

A systematic review of life cycle assessments for precision fermentation protein and microbial oil ingredients: bioprocess systems, methodological choices, and impact drivers

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2 **fermentation protein and microbial oil ingredients:**
3 **bioprocess systems, methodological choices, and impact**
4 **drivers**

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Abstract

Purpose: Precision fermentation enables microorganisms to produce functional food proteins and oils as lower impact alternatives to conventional animal-derived supply chains. However, their environmental impacts have not been comprehensively reviewed. This systematic review examines the life cycle assessment (LCA) evidence base for precision fermentation proteins and microbial oils, assessing the landscape of modeled bioprocess systems, LCA methodological choices and data quality, comparative environmental impacts, and key bioprocess and methodological impact drivers.

Methods: Following FLAVIA-LCT and STARR-LCA systematic review frameworks, separate searches for fermentation-derived protein and oil LCAs were conducted in Dimensions and Web of Science, supplemented by grey literature and citation chaining, covering publications from 2010 through 2025. Eligible studies included process-based LCAs published in English covering precision fermentation (PF) proteins or microbial oils for food or feed applications. Twenty-six studies were identified (13 PF protein and 13 microbial oil LCAs) spanning PF food ingredient proteins (dairy, egg, and meat) and production aid proteins (enzymes and growth factors), and microbial oil ingredients for omega-3 fish oil and vegetable oil applications, yielding 52 scenarios for analysis.

Results and Discussion: Comparative LCA results demonstrate consistent GHG and land use reductions for PF proteins over dairy and egg comparators. For microbial omega-3 oils over fish oil, GHG, acidification, and eutrophication reductions were consistent, while land use was higher in sugar-feedstock systems; however, marine living resource depletion, arguably the strongest environmental argument for fish oil alternatives, is not consistently measured in standard LCIA frameworks. Methodological heterogeneity in functional units, system boundaries, and LCIA methods limit cross-study comparison. Further, most inventories relied on prospective or pilot-scale data, with industrial-scale primary data represented in only two studies. Despite this, hotspot analyses across studies show convergence: carbon feedstock, process energy, and chemicals were the most frequently reported impact drivers, highlighting opportunities for renewable energy, sustainable feedstocks, and circular valorization.

Conclusion: Precision fermentation-derived proteins and microbial omega-3 oils demonstrate consistent environmental advantages, though performance depends on energy source, feedstock, and location. Solidifying this evidence-base requires consensus-based reporting guidance for fermentation-derived ingredient LCAs, improved primary process data, and integrated techno-economic and environmental assessment. Despite the robust landscape, evidence gaps remain for several commercially relevant ingredient categories along with comparative process representativeness, underscoring the need for continued LCA development and reporting as the precision fermentation sector matures.

Keywords: fermentation, precision fermentation, microbial oils, alternative protein, life cycle assessment, systematic review, environmental sustainability, novel foods

1. Introduction

Fermentation-derived alternative proteins and fats present a promising route for reducing the environmental footprint of conventional animal agriculture and marine fisheries. Precision fermentation enables microorganisms to produce specific functional proteins, such as dairy and egg proteins, that are nutritionally and functionally equivalent to their animal-derived counterparts (Eastham and Leman 2024), while microbial oil fermentation offers a terrestrial route to omega-3 and other lipids that conventionally depend on wild or farmed fish stocks (Qin et al. 2023). As both pathways expand commercially, understanding which production parameters most significantly influence environmental performance, alongside techno-economic feasibility, is essential to guiding the sector's development (David, Juliano, and Ridoutt 2025).

A foundational body of life cycle assessment (LCA) literature has begun to establish the environmental case for these pathways, including precision fermentation-derived egg protein (Järviö et al. 2021), dairy protein (Perfect Day 2021; Hamelin and Cellier 2024, 2022; Behm et al. 2022), and microbial oils (Bartek et al. 2021; Dutra et al. 2024; McKuin et al. 2022; Togarcheti and Padamati 2021), though with substantial variation in production context, comparator systems, and the magnitude of reported advantages.

What remains absent from the literature is a systematic, cross-study synthesis of this evidence base, a gap the present review intends to fill. Existing LCAs differ substantially in functional units, input data quality, system boundaries, impact assessment methods, and comparator systems, limiting the ability of stakeholders, including researchers, investors, and policymakers, to compare results or draw generalizable conclusions about where environmental impacts arise and what production choices most influence them. Prior systematic reviews have addressed single-cell protein (Fernández-López et al. 2024) and mycoprotein (Shahid et al. 2024) LCAs, but no equivalent synthesis exists for precision fermentation proteins or microbial oils, despite their growing commercial relevance and a comparably sized body of peer-reviewed evidence.

Beyond comparative impact synthesis, the bioprocess systems underlying these LCAs have not been systematically characterized or benchmarked against commercial practice, leaving open questions about how representative the existing evidence base is of real-world production. Methodological rigor, data quality, and data quality assessments also vary considerably across studies that directly affect the reliability and comparability of reported environmental outcomes.

This review also complements recent techno-economic landscaping of fermentation-derived ingredients (Good Food Institute 2025), supporting technology road-mapping that links environmental performance with economic feasibility. This pairing is increasingly recognized as essential for precision fermentation ingredient development (David, Juliano, and Ridoutt 2025), particularly given new international guidance for integrating environmental and economic assessments (ISO/TS 14076:2025).

This systematic review addresses three research questions: (i) what is the landscape of life cycle assessments for

precision fermentation proteins and microbial oils, including the bioprocess systems modeled and how they compare to commercial practice; (ii) what types and sources of data underlie these LCAs, and what data gaps, quality issues, or methodological inconsistencies might limit their comparability; and (iii) what are the environmental impacts of fermentation-derived proteins and oils, and what are the most significant drivers of those impacts?

2. Methods

2.1 Research strategy and search design

This systematic review followed the FLAVIA-LCT (Gulotta et al. 2023) and STARR-LCA (Zumsteg, Cooper, and Noon 2012) frameworks, applying the Product, Impact, Flow, and Type (PIFT) approach to identify eligible studies of food-relevant precision fermentation protein and microbial oil LCAs (Figure 1). Biomass fermentation LCAs were excluded from the search strategy given previously published reviews of single-cell protein (Fernández-López et al. 2024) and mycoprotein (Shahid et al. 2024) LCAs.

Separate searches for precision fermentation proteins and microbial oils were conducted in Dimensions (Digital Science) and Web of Science (Clarivate) between 25 July and 12 August 2025 using Boolean search strings reported in Supplementary Tables S1.1 and S1.2. The search covered publications from 1 January 2010 to 1 August 2025, with review articles excluded at the search stage; eligible studies identified after the initial search were appended through 1 November 2025. Extant literature, including grey literature and company-commissioned LCAs from the authors' existing library, was appended to the database search results, followed by citation chaining to identify additional eligible manuscripts.

2.2 Selection criteria and screening

Search results were screened for duplicates, then evaluated against the PIFT inclusion criteria following the FLAVIA-LCT approach (Gulotta et al. 2023). Three reviewers (KT, LE, AL) independently screened titles, abstracts, and full texts against the PIFT criteria. Studies out of scope were categorized by exclusion reason in Supplementary Table 1. Search and screening results are reported in the FLAVIA-LCT-inspired flow diagram (Figure 1) and in Supplementary Tables S1.3–S1.5.

Study eligibility included attributional or consequential process-based LCAs published between 2010 and 2025, in English, that were peer-reviewed, ISO-compliant, or ISO-conformant. Precision fermentation protein eligibility included food ingredient proteins (dairy, egg, meat) and process aid proteins (enzymes, growth factors). Microbial oil eligibility includes oil ingredients for food or feed applications. Both pathways were eligible across native or recombinant microbial systems, submerged or solid-state fermentation, and heterotrophic or autotrophic cultivation. Exclusions were applied to review articles, non-English manuscripts, non-food or non-feed applications such as pharmaceuticals and biofuels, and plant-based or cultivated systems (Figure 1).

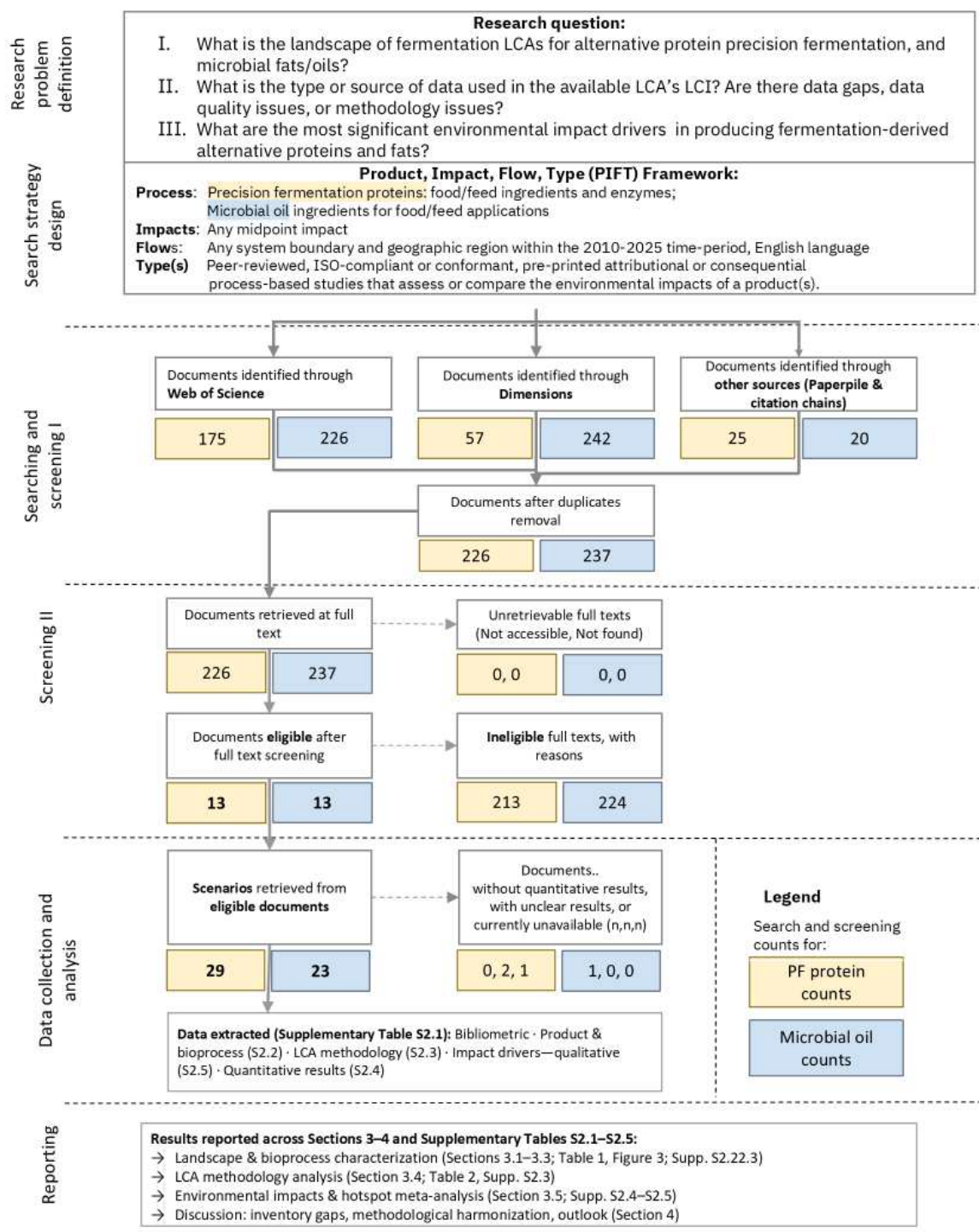


Fig 1 Systematic review of precision fermentation protein (yellow, dashed box) and microbially produced fats and oils (blue, solid box) LCAs following a FLAVIA-LCT inspired flow diagram detailing the search strategy and eligibility screening

2.3 Data extraction procedure

Data were extracted from eligible studies using a structured capture template expanded from the FLAVIA-LCT framework, spanning five categories: (1) bibliometric data, (2) product and bioprocess characteristics, (3) LCA methodology, (4) qualitative impact drivers, and (5) quantitative impact results (Supplementary Table S1.6). Extraction was conducted and validated manually by the authors, with text-analysis assistance used for cross-checking against extraction fields (ChatGPT-5.2, OpenAI; see Section 9). Ambiguous entries were resolved through follow-up with original study authors where possible. Full extracted data are reported in Supplementary Table S2.1. Commercial bioprocess boundaries for relevant microbial oil and protein ingredients were extracted from the FDA GRAS notice database for comparison (Supplementary Table S2.3; Cfsan 2024). Comparative quantitative LCA results were extracted from in-text data or supplementary tables and recorded in Supplementary Table S2.4. Environmental hotspots were identified through in-text attribution, figure interpretation, or supplementary material and mapped to sub-categories in Supplementary Table S2.5.

