusion level of worm or insect meal (from 3% to 100%), and FCR or PER (see Supplementary Data).

# Conclusions

Research on the environmental sustainability and conversion efficiency of bioconversion technologies is relevant from an early development stage to identify optimal substrates and processes. Compared to other insect species such as mealworms and crickets, BSFL is currently not allowed as food in the EU [1]. Nevertheless, it is by far the most studied technology. A key advantage in the context of waste-to-protein pathways is that BSFL are a non-disease vector with a high conversion rate on a large variety of different RB types [35]. In comparison, data on other insect species, worms, microalgae, fungi, and bacteria remains sparse. Hence, efforts to investigate underrepresented technologies should be intensified to close current research gaps. Furthermore, the large variability of RB and bioconversion technologies necessitates better methodological alignment to produce comparable results that collectively support decision-making. Nitrogen conversion factors should be aligned and documented to reduce uncertainty. Furthermore, a standardized approach for FCR and PER calculations should be defined. With respect to LCA, decisions on the FU and burdens associated with input materials and process substitution can strongly influence GWP, land, water, and energy use. Therefore, defining a common baseline for LCA studies of bioconversion technologies is strongly recommended.

From a nutritional perspective, the overall protein content of RB shows a positive correlation with the reported BSFL values, which vary significantly across substrates and studies. Contrastingly, the AA profiles of BSFL were consistent across studies and rearing substrates. They exceeded the dietary requirements of broiler chickens and fish, except for cysteine, glycine, methionine, and proline. RB can considerably influence the process efficiency of BSFL expressed by the FCR and PER. However, as with the other parameters, no significant difference between the feed- and non-feed-grade substrates was found. Therefore, these waste-to-protein pathways could be a relevant valorization strategy considering the lower economic value of non-feed-grade RB. Other nutritional and non-nutritional differences in substrates that influence the bioconversion process are beyond the scope of the present review. However, product safety remains a key priority and can incur additional costs. While this issue is raised by many studies, quantitative data are sparse for most technologies [1,2,17,31,40]. Investigations on BSFL did not find an accumulation of selected chemicals and toxins while pathogenic microbes were even reduced [35,43,49]. At the same time, BSFL accumulate heavy metals, which demands careful monitoring in the future [43,44,62]. Finally, products from bioconversion technologies, which are intended for direct human consumption, will have to overcome food neophobia and disgust among consumers. The rejection of insect-based products, for example, is particularly high in western countries and among older people [65]. Thus, it is likely that ingredients sourced directly from RB will evoke similar reactions among these consumer groups. Feed applications, on the other hand, result in animal-based products that are familiar to consumers and thus unlikely to be met with such reservations.

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# **CRediT** authorship contribution statement

Armin Siegrist: Conceptualization, Methodology, Calculations, Writing – original draft, Visualization, Writing – review & editing.

Ashley Green: Funding acquisition, Conceptualization, Methodology, Supervision, Writing – review & editing.

Moritz Gold: Writing – review & editing.

Alexander Mathys: Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

# **Declaration of Competing Interest**

The authors 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

All data used for this study is available in the supplementary material.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cogsc.2023.100833.

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- of special interest
- \*\* of outstanding interest
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A comprehensive review article, which provides an excellent overview on waste-to-nutrition pathways. The authors document existing and emerging conversion technologies, describe their applications in the utilization of various organic waste streams, and propose a classification according to the applied mechanisms.

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The article documents the conversion of carrot pomace, a side-product from carrot juice production, into single cell protein by means of fermentation. Furthermore, the researchers assessed how supplemented protein effects the nutritional and cooking properties of noodles. Thereby, it is the only identified study that reports a direct food application of bioconverted protein as opposed to animal feed supplements.

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   black soldier fly larvae (*Hermetia illucens* L.). Sci Total Environ
   2021, 750:141656. https://doi.org/10.1016/
   j.scitotenv.2020.141656.

The authors present a material flow and life cycle analysis for food waste bioconversion by black soldier flies based on a full-scale operational facility treating 15 tons of biowaste per day. Compared to common practice of relying on literature data or upscaling from pilot plant operations, this study is based on direct measurements of inventory flows including gaseous emissions for all processes within the factory gates.

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Taking a life cycle assessment approach, the authors investigate the sustainability of microalgae production in an operating production

facility. An extensive scenario analysis was conducted to investigate the influence of various nutrient and carbon sources on the overall process performance.

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