3. Results

3.1 Bibliometric findings

From the systematic review framework, 26 eligible publications were identified: 13 precision fermentation protein studies and 13 microbial oil studies (Figure 1; Supplementary Table S1.5). Separate searches returned a combined total of over 480 records before duplicate removal, drawn from Web of Science, Dimensions, and existing literature libraries and citation chains. Following duplicate removal and full-text screening, the large majority of records were excluded for not being process-based LCAs; exclusion reasons are detailed in Supplementary Tables S1.3 and S1.4.

Of the 13 eligible microbial oil publications, 23 distinct scenarios were extracted for analysis. Of the 13 eligible precision fermentation protein publications, 29 distinct scenarios were extracted for analysis. This total includes two LCA publications by Hamelin and Cellier (2022, 2024) evaluating Verley's (formerly Bon Vivant) whey protein where the 2024 update superseded the 2022 study; the earlier version is no longer publicly available but was retained in the authors' literature archive. Two lactoferrin LCAs identified during manuscript preparation (Zhang et al. 2026; David et al. 2026) fall outside the defined search window and are noted as emerging evidence but excluded from the systematic synthesis.

Geographic distribution of eligible publications was concentrated in Europe and the United States for both searches (Figure 2a), as observed for single-cell protein LCAs (Fernández-López et al. 2024) and consistent with their status as leading global hubs for fermentation-made ingredient research and development, startups, and commercial production (Good Food Institute 2023). Modeling activity for both platforms has increased over the past 15 years, reflecting growing attention to food system sustainability (Figure 2b). Academic and public institutions were the primary contributors for both product classes, though precision fermentation studies showed greater representation from private companies and consultancies (Figure 2c), driven by the industry's need to demonstrate environmental

competitiveness for investment, regulatory, and commercial purposes.

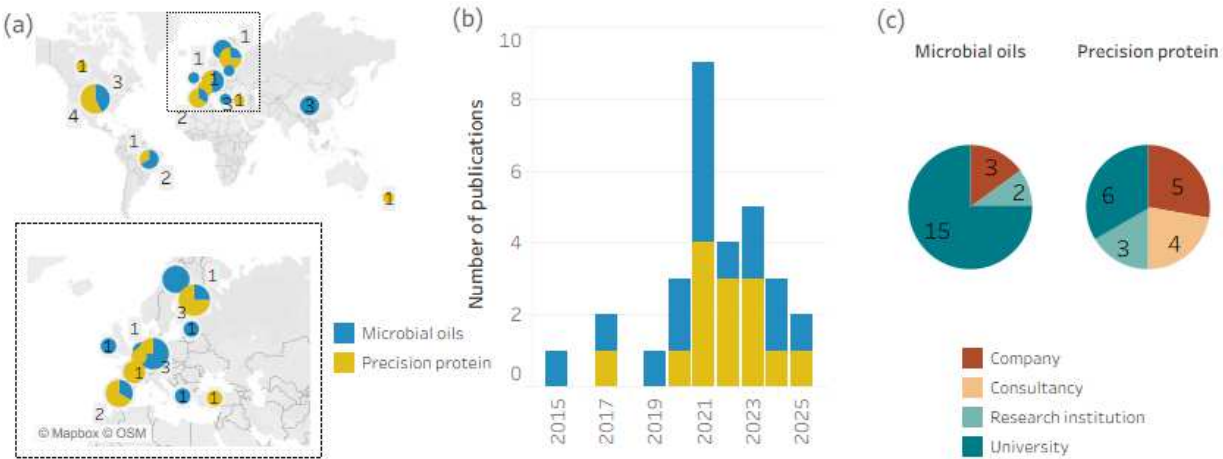


Fig 2 (a) Geographic distribution of LCAs studies for microbial oils (blue) and precision fermentation (yellow), (b) Number of reports published per year or microbial oils (blue) and precision fermentation (yellow), (c) Types of institutions publications were published under. Source data available in Supplementary Table S2.1

3.2 Ingredient bioprocess systems

3.2.1 Microbial oils: Bioprocess

Microbial oil publications modeled two distinct triacylglycerol (TAG) oil ingredient classes (Table 1). Polyunsaturated fatty acid (PUFA)-rich oils targeting omega-3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (n = 7 publications) are intended primarily for aquafeed or human nutrition as replacements for wild or farmed fish oils. Broader monounsaturated/saturated fatty acid (MUFA/SFA)-rich bulk oils (n = 7 publications) target replacement of conventional vegetable oils (T. These classes differ in target product, comparator system, and predominant microorganism, and are distinguished where relevant in the methodology and impact discussions that follow.

Organisms across both lipid classes were exclusively oleaginous microalgae and yeasts, with heterotrophic aerobic submerged or photoautotrophic cultivation as the primary distinguishing modes (Table 1). All PUFA-rich omega-3 oil studies used microalgal strains. *Schizochytrium* sp. grown heterotrophically dominated, serving as the model organism in five publications, consistent with commercial practice (Table 1, Figure 3). *Cryptocodinium cohnii* and *Nitzschia laevis* represented the remaining heterotrophic strains; Dutra et al. (2024) modeled both to assess a blended DHA/EPA oil. Photoautotrophic cultivation was used in two PUFA-rich studies: *Nannochloropsis oculata* (Deprá et al. 2020) and *Phaeodactylum tricornutum* (Togarcheti and Padamati 2021). For bulk MUFA-rich lipids, aerobic submerged fermentation was dominant, using oleaginous yeasts including *Cutaneotrichosporon oleaginosus*, *Rhodospiridium toruloides*, *Rhodotorula babjevae*, and *Yarrowia lipolytica*, as well as *Chlorella* sp.

158 and *Chlorella vulgaris* grown heterotrophically or photoautotrophically (Table 1; Figure 3).

Table 1 Landscape of eligible LCA publications for fermentation-derived food and processing aid ingredients by product subcategory, production organism, and modeled carbon feedstock

		◀ Gen1 feedstocks				Gen2 feedstocks ▶		
Product type / fermentation system	n	Glucose	Sucrose	Glycerol	Other G1†	CO ₂ / light	Cellulose	Waste str.‡
Microbial oils: heterotrophic and photoautotrophic submerged fermentation								
PUFA omega-3 rich oil (DHA, EPA) <i>Heterotrophic: Schizochytrium sp., C. cohnii, N. laevis</i> <i>Photoautotrophic: N. oculata, P. tricornutum</i>	7	4	2	-	-	2	-	1 VFAs
MUFA/SFA-rich oil: yeast <i>C. oleaginosus, R. babjevae, R. toruloides, Y. lipolytica</i>	4	2	1	-	1	-	1	1 VFAs
MUFA/SFA-rich oil: microalgal <i>Chlorella sp., Chlorella vulgaris</i>	3	1	1	-	-	3*	-	-
Precision fermentation proteins: aerobic submerged fermentation and solid-state fermentation								
β-Lactoglobulin (whey) <i>T. reesei (n=2); A. oryzae† (n=2)</i>	4	3	3	-	-	-	-	-
Casein (dairy protein) <i>NR</i>	1	1	1	-	-	-	-	-
Ovalbumin (egg protein) <i>T. reesei (n=2)</i>	2	1	-	-	-	-	1	1 Whey
Myoglobin (meat protein) <i>K. phaffii†</i>	1	-	-	1	-	-	-	-
Enzymes (cellulase, xylanase, β-Gal.) <i>T. reesei (n=1); S. cerevisiae, fungi NR</i>	4	-	-	-	1	-	1	3
Growth factors (FGF, TGF-β, PDGF) <i>E. coli; S. cerevisiae</i>	1	-	-	1	-	-	-	-
n = number of publications; Feedstock numbers = count of publications using that feedstock for that product; one publication may span multiple feedstock columns. *Lu Y et al. 2021 (DHA) and Togarcheti et al. 2021 include both hetero (glucose) and photoauto (CO ₂) scenarios; Lu Y 2021 (MUFA) counted in microalgal row. **Other Gen1 = glucose/acetate scenario (Masri et al. 2019), malted barley (Çimenlik 2021). ‡Waste streams: volatile fatty acids (VFAs) from anaerobic digestion, sugarcane molasses, whey waste, coffee husk; †organism inferred from patent or GRAS notice.								

159 Carbon feedstock selection across both microbial oil and precision fermentation protein publications spans first-
 160 generation (Gen1) and second-generation (Gen2) carbon sources, as summarized in Table 1. Gen1 feedstocks,
 161 representing the current commercial baseline for industrial fermentation, are crop-derived sugars and starches,
 162 principally sucrose from sugarcane and sugar beet, or glucose from corn and wheat. Gen2 feedstocks, as applied in
 163 this review, encompass carbon sources that do not directly compete with food supply, including lignocellulosic
 164 sugars, organic waste digestate, and food processing byproducts such as cheese whey permeate, sugarcane molasses,
 165 and starchy residues (Lips 2022). Photoautotrophic cultivation using CO₂ and light as the sole carbon and energy
 166 source is also classified as Gen2 here, though this categorization is not universally applied.

167 For microbial oil studies, Gen1 glucose and sucrose were the most frequently modeled feedstocks across both oil
 168 classes (Table 1). CO₂-based photoautotrophic systems were well-represented (n = 5), while Gen2 waste-derived
 169 feedstocks, including cellulosic (n = 1) and volatile fatty-acids (VFAs) (n = 2), were modeled in a smaller subset
 170 (Table 1). This reflects current commercial practice but represents a notable gap given the potential of Gen2
 171 feedstocks to improve the environmental profile of microbial oil production at scale

Microbial oil downstream processing (DSP) followed a general cell separation, extraction, and refining sequence organized around two archetypes: (i) drying before extraction and (ii) wet extraction (Figure 3; Supplementary Table S2.2). For dry extraction, cell biomass is dried by fluid bed, spray, or lyophilization prior to extraction using hexane, supercritical CO₂, or other solvent-based methods. In the wet extraction pathway, cell disruption precedes lipid liberation without a dedicated drying step, using high-pressure homogenization or mechanical pressure lysis, enzymatic hydrolysis, or acid thermolysis, followed by hexane-based solvent extraction (n=5) or solvent-free microwave extraction (Figure 3; Supplementary Table S2.2). One study modeled whole-cell SCO biomass as the functional product, bypassing extraction entirely (Davis et al. 2021). Cell separation preceding both pathways was predominantly by centrifugation (n = 8), with flocculation preceding centrifugation in several microalgal systems (n = 5). Oil refining (degumming, bleaching, deodorizing) was included in only two studies (McKuin et al. 2022; Togarcheti and Padamati 2021) and final formulation was described in one study (McKuin et al. 2022, blending with canola oil).

Comparison with FDA GRAS-noticed commercial microbial oil products (Supplementary Table S2.3) reveals representativeness gaps. Wet enzymatic extraction using alkaline protease lysis, a dominant commercial archetype for microalgal EPA and DHA oils, appears in only two eligible LCAs (Masri et al. 2019; Lu et al. 2021), suggesting the literature may overrepresent dry extraction pathways and their associated drying energy burdens. Additionally, fungal arachidonic acid (ARA) oils, produced predominantly via dry extraction commercially, are absent from the eligible LCA literature entirely. Finally, oil refining is standard in most GRAS-noticed microbial oil products yet appears in only 2 of 13 eligible LCAs (McKuin et al. 2022; Togarcheti and Padamati 2021), representing a systematic inventory gap with implications for hotspot attribution (Section 4.1).

3.2.2 Precision fermentation proteins: Bioprocesses

The eligible precision fermentation protein publications span four product subcategories among food ingredients: dairy whey protein (β -lactoglobulin; n = 4), dairy casein (n = 1), egg white protein (ovalbumin; n = 2), and meat protein (myoglobin; n = 1), alongside five production and processing aid studies covering enzymes (n = 4) and growth factors (n = 1) (Table 1, Figure 3, Table 2). The eight food-ingredient studies, comprising 22 of the 29 total extracted scenarios, are the primary focus of the comparative bioprocess characterization below.

All food ingredient studies used aerobic submerged fermentation (SmF). Gen1 feedstocks dominated across food-ingredient studies (Table 1), with 7 of 8 publications modeling glucose and sucrose in whey and ovalbumin production and glycerol in the myoglobin study (Planet 2023). Leino et al. (2025) is the only food-ingredient precision fermentation study to assess Gen2 feedstocks, evaluating lignocellulosic side-streams and low-quality whey waste (lactose-rich) as alternative scenarios within an expanded Järviö et al. (2021) inventory (Table 1). Although Behm et al. (2022) includes glucose and sucrose feedstock scenarios, both are Gen1 sources. No direct Gen1 versus Gen2 feedstock comparison exists for PF food-ingredient production beyond the Leino et al. (2025) extension. Gen1 dominance in PF food-ingredient LCAs mirrors current industrial practice but highlights a meaningful modeling gap given the sector's stated Gen2 ambitions

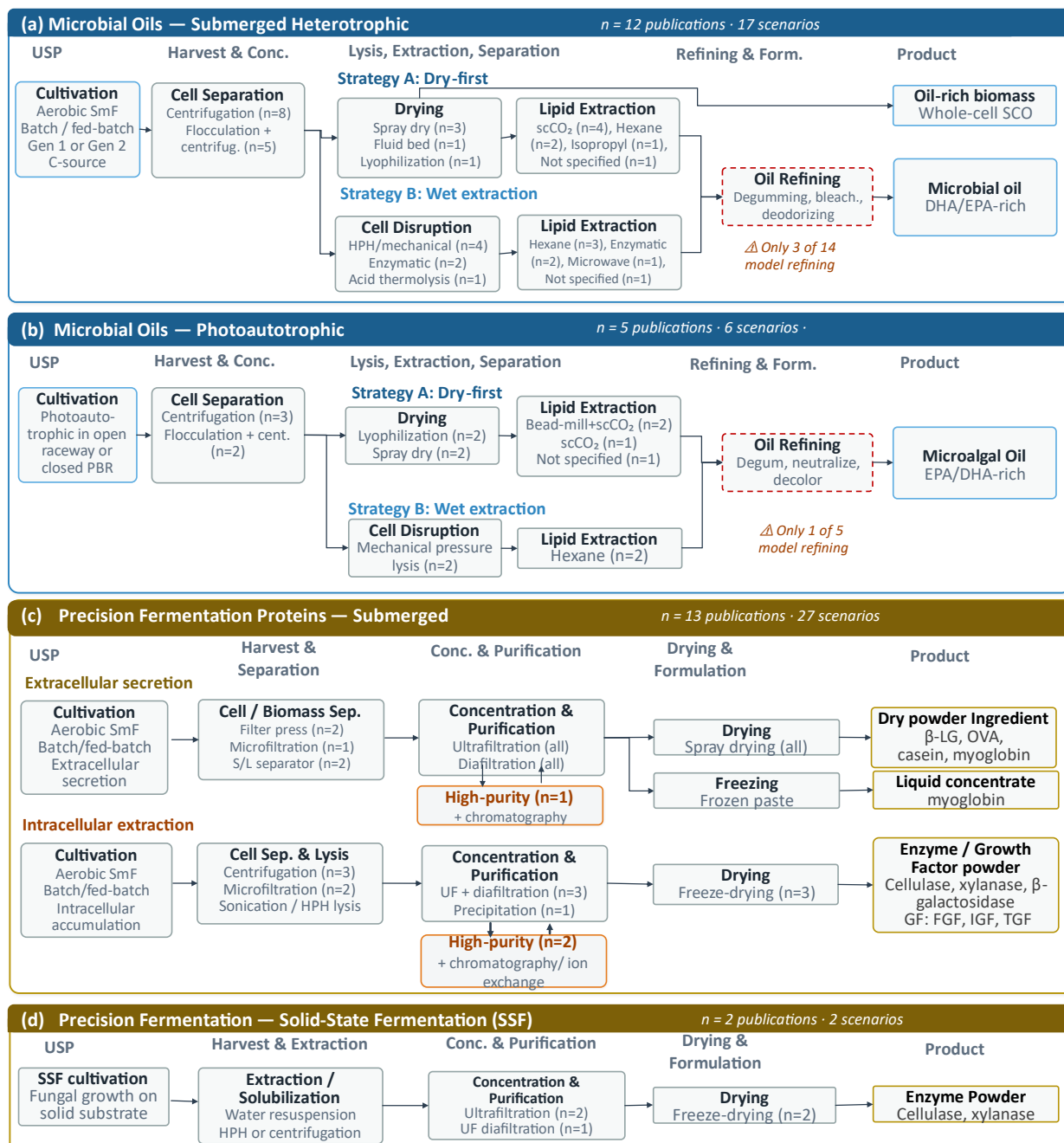


Fig 3 Modeled bioprocess systems for microbial oils (a) submerged heterotrophic and (b) photoautotrophic bioprocesses, and precision fermentation proteins (c) submerged and (d) solid-state bioprocesses with summary of strains, feedstocks, and DSP unit operations. Counts represent scenario counts. scCO₂: supercritical CO₂ extraction; HPH: high-pressure homogenization; UF/DF: ultrafiltration/diafiltration; SmF: submerged fermentation; SSF: solid-state fermentation; Gen1 / Gen2: first/second-generation feedstocks; Dashed orange border: data gap or infrequently modeled step; Dashed chromatography border: step significantly increases energy/material burden. n = number of publications or scenarios; scenario counts may exceed publications. See Section 3.3 and Supplementary Table S2.

For organisms, *Trichoderma reesei* was the only explicitly named production host across food ingredient studies, used in four publications covering whey protein (Perfect Day 2021; Behm et al. 2022) and ovalbumin (Järviö et al. 2021; Leino et al. 2025) production (Table 1). The production host was not reported in the remaining four food-ingredient publications. The Hamelin and Cellier (2022, 2024) LCAs conducted on behalf of Verley (formerly Bon Vivant) do not disclose the production host beyond fungi system; however, Verley's FDA GRAS notice (GRN 1241) identifies *Aspergillus oryzae* for β -lactoglobulin production (Cfsan 2024). The casein study (Bhandari et al. 2021) similarly does not report the production host. The myoglobin LCA commissioned by Planet A Ventures on behalf of Paleo identifies a yeast host without specifying the strain; however, glycerol as the modeled carbon source and Paleo's patent together suggest *Komagataella phaffii*, a widely used recombinant protein production platform (Sanctorum and de Jong 2022; Planet 2023; Eastham and Leman 2024).

Downstream processing for food-ingredient precision fermentation proteins followed a consistent standard purification archetype (70–90% protein purity): cell separation, concentration and purification by ultrafiltration and diafiltration, and spray drying (Figure 3; Eastham and Leman 2024). In extracellular systems, cell separation was by filter press, microfiltration, and/or solid-liquid separation (Figure 3; Supplementary Table S2.2). Concentration and purification relied predominantly on ultrafiltration and diafiltration across all food ingredient studies, consistent with commercial practice for food-grade protein ingredients. A notable exception is Behm et al. (2022), which modeled an additional chromatography step for a higher-purity scenario, substantially increasing material and energy burdens relative to the base case (Figure 3C). Spray drying was the final step in all food ingredient studies where drying was specified (Table 2; $n = 5$), consistent with FDA GRAS disclosures for commercial β -lactoglobulin and ovalbumin ingredients (Supplementary Table S2.3; Cfsan 2024). Plant A GmbH (2023) instead assessed myoglobin drying versus freezing of a liquid concentrate, in line with commercial myoglobin and leghemoglobin processes (GRN737, GRN1001; Cfsan 2024). DSP was not specified in one food ingredient study (Bhandari et al. 2021).

FDA GRAS-noticed commercial precision fermentation protein processes (Supplementary Table S2.3) broadly support the extracellular standard purification archetype as the dominant commercial pathway, consistent with the LCA literature. However, a high-purity extracellular archetype incorporating ion exchange chromatography, spanning β -lactoglobulin, ovalbumin, lactoferrin, and brazzein products, is well-represented among GRAS notices yet largely absent from the LCA evidence base, with Behm et al. (2022) as the only literature representation. Intracellular production remains a minority commercial pathway and is similarly underrepresented. Impossible Foods' leghemoglobin (GRN 1202) is the primary commercial example of an intracellular precision fermentation protein process, yet no standalone process-based LCA of leghemoglobin production exists to date beyond its appearance as an inventory component in Impossible's plant-based meat LCA (Khan et al. 2019).

Beyond technical representativeness, several commercially relevant precision fermentation protein ingredient classes remain without a robust published process-based LCA (Eastham and Leman 2024). Casein is represented by a single study (Bhandari et al. 2021), though its generic proxy-based inventory limits its utility as a process characterization.

Collagen and sweet proteins lack any published LCA entirely. Two lactoferrin LCAs identified during manuscript preparation (David et al. 2026; Zhang et al. 2026) fall outside the defined search timeline and are noted as emerging evidence but excluded from the systematic synthesis. These gaps are meaningful given the commercial activity in these ingredient categories and underscore the need for continued LCA development as the precision fermentation sector matures.

The five enzyme and growth factor studies used aerobic submerged ($n = 3$) or solid-state ($n = 2$) fermentation across bacterial, fungal, and yeast hosts, predominantly with second-generation feedstocks including coffee husk (Catalán and Sánchez 2020), cheese whey (Feijoo et al. 2017), and sugarcane molasses (de Lima et al. 2022), the latter being the only study across the eligible literature to model molasses despite its commercial relevance as an abundant sugar crop byproduct. DSP in these studies departs from commercial food-grade enzyme practice in the final drying step: LCA studies predominantly apply freeze-drying, while industrial enzyme production typically yields liquid concentrates or spray-dried forms; cell disruption and chromatographic purification, by contrast, are commercially appropriate for many enzyme classes. These process differences preclude direct impact comparison with the dairy and egg protein studies, though the publications contribute to the hotspot meta-analysis (Section 4.1) given shared upstream fermentation and feedstock characteristics. The absence of commercially relevant DSP archetypes in enzyme and growth factor LCAs may reflect process-specific complexity, limited standardized reporting, and the search strategy's focus on food ingredient rather than enzyme-specific terminology.

3.3 LCA methodology

This section reports methodological choices across three LCA phases: (i) goal and scope, (ii) life cycle inventory, and (iii) life cycle impact assessment. Table 2 summarizes the LCA characteristics and methodological choices of microbial oil and precision fermentation protein studies.

3.3.1 Goal and scope

Study motivations across both product categories centered on environmental sustainability assessment of emerging fermentation ingredients relative to conventional alternatives. Microbial oil studies were primarily motivated by global demand for sustainable omega-3 and vegetable oil alternatives, geographic and technological feasibility constraints, and effects on biodiversity. Precision fermentation protein studies were motivated by growing protein demand, displacement of animal-derived products, and environmental and economic sustainability assessment of emerging technologies (Supplementary Table S2.1).

Table 2 Characteristics of eligible LCAs for fermentation-derived food and processing aid ingredients: functional unit, system boundary, allocation, and comparator by product subcategory

Publication	Product	Functional unit	SA / UA	Allocation	Comparator	Loc.
Microbial oils — omega-3 / PUFA (n = 7)						
Bartek et al., 2021	DHA-rich oil	1 t oil (DHA)	✓ / ✓	SE	Fish oil	DE
Davis et al., 2021	DHA-rich powder DHA-rich oil	1 kg omega-3 fatty acids	✓ / —	Econ, energy	Fish oil	BR
Deprá et al., 2020	DHA-rich oil EPA-rich oil	1 kg oil (DHA) 1 kg oil (EPA)	— / —	—	No conv. comp. (intra-study)	NR
Dutra et al., 2024	DHA-rich oil EPA-rich oil	0.11 kg/pers-yr	— / —	—	Fish oil	NR
Lu Y et al., 2021‡	DHA-rich oil	1 t oil (DHA)	✓ / ✓	—	No conv. comp. (intra-study)	CN
McKuin et al., 2022	DHA-rich oil	1 kg <i>Schizochytrium</i> oil	✓ / ✓	Econ	Fish oil	BR/US
Togarcheti et al., 2021	EPA/DHA-rich oil	1 kg oil (omega-3)	— / —	—	Fish oil (farmed)	EU
Microbial oils — bulk MUFA / TAG (n = 7)						
Abelleira et al., 2024	Yeast oil	1 kg oil	— / —	Econ	Vegetable oils	ES
Bonatsos et al., 2020	Yeast oil	1 tonne oil	— / —	SE	Vegetable oils	NR
Lu Y et al., 2021‡	Microalgal oil	1 tonne oil	✓ / ✓	—	No conv. comp. (intra-study)	CN
Masri et al., 2019	Yeast oil	1 kg oil	✓ / —	—	Vegetable oils	DE
Sigtryggsson et al., 2025	Yeast oil	1 tonne oil	✓ / —	Econ	Rapeseed oil	SE
Smetana S et al., 2017	Microalgal oil	1 kg oil (FU4)	✓ / —	SE	No conv. comp. (co-product)	DE
Zhang et al., 2022	Microalgal oil	1 kg of products	— / —	—	No conv. comp. (intra-study)	CN
Precision fermentation — dairy protein (n = 5)						
Behm et al., 2022	Whey beta-lac.	1 tonne whey (dry, 90%)	✓ / ✓	SE	Dairy protein	Multi
Perfect Day 2021	Whey beta-lac.	1 kg protein (β-LG)	✓ / —	Mass	Dairy protein	US
Hamelin & Cellier 2022	Whey beta-lac	1 tonne protein	✓ / —	SE	Cow milk	FR
Hamelin & Cellier 2024	Whey beta-lac	1 tonne protein	✓ / ✓	SE	Cow milk	FR
Bhandari et al., 2021	PF casein	1 kg casein	✓ / ✓	—	Dairy protein	US/IN
Precision fermentation — egg protein (n = 2)						
Järviö et al., 2021	Ovalbumin	1 kg OVA protein (dry, 92%)	✓ / ✓	Mass, Econ	Egg white powder	FI
Leino et al., 2025	Ovalbumin	1 kg OVA protein (dry, 92%)	— / —	—	Egg white powder	FI
Precision fermentation — meat protein (n = 1)						
Plant A GmbH 2023	Myoglobin	1 kg myoglobin	✓ / —	—	Animal proteins	BE
Precision fermentation — enzymes & growth factors (n = 5)						
Catalán and Sánchez 2020	Enzyme	1 kg cellulase	✓ / —	Econ	SmF vs SSF	NR
de Lima et al., 2022	Enzyme	1 kg protein (mix)	— / —	—	Enzyme	BR
Çimenlik et al., 2021	Enzyme	1 kg xylanase	— / —	—	SmF vs SSF	TR
Feijoo et al., 2017	Enzyme	1 kg β-Gal.	✓ / —	—	Process	NR
Trinidad et al., 2023	Growth factor	1 mg GF	✓ / —	—	GF variants internal	US
All studies: cradle-to-gate system boundary. SA/UA = sensitivity / uncertainty analysis (✓ = conducted; — = not reported). SE = system expansion; Mass = mass allocation; Econ. = economic allocation; — = no allocation applied. Loc. = model location by country, NR = not reported. Intra-study = no conventional comparator; study evaluates microbial oil scenarios only (process hotspot LCA). SmF = submerged fermentation; SSF = solid-state fermentation. ‡Lu Y et al. 2021 evaluated DHA-rich (<i>Schizochytrium</i> sp.) and MUFA-rich (<i>Chlorella</i> sp.) oil scenarios independently; listed in both MO subsections, 26 unique publications total.						

Microbial oil studies predominantly used functional units of 1 kg or 1 tonne of oil product, with two exceptions: Dutra et al. (2024) used a dietary intake per-person-per-year basis, and Davis et al. (2021) evaluated 1 kg DHA-rich biomass powder. All studies were assessed on a cradle-to-gate basis (Table 2). Nine studies performed comparative assessments: five compared omega-3 oils against fish oils and four compared bulk lipids against vegetable oils; the remaining four benchmarked absolute production impacts without a conventional comparator. Precision fermentation protein studies used functional units of 1 kg protein or 1 tonne product, all cradle-to-gate. For comparators, dairy protein studies used bovine dairy products, ovalbumin studies used egg-white powder, and the myoglobin study used conventional animal proteins; enzyme studies compared submerged versus solid-state fermentation processes rather than product-based comparators (Table 2).

Allocation and co-product handling varied considerably across studies, with implications for cross-study comparability. ISO 14044 recommends avoiding allocation where possible, with system expansion preferred; studies applying either approach are therefore more closely aligned with ISO guidance than those using economic or mass-based allocation. Six microbial oil and nine precision fermentation protein studies did not include allocation (Table 2). Three microbial oil studies (Bartek et al. 2021; Bonatsos et al. 2020; Smetana et al. 2017) and three protein studies (Hamelin and Cellier 2022, 2024; Behm et al. 2022) applied system expansion. Hamelin and Cellier (2022, 2024) additionally used system expansion to establish functional equivalence between the precision fermentation protein system and its conventional cow milk comparator, a methodologically significant choice discussed in Section 4.2. Three microbial oil studies used economic allocation, and two precision protein studies used mass-based allocation (Table 2). The Perfect Day LCA illustrates allocation sensitivity directly, presenting both allocated and unallocated impact results to show the range of outcomes (Perfect Day 2021).

3.3.2 Life cycle inventory

LCA accuracy fundamentally depends on the type and quality of foreground data used in its life cycle inventory (LCI), which is a particular challenge for novel fermentation processes where technological maturity is low and industrial-scale primary data are scarce. Most studies are prospective, projecting commercial performance from lab, pilot, or literature data. Consequently, findings are sensitive to data representativeness from extrapolation accuracy, and bioprocess and yield assumptions (Tuomisto 2022).

The studies employed various data handling methods. Three precision fermentation protein studies extrapolated pilot-scale or company-run data to industrial scale (Järviö et al. 2021; Behm et al. 2022; Perfect Day 2021), and one simulated a full-scale plant from microbial kinetics data (Feijoo et al. 2017). Seven microbial oil studies similarly extrapolated from smaller scales using spreadsheet or process simulation models ($n = 7$; Supplementary Table S2.1). Scale-matched approaches appeared in five pilot or lab-scale studies ($n = 5$; Supplementary Table S2.1). Only two studies across both pathways used industrial-scale primary data (Davis et al. 2021; Lu et al. 2021), representing one of the most significant data quality limitations in the fermentation LCA landscape (Figure 4).

The distribution of foreground data sources by author affiliation further illustrates these differences (Figure 4).

Academic and research institution-affiliated studies showed the highest reliance on literature-modeled foreground data, while industry and consultancy-affiliated studies more frequently used pilot-scale primary data. Industry-affiliated LCAs also more frequently relied on confidential foreground inventories, including proprietary TEA-derived data and unspecified company data (Hamelin and Cellier 2022, 2024; Perfect Day 2021; Planet 2023), constraining independent validation and reproducibility.

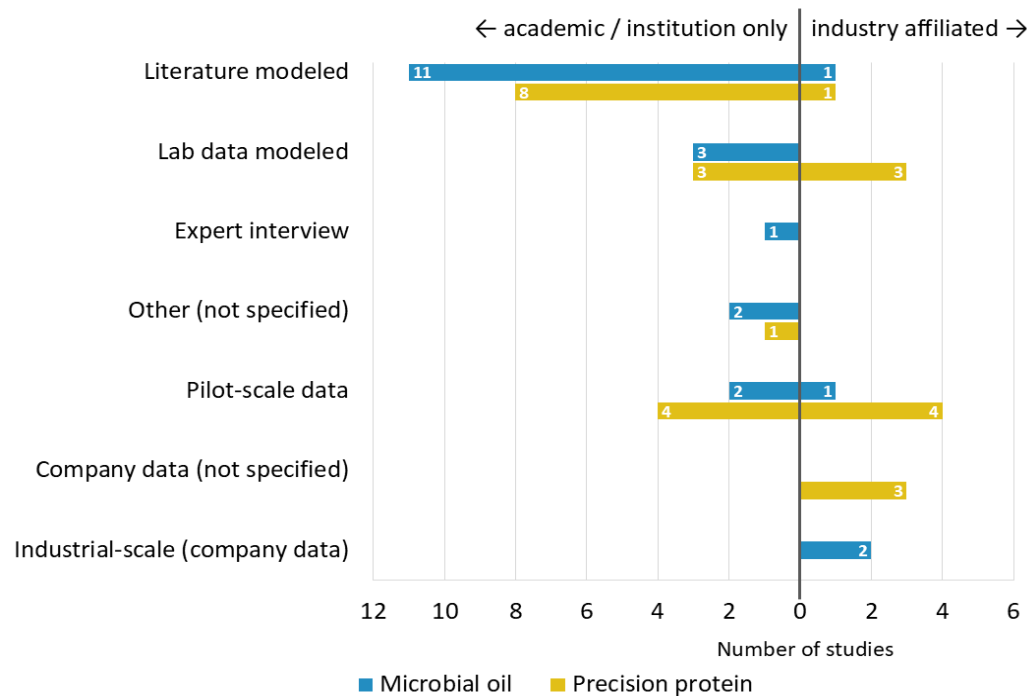


Fig 4 Foreground data sources for microbial oil (blue) and precision fermentation protein (yellow) publications broken down by author affiliation type as academic/research institution only (left axis), or industry affiliated (right axis), which is designated by any including industry or consultancy partners. Multiple data sources per publication reflected in the total counts

Background databases provide standardized inventory data for inputs that cannot be sourced directly; their geographical, technological, and temporal representativeness is a key determinant of LCA result quality. Proxy processes may substitute where standard entries are unavailable, if appropriately justified (Trinidad et al. 2023). Ecoinvent was the dominant background database, serving as the sole or primary database in 12 of the 13 protein studies and 9 of the 13 microbial oil studies. GaBi was used in three microbial oil and one protein study; several publications supplemented with regional databases including Agrifootprint, CLCD, USLCI, and WFLDB. Background database choice is itself a source of result sensitivity: Järviö et al. (2021) showed that switching glucose inventory from WFLDB to Ecoinvent produced materially different results, and Davis et al. (2021) used fish oil inventories from both Agrifootprint and Ecoinvent as separate comparators. SimaPro was the most widely used LCA software across both pathways, followed by openLCA; additional platforms included Brightway2, GaBi, CCaLC, and eFootprint-CLCD. Full software and database assignments by study are in Supplementary Table S2.1.

Data quality remains a major challenge in LCA. ISO14044 requires that data quality be defined in the goal and scope and evaluated during interpretation; however, the reviewed studies varied in both transparency and rigor. Most studies addressed uncertainty through scenario and sensitivity analysis rather than probabilistic approaches or systematic data quality assessment, and only a subset applied formal pedigree matrices or data quality indicators (Hamelin and Cellier 2022, 2024; Perfect Day 2021). Sensitivity analyses were conducted in 7 of 13 microbial oil and 10 of 13 precision fermentation protein studies, but only 3 of 26 publications conducted formal probabilistic uncertainty analyses such as Monte Carlo simulation (Järviö et al. 2021; Hamelin and Cellier 2024; McKuin et al. 2022). Sensitivity analysis serves a dual function, identifying process hotspots and dominant inventory contributions while checking the robustness of results to data uncertainty, yet its application is inconsistent across the eligible literature.

3.3.3 Impact assessment methods

Global warming potential (GWP) was the only impact category assessed in all eligible publications. Acidification and eutrophication were the next most frequently reported (~69%), followed by ozone depletion, land use, photochemical ozone formation, and water use (~46–54%). A higher frequency for ozone depletion may reflect its inclusion as a standard output in ReCiPe 2016, Environmental Footprint (EF), and CML 2001 methods. Ecotoxicity, energy demand, human toxicity, particulate matter, abiotic depletion, and ionizing radiation were less frequently reported (23–38%) (Figure 5A). While eutrophication was assessed in most studies, the reported categories (freshwater, marine, terrestrial, or combined non-disaggregated) varied by LCIA method family, reflecting a methodological shift between older single-category and disaggregated frameworks that constrain cross-study comparisons.

Coverage of water scarcity indicators was concentrated in precision fermentation protein studies (4 of 13 PF vs. 1 of 13 MO), with two distinct scarcity-weighted approaches: the Available Water Remaining (AWARE) method (Järviö et al. 2021; Behm et al. 2022) and the EF water deprivation method (Hamelin and Cellier 2022, 2024; Davis et al. 2021). The higher AWARE adoption in PF protein LCAs likely reflects the method's more recent development (Boulay et al. 2018) and more recent publication of PF studies (Figure 2; Figure 5A). While all studies reported midpoint impact categories, only Bartek et al. (2021) reported endpoint ecosystem damage results. Further, only McKuin et al. (2022) measured biotic resource use for comparison to marine fishery extraction. Impact category coverage by study is shown in Figure 5A and Supplementary Table S2.4.

ReCiPe-based methods were the most applied characterization framework across both pathways (n = 11 publications; Supplementary Table S2.1). The EF method appeared in five studies (n = 3 PF; n = 2 MO), all involving EU-based companies or EU-funded research, consistent with the EF method's status as the EU-recommended framework (EU Commission 2021). CML 2001-based methods appeared in three microbial oil studies (n = 3 MO; Table 2). TRACI 2.2 was used by Lu et al. (2021), and Zhang et al. (2022) applied a Chinese national platform (eFootprint).

The diversity of LCIA methods across studies limits direct cross-study comparison. Even within the same method family, version differences introduce incompatibility: ReCiPe 2008 and ReCiPe 2016 midpoint results cannot be directly compared due to substantive methodological differences. Comparisons across method families carry additional uncertainty from variable characterization factors, even where functional units and system boundaries are similar (Rybczewska-Błażejowska and Jezierski 2024), highlighting an opportunity for harmonization in the fermentation-derived ingredient sector.

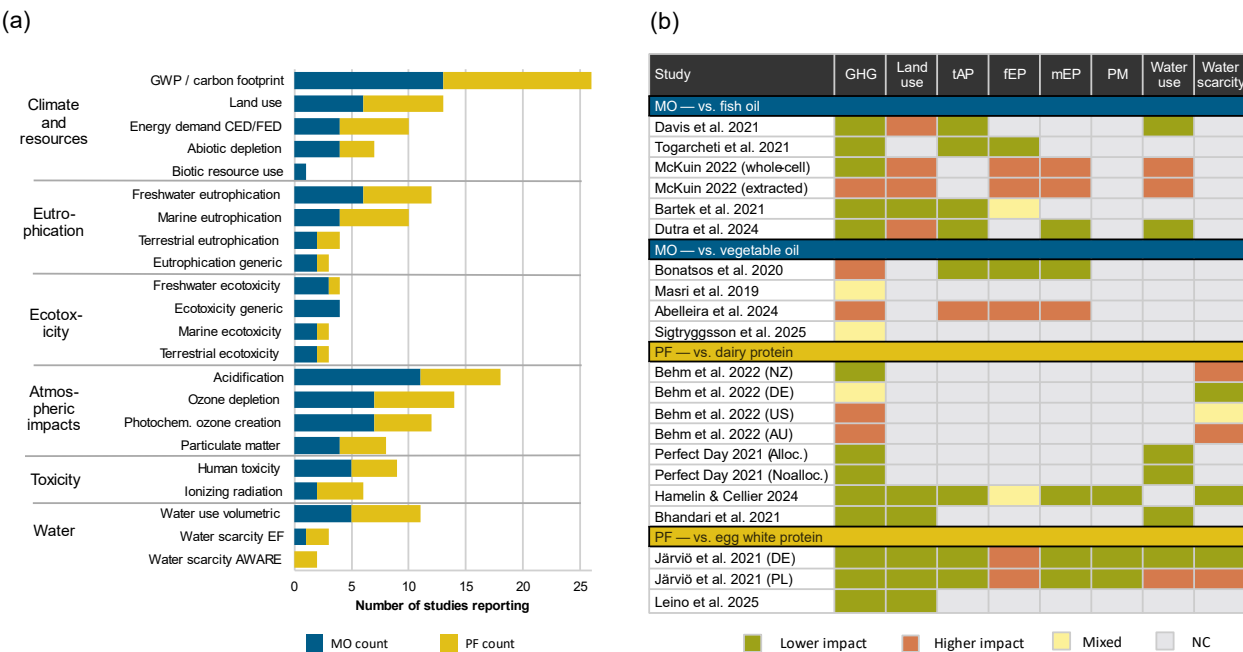


Fig 5 (a) Frequency of LCA publications reporting each midpoint impact category, by product pathway (microbial oils, blue; precision fermentation protein, yellow). (b) Directional environmental impact comparison of fermentation-derived ingredients versus in-study conventional comparators; Within-study impact comparisons only: green = lower than comparator, red = higher than comparator, beige = not significant or mixed across scenarios, gray = not compared (NC). Abbreviation: tAP = terrestrial acidification potential, fEP = freshwater eutrophication, mEP = marine eutrophication, PM = particulate matter; Studies without a conventional comparator are excluded. Impact magnitudes in Supplementary Table S2.5

3.4 Comparative impacts

Methodological heterogeneity across studies, spanning functional units, system boundaries, inventory assumptions, and LCIA methods, limits direct quantitative comparison of reported midpoint results. Accordingly, comparative impact results are reported at the study level within product class subgroups, drawing on within-study comparisons only (Supplementary Table S2.5; Figure 5B).

3.4.1 Omega-3 oils vs. fish oil

Five eligible studies directly compared microbial omega-3 DHA/EPA oil production with fish oil comparators,

covering GHG emissions, land use, water use, eutrophication, acidification, and biotic resource use across heterotrophic and photoautotrophic production systems (Supplementary Tables S2.4, S2.5).

GHG emissions were consistently lower for microbial oil across all five comparative studies (Figure 5b). Sugar-feedstock studies reported reductions of 29–76% against wild-caught fish oil comparators (Davis et al. 2021; Dutra et al. 2024), while Bartek et al. (2021), modeling food-waste-derived feedstock under system expansion, reported substantially greater reductions. Togarcheti et al. (2021), the only comparative LCA against farmed fish oil, reported approximately 99% lower GHG, eutrophication, and acidification, driven primarily by the energy intensity of fish farming and aquaculture feed. Acidification and eutrophication were reduced across all comparative studies, reflecting the absence of fishing vessel emissions and aquaculture feed-related nutrient pollution.

Land use and water use show a consistent trade-off in sugar-feedstock fed oil studies, driven by crop cultivation for the carbon source. This narrows with waste-derived or gas-based feedstocks (Bartek et al. 2021), reflecting a characteristic of terrestrial fermentation rather than a process-specific inefficiency.

A notable limitation across all five comparative studies is the near absence of biotic resource use metrics, the impact category most directly relevant to fish oil displacement. Only McKuin et al. (2022) reported biotic resource use, finding microalgal DHA approximately 96% lower than fish oil; only Bartek et al. (2021) reported endpoint ecosystem damage (32% total reduction). The methodological implications of this gap are discussed in Section 4.2.

3.4.2 Bulk lipid oils vs. vegetable oils

Four publications benchmarked microbial bulk oils against conventional vegetable oil comparators, with considerable variation in reference systems and impact category coverage. Two studies compared GHG emissions only (Masri et al. 2019; Sigtryggsson et al. 2025); while Bonatsos et al. (2020) and Abelleria et al. (2024) additionally reported eutrophication and terrestrial acidification, leaving many midpoint categories unassessed.

GHG results were higher than vegetable oil comparators in most cases, but with notable sensitivity to comparator choice. Masri et al. (2019) illustrates this directly: yeast oil GWP ranged from 53% lower than peanut oil and 21% lower than soybean oil, to 23% higher than rapeseed oil. Bonatsos et al. (2020) reported higher GWP than vegetable oil comparators under both feedstock scenarios, though acidification and eutrophication were lower than most comparators. In contrast, Abelleira et al. (2024) found higher impacts across all assessed categories, noting a 74% GWP reduction would be required to reach soybean oil parity (Supplementary Table S2.5). These variable findings reflect the limited impact category coverage, reliance on unoptimized literature- and lab-scale data (Figure 4), and early-stage bioprocess assumptions across these studies, underscoring the need for more robust comparative assessments of bulk microbial oils against vegetable oils.

A notable gap in the comparative landscape is the absence of LCAs targeting high-value specialty fats such as cocoa butter and animal tallow, despite their commercial relevance as displacement targets for oleaginous strains. Existing comparators are limited to commodity vegetable oils (palm, soybean, rapeseed, sunflower). Recent techno-economic

analysis suggests microbial TAG minimum selling prices of approximately \$10/kg from waste-derived feedstocks may be competitive with premium fats such as cocoa butter (Ivanisevic et al. 2026), underscoring the need for LCA evidence in this product space.

3.4.3 Precision fermentation dairy protein vs. conventional dairy

Four studies compared PF β -lactoglobulin production against conventional dairy protein comparators across multiple locations, carbon sources, and production scenarios (Supplementary Table S2.5). Two additional studies are noted but excluded: Bhandari et al. (2021) reported absolute values under widely varying proxy assumptions that preclude reliable comparative interpretation, and Gorman et al. (2023) conducted a product-level footprint (out of scope) rather than a process-based LCA.

The Perfect Day (2021) LCA compared US-produced PF β -lactoglobulin against bovine milk protein across a global literature range of dairy comparators. Under mass allocation, GHG reductions of 91–97% and blue water reductions of 96–99% were reported. A sensitivity analysis allocating 100% of burdens to the whey protein still yielded GHG reductions of 62–85% and water reductions of 84–94%, confirming robustness across allocation assumptions. Land use and eutrophication were not reported.

Behm et al. (2022) modeled PF β -lactoglobulin across four production locations (NZ, US, DE, AU) and two carbon sources (glucose, sucrose), comparing against New Zealand (NZ) dairy protein, which is among the lower-impact conventional dairy benchmarks globally. In the NZ production scenario, GHG reductions ranged from –4% to –45% relative to the NZ average dairy benchmark, averaging –17% for glucose-fed and –45% for sucrose-fed scenarios. Water scarcity ranged from –0.6% to +111% in the NZ glucose scenario but ranged from –82% to +939% across all scenarios and geographies. Their results highlight that performance is highly sensitive to grid carbon intensity, feedstock type and origin, local watershed stress, and co-product treatment. Land use was not assessed (Figure 5B; Supplementary Table S2.5).

Hamelin and Cellier (2024), on behalf of Verley, assessed French-produced PF β -lactoglobulin against a global (GLO) cow milk protein system using system expansion for co-product equivalence (Section 4.2). The baseline scenario showed GHG reductions of 69%, land use reductions of 99%, water scarcity reductions of 81%, and acidification reductions of 71%. A sensitivity scenario improved GHG reductions to –84% and water scarcity to –96%. Freshwater eutrophication showed the highest variability, ranging from +63% in the baseline to –103% in the sensitivity scenario, driven by marginal supplier assumptions for compensatory canola oil co-products required to establish functional equivalence rather than the fermentation process itself.

3.4.4 Precision fermentation egg white protein vs. conventional egg white

Two studies compared PF ovalbumin against conventional egg white powder using the same *T. reesei* production system. Järviö et al. (2021) compared recombinant ovalbumin (Tr-OVA) against egg white in Germany and Poland. The Germany scenario showed GHG –55%, land use –87%, blue water –69%, water scarcity –86%, and terrestrial

acidification –77% relative to egg white powder. The Poland scenario showed more modest GHG reductions (–31%) and consistent land use reductions (–89%), but blue water (+53%) and water scarcity (+26%) reversed direction. This reversal is driven by egg comparator feed sourcing assumptions and AWARE characterization factors rather than Tr-OVA's own footprint, a finding with direct implications for location-sensitive LCA interpretation (Section 4.1). Freshwater eutrophication was higher in both scenarios (+124% Germany; +153% Poland), reflecting a partial burden shift from agricultural to industrial inputs (Supplementary Table S2.5).

Leino et al. (2025) extended the Järviö et al. (2021) inventory to second-generation feedstocks across Finnish energy scenarios compared against Finnish egg production. GHG reductions of –57 to –74% (including LULUC) and –35% to –58% (excluding LULUC) were reported, with land use reductions of –88% to –94%. The best results combined low-carbon electricity with lignocellulosic side-stream feedstocks, confirming feedstock decarbonization as the dominant improvement lever for the ovalbumin pathway.

Across both dairy and egg white protein studies, impact categories beyond GHG, land use, and water use were inconsistently reported, limiting synthesis for acidification, eutrophication, and toxicity. Where reported, acidification and marine eutrophication generally showed reductions consistent with the elimination of livestock inputs; ionizing radiation and human toxicity were elevated in some PF scenarios, reflecting a shift from agricultural to industrial burden sources. The myoglobin study (Plant A GmbH 2023) provides absolute impact values but no modeled conventional comparator, precluding percentage reporting. Enzyme and growth factor studies are similarly excluded given their distinct functional units and absence of product-based comparators.

3.5 Reported process impact drivers

To complement the within-study comparative results above, a cross-study synthesis of environmental impact drivers was conducted by extracting the leading process contributors from each eligible scenario and aggregating them into harmonized hotspot categories (Supplementary Table S2.6), providing a production-focused perspective on recurring impact drivers regardless of comparator system or functional unit.

Across both production platforms, impact drivers concentrate across three recurring areas: carbon feedstock supply, process energy demand, chemicals and consumables (Figure 6A). Location (grid/energy mix, climate) and bioprocess performance (yield, productivity, titer) remain influential but are often embedded in background assumptions or tested only through sensitivity analyses and may therefore be underrepresented in hotspot rankings.

At the scenario level, carbon feedstock production and energy demand dominate. Carbon feedstock production appears as a top three hotspot in most modeled scenarios, while energy demand, disaggregated across upstream electricity, downstream processing energy, and total process energy, similarly emerges as a leading contributor (Figure 6B). In contrast, nutrients, cleaning, sterilization, and solvents appear far less frequently as top-ranked hotspots, despite clear evidence from individual studies that they can contribute materially when explicitly modeled. This disparity reflects differences in inventory resolution and reporting conventions rather than intrinsic process

importance. Less frequently reported drivers should not be interpreted as low importance; their visibility in hotspot rankings depends on scenarios, sensitivity analysis, and whether inventories disaggregate these flows or absorb them into broader inventory categories (Figure 6B).

Hotspot sub-category distributions differ by production platform. In microbial oil LCAs, upstream electricity demand is especially prominent, reflecting cultivation energy requirements and, in some systems, the added burden of extraction and drying. In precision fermentation protein LCAs, hotspots more often aggregate to overall energy demand and background electricity supply, reflecting the more common use of aggregated inventories spanning fermentation, downstream recovery, and cleaning operations (Figure 6B). These differences show that hotspot attribution is shaped not only by process design but also by inventory resolution and system configuration.

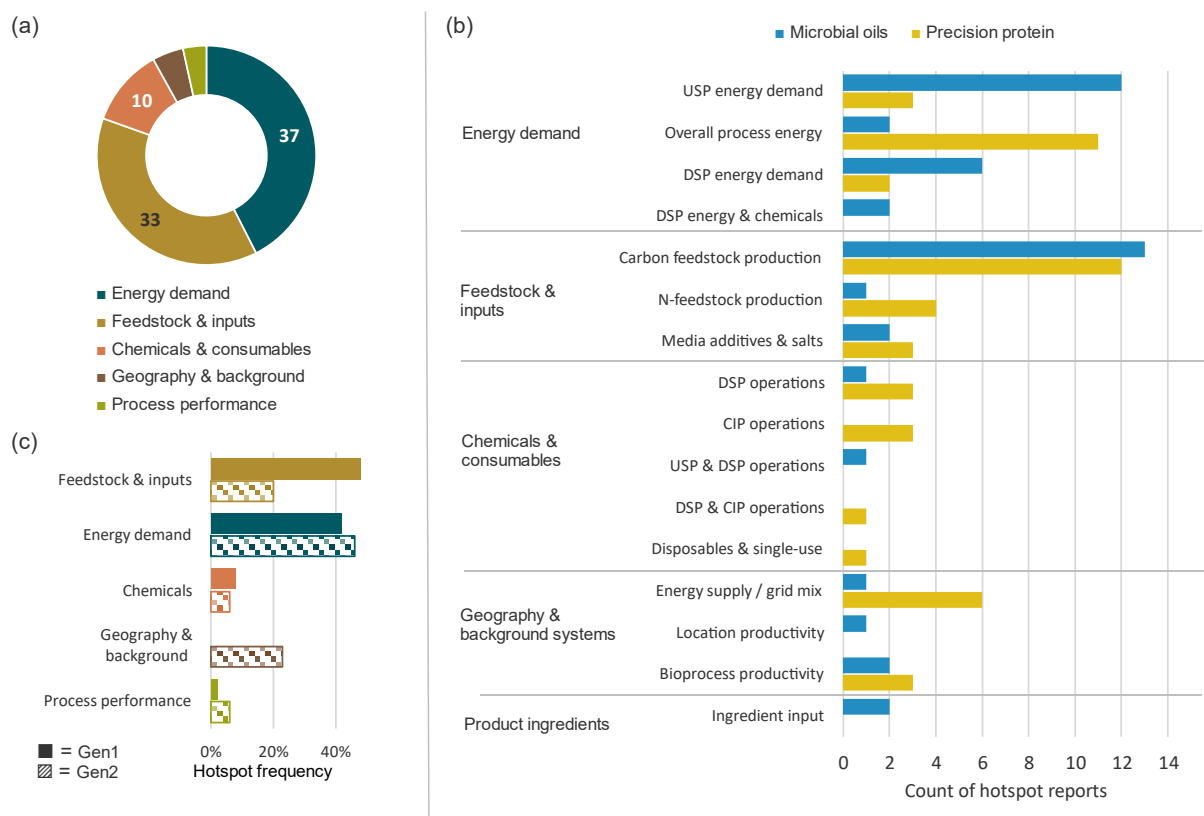


Fig 6 Frequency and categorical structure of top-ranked LCA process hotspot drivers across microbial oil and precision fermentation protein publications. (a) Distribution of top 3 categorical hotspot rankings deduplicated at publication level (n = 87). (b) Sub-categorical hotspot driver frequency by product type, reporting top 4 rankings deduplicated within publications at scenario level (n = 98) (c) Percentage distribution of top 3 categorical hotspot drivers by modeled feedstock generation (Gen1, solid fill; Gen2, diagonal hatching). Hotspot frequencies reflect the most reported scenario-level contributors; drivers such as grid and heat mix, climate, and bioprocess productivity are often modeled as background assumptions or explored only in sensitivity analyses and may therefore be underrepresented

4. Discussion

4.1 Bioprocess and methodology intersection

The following sections examine how bioprocess design choices and modeling assumptions shape reported hotspot outcomes, with emphasis on parameters most likely to influence environmental performance in commercial systems. Hotspot rankings reflect what is modeled within each study rather than intrinsic process importance; location and bioprocess productivity are often embedded in background assumptions rather than foregrounded as scenario parameters and are therefore discussed separately below.

4.1.1 Feedstock selection

Across both microbial oils and precision fermentation proteins, carbon feedstock production is the most consistently reported hotspot driver, which is expected given the scale of carbon input required for microbial production and commodity sugar production inputs.

Where studies assess alternative Gen2 carbon feedstocks, a consistent pattern emerges. Shifting away from Gen1 feedstocks tends to reduce the relative contribution of crop-based feedstock production and associated agricultural inputs, while increasing the prominence of process energy demand and upstream preprocessing burdens. The latter are interrelated: Gen2 feedstocks can require additional processing steps or process energy intensity increasing overall energy demand that offset feedstock-related gains. Scenarios utilizing Gen1 feedstocks most frequently report carbon feedstock production as the top hotspot, whereas Gen2 feedstock scenarios more frequently elevate process energy demand as the leading driver (Figure 6C). Smetana et al. (2017) and Togarcheti and Padamati (2021) illustrate this for photoautotrophic CO₂-fed cultivation, which can be more energy intensive than conventional sugar-fed systems. In some cases, this burden-shifting can be an effective mitigation strategy, but it does not remove the need to address energy-intensive upstream and preprocessing steps that may become dominant as feedstock impacts decline.

However, this interpretation is constrained by two structural limitations. First, few studies model both Gen1 and Gen2 feedstocks in parallel under harmonized assumptions, limiting direct opportunity cost comparisons. Second, even where Gen2 feedstocks are modeled, inventory resolution is often insufficient for process-level decomposition. Leino et al. (2025), the most detailed Gen2 scenario in the precision fermentation literature, does not provide granular process-level impact breakdowns, reducing interpretation to feedstock as a categorical driver. As a result, Figure 6C reflects commonly reported hotspot rankings rather than a controlled comparison of feedstock pathways.

This gap extends beyond LCA literature. Techno-economic analyses similarly report limited direct Gen1 versus Gen2 feedstock comparisons, while pointing to a potential burden shift from feedstock costs to preprocessing and capital requirements when transitioning to Gen2 systems (GFI 2025). Nadar et al. (2025) partially address this gap by developing a detailed ex-ante fermentation process model comparing sucrose with multiple Gen2 feedstocks, providing a foundational dataset for future TEA and LCA studies.

Even within Gen1 systems, results are highly sensitive to inventory assumptions and geographic context. Studies vary substantially in how they represent the feedstock supply chain: Järviö et al. (2021) show that substituting glucose background inventories significantly alters land use and terrestrial acidification; Behm et al. (2022) illustrate how aggregated global datasets obscure regional variability; Davis et al. (2021) extend scope to farm-gate level for supplier-specific sugarcane; Bonatsos et al. (2020) and McKuin et al. (2022) similarly demonstrate sensitivity through agricultural scope inclusion and sugarbeet co-product allocation respectively. Together these studies show that feedstock impacts are not intrinsic to sugar itself but depend critically on how agricultural production and refining are represented in the inventories used.

As fermentation scales and feedstock sourcing diversify, including supplier-specific refinements and emerging low-emission sugar production pathways (Jakel et al. 2017), the development of geographically and technologically representative inventories will become increasingly important. Accurate, context-specific feedstock inventories are essential for fermentation LCAs, particularly when informing site selection, procurement strategy, process design, or environmental product declarations.

4.1.2 Energy demand and energy supply

Energy demand is one of the most consistently reported hotspot drivers across both pathways, reflecting the energy-intensive nature of aerobic cultivation, product recovery, and downstream processing. In microbial oil LCAs, upstream electricity demand is frequently a dominant hotspot due to cultivation aeration and mixing; PF protein LCAs more often report aggregated process energy demand spanning fermentation and downstream recovery. Both the magnitude and ranking of energy-related hotspots are highly sensitive to electrical grid and thermal energy source assumptions.

This sensitivity is evident in Gen2 feedstock scenarios (Figure 6), where increased energy demand often coincides with greater prominence of background energy systems as hotspot drivers. Järviö et al. (2021) model explicit geographic scenarios allowing grid mix to function as a primary driver; Behm et al. (2022) pair process scenarios with location-based sensitivities, demonstrating that electricity supply can materially shift results. Industry-informed studies add nuance: the Perfect Day (2021) LCA identifies grid mix as a key contributor under fossil-intensive conditions; Hamelin and Cellier (2022, 2024) flag electricity and steam as important contributors; and Smetana et al. (2017) show that climate context affects process energy demand directly through open-pond heating requirements.

Taken together, energy demand and energy supply should be interpreted as coupled drivers requiring parallel mitigation strategies. On the process side, aeration and mixing efficiency, heat recovery and integration, and DSP optimization represent the highest-leverage engineering targets for reducing inherent energy intensity. On the supply side, renewable energy procurement and site selection for grid carbon intensity represent procurement and investment decisions with comparable or greater influence on LCA outcomes. Current LCAs frequently treat both as background assumptions rather than explicit scenario parameters, understating their practical relevance as improvement levers for the sector.

4.1.3 Bioprocess performance

Energy demand and resource efficiency are inherently linked to bioprocess performance. Key fermentation process metrics—yield, productivity, and titer (YPT)—govern process efficiency by influencing raw material efficiency, energy consumption, DSP operations, and water demand. Yet YPT is rarely foregrounded as a hotspot driver, as it is typically embedded in inventory assumptions or explored only through sensitivity analyses (e.g., Lu et al. 2021; Togarcheti and Padamati 2021; Behm et al. 2022). Proprietary studies often obscure YPT for confidentiality reasons (Perfect Day 2021; Hamelin and Cellier 2022, 2024; Planet 2023).

YPT reporting is sparse and inconsistent across both pathways. Precision fermentation protein LCAs report yield and titer in only 38% and 23% of cases respectively, while microbial oil publications report these metrics more frequently (76%) but with inconsistent units and definitions. Among PF protein LCAs, only Behm et al. (2022) report a product titer (50 g/L), while Järviö et al. (2021) provide annualized production volumes and bioreactor specifications from which batch productivity can be approximated but not directly extracted. Among bulk lipid LCAs, Bonatsos et al. (2020) reported 72 g/L biomass and Masri et al. (2019) reported 240 g/L biomass at 87% lipid content; PUFA-oil studies report biomass titer more frequently but with inconsistent units and definitions. Whether other modeled inventories reflect commercially representative process performance remains unclear from available data.

The infrequent naming of productivity as a hotspot reflects a reporting limitation rather than low importance. Improving YPT remains one of the highest-leverage strategies for reducing per-unit energy demand and resource intensity, particularly as grid decarbonization and next-generation feedstocks reduce upstream burdens. Future LCAs should harmonize reporting of key performance metrics, as a similar gap exists in the TEA landscape (GFI 2025). At minimum, annualized volumetric productivity should be reported to enable cross-study comparison and link environmental outcomes to production maturity.

4.1.4 Cleaning and sterilization

Cleaning-in-place (CIP) involves sequential alkaline and acid wash cycles to remove biological and chemical residues from bioreactor vessels and piping in situ, generating chemical and wastewater treatment burdens. Sterilization-in-place (SIP) uses pressurized steam to sterilize vessels and culture media, contributing thermal energy demand. Together, CIP and SIP can materially affect LCI through water use, energy demand, chemical consumption, and wastewater treatment, yet their inclusion in fermentation LCA inventories is inconsistent. Precision fermentation studies more often model CIP than SIP, whereas microbial oil studies more frequently include SIP than CIP, and only a few model both (Supplementary Table S2). Industry-affiliated studies are more likely to explicitly include both, suggesting that access to commercial-scale data improves cleaning-related inventory resolution.

Where disaggregated, CIP contributions can be substantial: Järviö et al. (2021) identify cleaning as contributing

approximately 20–40% across several impact categories in their ovalbumin LCA, and Feijoo et al. (2017) report significant contributions from CIP-related chemicals. Hamelin and Cellier (2022) identify CIP as meaningful through sensitivity analysis, leading to more explicit representation in their 2024 study. In contrast, Behm et al. (2022) incorporate CIP across material, electricity, and process water inventories, preventing it from emerging as a distinct hotspot.

The absence of CIP or SIP as a reported hot spot should not be interpreted as a negligible impact, but as a function of inventory granularity and reporting practice. Improving transparency in cleaning-related inventories would enhance comparability and clarify whether these operations represent meaningful levers for environmental improvement alongside feedstock and energy.

4.1.5 Downstream processing and drying

DSP contributes to environmental hotspots through energy demand and material inputs associated with separation, purification, and drying, with magnitude depending strongly on modeled unit operations and product format. DSP-related impacts are often obscured by limited inventory resolution or inflated by non-commercially representative unit operations.

For proteins, purification steps can introduce material and chemical hotspots. Behm et al. (2022) show that chromatographic purification for higher-purity scenarios increases both carbon and water footprints through resin production, chemical inputs, and additional filtration. Feijoo et al. (2017) similarly identify downstream chemicals (NaOH, Tris-HCl) as major contributors when explicitly modeled. However, chromatography-intensive purification is not representative of most commercial PF food-protein production, where ultrafiltration and diafiltration are sufficient (Eastham and Leman 2024; Supplementary Tables S2.2 and S2.3).

Spray drying is consistently the final formulation step across food ingredient LCAs and is commercially representative. Where disaggregated, Järviö et al. (2021) find it contributes relatively little compared to cultivation and separation energy. Lyophilization, by contrast, is a major energy driver in enzyme and growth factor studies (Catalán and Sánchez 2020; Feijoo et al. 2017; Trinidad et al. 2023) but is not representative of large-volume food protein production (Haas et al. 2024), reflecting pilot-scale rather than commercial assumptions. Liquid or frozen formats reduce drying energy demand, as shown by Plant A GmbH (2023), consistent with commercial myoglobin and leghemoglobin practice (GRN 737; GRN 1001).

For microbial oils, the extraction pathway dominates DSP impacts. Solvent-based extraction creates combined thermal and chemical burdens from biomass drying and solvent distillation. Wet enzymatic extraction can bypass the drying step but remains underrepresented relative to commercial practice (Section 3.2.1). Oil refining is similarly absent from most LCA inventories despite being standard commercially, masking secondary hotspots in later processing stages. DSP impacts must therefore be interpreted against commercially realistic processing pathways rather than modeled assumptions alone.

4.1.6 Waste stream valorization

Microbial protein and oil bioprocesses generate residual biomass streams that contain a substantial share of carbon and resource inputs. Several studies explore valorization pathways including fertilizer, biogas, nutrient recovery, animal feed, and water recycling, providing meaningful credits through system expansion or reducing allocation burdens. Whether allocated, credited through substitution, or excluded, these choices can substantially influence life cycle results (Section 3.4).

Microbial oil LCAs show particular diversity in valorization approaches, including biomass combustion or anaerobic digestion for on-site energy (Lu et al. 2021; Bartek et al. 2021), hydrolysate recycling for upstream enzyme production (Masri et al. 2019), wastewater return to sugarcane fields as fertilizer (Davis et al. 2021), and water recycling to reduce scarcity impacts (McKuin et al. 2022). Bonatsos et al. (2020) exclude residual biomass due to limited data on feed displacement potential. These varied approaches provide different material and energy credits, contributing to cross-study variability that cannot be attributed to process design differences alone.

For PF protein studies, some studies allocate directly between product and biomass co-products: the Perfect Day (2021) LCA assigned ~78% of burdens to residual biomass and ~22% to the target protein, illustrating how allocation choice can materially shift apparent product burden. Other studies apply system expansion to model fermentation biomass as animal feed, showing substantial reductions in carbon footprint and water scarcity when avoided feed production is credited (Behm et al. 2022; Hamelin and Cellier 2022; 2024). In contrast, Järviö et al. (2021) treat fungal biomass as biowaste while excluding ultrafiltration wastewater treatment from the system boundary, affecting inventory completeness. Since these impacts are mediated through allocation choices or system expansion credits, they may not consistently emerge in the qualitative hotspot meta-analysis despite materially influencing overall results.

4.1.7 Location and background systems

Geographic context and background system assumptions can significantly influence hotspot outcomes through the representativeness of electricity, feedstock, and chemical inventories, as well as co-location factors such as access to low-carbon energy and industrial infrastructure.

The Järviö et al. (2021) geographic scenarios demonstrate this directly: holding PF egg white production constant, water scarcity reversed direction between Germany and Poland, driven primarily by differences in egg white comparator feed sourcing and regional water stress rather than the production system (Section 3.4.4). Behm et al. (2022) illustrate this through explicit geographic scenarios across four production locations, demonstrating that grid mix, feedstock origin, and regional water stress each materially shift results. Comparator selection compounds this further: benchmarking against New Zealand dairy protein, one of the lowest-impact conventional dairy systems globally, substantially narrows the apparent GHG advantage relative to what the same process might show against global or European dairy benchmarks (Section 3.4.3).

In some systems, location also affects process performance directly through climate-dependent heating and cooling requirements or cultivation conditions (Humbird, Davis, and McMillan 2017; Smetana et al. 2017). Commercial-scale fermentation systems show further variability in energy and water use across facilities due to site-specific utility systems, linking local climate and infrastructure to process environmental outcomes. Across geographic scenario definition, comparator selection, and site-specific process conditions, the reported performance range reflects scenario design as much as process reality.

4.2 Methodological gaps and recommendations

While the existing body of fermentation LCAs is substantial, rigorous, and influential, specific refinements are necessary to enable more reliable comparisons across studies. Three primary areas for enhancement have been identified: (1) comparability, currently limited by varied methodological approaches and gaps; (2) credibility, affected by lack of primary data, reliance on proxy datasets, and lack of robust uncertainty modeling; and (3) decision relevance, constrained by operational assumptions and insufficient integration between TEA and LCA.

Harmonization of impact assessment methods and reporting categories would substantially improve the comparability of fermentation LCA results. A global team of LCA experts (Blackstone et al. 2025) recently published such harmonization guidelines for cultivated meat LCAs. Similar guidance for fermentation-derived ingredient LCAs would be a valuable contribution to the field. Variation in functional units, system boundaries, and allocation approaches remains the most pervasive source of cross-study incomparability. While ISO 14040/44 permits this flexibility, structurally different modeling choices produce divergent outcomes that are difficult to attribute to process differences alone (Fox-Chapman and Bess 2025; Tuomisto 2022). The Verley LCAs (Hamelin and Cellier 2022; 2024) illustrate this most sharply, applying system expansion under a functional equivalence framework that matches the full nutritional co-product profile of conventional cow milk by including compensatory ingredients. This is robust and defensible for comparing precision fermentation protein against a nutritionally equivalent dairy system but limits direct comparability with other precision fermentation protein LCAs. Studies using a dried protein ingredient mass as the functional unit are equally defensible: high-purity, shelf-stable ingredients are the actual commercial form of these products, and their intended applications as functional food ingredients do not require replication of the full dairy or egg nutritional matrix. The same logic applies to microbial oil LCAs, where a per-kg DHA-rich oil functional unit correctly reflects the commercial form used in nutritional supplementation, food formulation, and aquafeed. Greater convergence around functional unit definitions and multifunctionality handling, without requiring full standardization, would substantially strengthen cross-study interpretation.

Impact category coverage and divergent LCIA methods both warrant attention as sources of cross-study incomparability. At minimum, fermentation-derived ingredient LCAs should routinely include GWP, water use, land use, eutrophication, and acidification (Chapman and Murray 2023), yet only a few impact areas are evaluated across studies (Figure 5A). For microbial oil LCAs compared against fish oil, a structural gap exists for biodiversity and marine impacts. Only McKuin et al. (2022) measured biotic resource use and only Bartek et al. (2021) reported

endpoint ecosystem damage, yet overexploitation of living resources remains largely absent from all operational LCIA methods (Damiani et al. 2023), and exploitation-focused methods that do exist rely on stock-specific characterization factors requiring regular recalculation (Bergman et al. 2025). This creates an asymmetric comparison: impact categories where microbial oil production often incurs costs, such as land use, are routinely quantified, while the fisheries overexploitation liability of fish oil falls largely outside standard LCIA frameworks, limiting the ability of comparative LCAs to fully capture the biodiversity benefit of microbial alternative fish oils. The environmental case for microbial omega-3 oils is therefore likely stronger than headline comparative figures suggest.

Data quality and process representation are equally limiting. Most studies rely on literature-derived or proxy inventories and inconsistently report key bioprocess performance parameters, such as yield, titer, productivity, and DSP recovery, that are central to both environmental and economic outcomes. Sensitivity analyses are common, but formal probabilistic uncertainty analysis and data quality assessments of technological representativeness, geographic and temporal fit, and data completeness remain infrequent despite being required under ISO 14044. More broadly, results remain sensitive to process design, scale, location, and background energy assumptions, reinforcing the need for bioprocess-grounded scenarios and location-specific inventories.

Process representation is a related concern. Some modeled unit operations, such as lyophilization or chromatography-intensive purification, may not be reflective of most commercial PF protein processes, potentially misattributing hotspots and limiting the relevance of prospective results to actual commercialization pathways. A parallel issue exists for biomass and wastewater valorization. Several studies model system expansion credits or allocation for animal feed, fertilizer, anaerobic digestion, or water recycling; yet these pathways are often not implemented in early commercial or contract manufacturing operations where the primary focus is proving the ingredient. Further, genetically modified spent microbial biomass may face regulatory, cost, and perception challenges (Garnica-Garcia, Lal, and Marcellin 2026). Modeled valorization credits may therefore overstate the environmental performance achievable at current commercialization stages. This points to both a development need, as valorization infrastructure represents a meaningful improvement pathway for commercial facilities and a modeling recommendation where valorization approaches are better represented as sensitivity scenarios exploring improvement potential rather than baseline assumptions. Closer public-private collaboration and access to representative, scale-appropriate process data, including through aggregated or non-confidential data sharing, remains essential to improving data transparency and accuracy across the field.

Finally, integration between TEA and LCA remains limited. To ensure precision fermentation delivers on its sustainability potential, economic and environmental viability must be pursued iteratively from the outset (David, Juliano, and Ridoutt 2025). Most studies evaluate environmental and economic performance independently despite relying on shared process assumptions, such as yield, titer, scale, and feedstock sourcing, that jointly determine both cost and environmental outcomes. Only a few studies identified here demonstrate combined approaches, such as Abelleira et al. (2024) with life-cycle costing, and Masri et al. (2019) and Bonatsos et al. (2020) with TEAs. The

recently published ISO/TS 14076:2025 on environmental techno-economic assessment (eTEA) provides a standardized framework for integrating environmental and economic dimensions simultaneously. Wider adoption of eTEA, alongside the harmonization alignment recommended here, would improve both the consistency and decision-relevance of fermentation LCAs as the field moves toward commercial scale and a more sustainable food system.

5. Conclusions

This systematic review addresses three research questions: (i) what is the landscape of life cycle assessments for precision fermentation proteins and microbial oils, including the bioprocess systems modeled and how they compare to commercial practice; (ii) what types and sources of data underlie these LCAs, and what data gaps, quality issues, or methodological inconsistencies limit their comparability; and (iii) what are the environmental impacts of fermentation-derived proteins and oils, and what are the most significant drivers of those impacts.

This systematic review identified robust landscape LCAs evaluating precision fermentation proteins ($n = 13$) and microbial oils ($n = 13$), extracting 52 scenarios for environmental impact and hotspot analysis. Systematic characterization of the modeled bioprocess systems and benchmarking against FDA GRAS commercial practice demonstrated robust modeling yet revealed meaningful representativeness gaps. For example, wet enzymatic extraction and oil refining are standard commercial practice for microbial oils yet largely absent from LCA inventories, and high-purity chromatographic purification archetypes are underrepresented for PF proteins. Methodological heterogeneity across studies, including inconsistent functional units, allocation approaches, and LCIA methods, limits direct cross-study comparison. Foreground data quality, sparse YPT reporting, and infrequent probabilistic uncertainty analysis are the primary limitations constraining LCA credibility for these emerging systems.

Overall, precision fermentation-derived proteins and oils may offer environmental benefits over many conventional ingredients. Across the reviewed studies, PF proteins consistently showed lower GHG emissions and land use than conventional dairy and egg protein under most modeled scenarios, though the magnitude of advantage was highly sensitive to energy grid, feedstock, and comparator assumptions. Microbial omega-3 oil studies reported GHG and pollution reductions relative to fish oil comparators in most cases, with a consistent land use trade-off from crop-based feedstocks; however, the near-absence of biotic resource use metrics in standard LCIA frameworks means the full environmental picture for fish oil displacement remains incomplete. Bulk lipid microbial oils showed higher impacts than conventional vegetable oil comparators at current production scales, with energy intensity and feedstock production as dominant drivers. These findings are drawn from a predominantly prospective evidence base, and results remain sensitive to scale, location, and modeling assumptions.

Since most analyzed processes remain at low to mid technology readiness levels, the associated LCAs are primarily prospective. While prospective LCAs require careful communication of uncertainty and rely on assumptions about future scale, configuration, and performance, they remain a critical tool for prioritizing research directions and

identifying environmental improvement levers in emerging bioprocesses. This review demonstrates that environmental performance of fermentation-derived ingredients is not automatic: energy source, feedstock choice, location, and bioprocess productivity are each decisive, and their influence is often obscured by current modeling conventions (Blackstone et al. 2025; Fox-Chapman and Bess 2025).

Realizing the sector's environmental potential therefore depends not only on process improvement but on addressing barriers that are often economic, regulatory, supply chain, and infrastructure-related rather than scientific. Rigorous and accessible LCA evidence is an important tool for navigating these challenges and guiding investment, commercialization, and policy decisions. Advancing the field will require harmonized reporting standards, improved primary process data through public-private collaboration, and integrated TEA-LCA approaches. Investment in renewable energy infrastructure, next-generation feedstocks, and open-access R&D will simultaneously reduce both cost and environmental impact, accelerating the path to commercially viable, low-impact fermentation-derived ingredients. Implementation of fermentation-derived ingredient supply chains represents not only an environmental opportunity but also a strategy for protein diversification and supply chain resilience in the face of growing global disruptions to conventional food systems.

6 Author Contributions

Lucas Eastham: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing: Original Draft, Writing: Second draft, review, and editing, Project Administration

Kirsten Trinidad: Methodology, Investigation, Data Curation, Formal Analysis, Writing: Original Draft (Methods, LCA methodology results, and tables), Writing: Review and Editing.

Adam Leman: Conceptualization, Resources, Writing: Review and Editing, Project Administration

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8. Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. All systematic search and result data along with extracted meta-data are found in the supplementary data files. Supplementary Table 1: Systematic literature search and screening; Supplementary Table 2: Literature data extraction and analysis.

This study includes literature search data derived from **Dimensions**, a Digital Science platform, accessed under

license agreement with the Good Food Institute (<https://app.dimensions.ai>). This study also includes literature search data derived from **Clarivate™ Web of Science™**, accessed under license agreement with Tufts University. © Clarivate 2025. All rights reserved.

9. Declaration of AI use

During the systematic review, KT and LE conducted data extraction manually with text-analysis assistance (ChatGPT-5.2, OpenAI) used to support validation of manually extracted data. No automated data extraction was conducted. All extracted data were entered, reviewed, validated, and finalized by the authors. During the preparation of this manuscript Claude Sonnet 4.6 (Anthropic) was used to review sections of text and propose edits to reduce content length, improve readability, and highlight key messages. After using these tools, the authors reviewed and edited the content and take full responsibility for the content of the published article. All analytical conclusions, interpretations, and intellectual contributions presented in this manuscript are solely those of the authors.

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