

Building a Knowledge Base for Biomass Fermentation in Alternative Proteins

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Introduction

The alternative-protein sector is expanding rapidly, driven by the urgent need to develop sustainable and nutritious alternatives to animal-derived foods. Among the technologies being explored, microbial biomass fermentation, through submerged fermentation (SmF) and solid-state fermentation (SSF), offers significant potential to produce protein-rich ingredients with nutritional, functional, and sensory desired properties. Despite this promise, the field remains highly fragmented.

To the best of our knowledge, there is no open, comprehensive dataset that consolidates prior work in biomass fermentation. Critical details such as microbial strains, substrate types, fermentation conditions, titer and yield, nutritional outcomes, functional properties (like solubility, emulsification, water-holding capacity), and organoleptic characteristics (texture, flavor, mouthfeel) are dispersed across numerous studies. As a result, research teams in academia, startups, and industry frequently duplicate efforts, repeat unsuccessful experiments, and face unforeseen regulatory or technical challenges. This inefficiency not only slows scientific progress but also delays the commercialization of fermentation-derived proteins capable of replacing animal ingredients at scale.

This report addresses that gap by systematically reviewing the scientific literature on biomass fermentation for food applications, with a focus on meat alternatives. By mapping both SmF and SSF processes across microorganisms, feedstocks, and reported outcomes, the analysis aims to identify methodological gaps, highlight emerging patterns, and provide a foundation for a consolidated, validated knowledge base. The hope is that such a resource would reduce redundancy, reveal true knowledge gaps, and guide targeted experimentation and collaboration. Ultimately, the goal is to accelerate innovation and de-risk development for companies and researchers working to bring microbial protein solutions to market.

Methods

To map the current scientific landscape of biomass fermentation for food applications, specifically meat alternatives, a structured literature review was conducted- focused on both submerged fermentation (SmF) and solid-state fermentation (SSF) processes. The goal was to identify studies that utilize microbial biomass for direct human consumption as an alternative protein source.

Search Strategy

Google Scholar was searched using combinations of relevant keywords, including:

- *biomass+ meat replacement*
- *biomass+ meat analogue*
- *biomass+ alternative protein*
- *solid state fermentation+ meat replacement*
- *liquid fermentation+ meat replacement*
- *submerged fermentation+ meat replacement*
- *solid state fermentation+ alternative protein*
- *liquid fermentation+ alternative protein*
- *submerged fermentation+ alternative protein*
- *biomass fermentation + meat analogue/substitute*
- *fungal fermentation + meat analogue/substitute*

In addition, the citation trails of relevant papers were reviewed in order to identify other significant studies, both forward and backward (papers citing the source and papers cited by the source). Systematic reviews and thematic reviews were also included. Patents and PCTs related to biomass fermentation were reviewed but excluded, as they were broad and lacked specific experimental details such as exact substrates, strains, or measurable outcomes.

Inclusion Criteria

Only papers that met the following conditions were included:

- Focused on biomass production for human food, particularly as a meat substitute or alternative protein.
- Involved microbial biomass such as bacteria, yeast or filamentous fungi.
- Described processes involving SmF or SSF technologies.
- Microbial fermentation of plant-based substrate, when the goal is to enhance nutritional content for a meat alternative for human consumption.

Exclusion Criteria

The following types of studies were excluded:

- Traditional fermented foods (koji, tempeh, ancom, etc.)
- Biomass fermentation for animal feed.
- Biomass fermentation by microalgae.
- Fermentations focused on the production of metabolites, enzymes, secondary metabolites or specialty ingredients (vitamins, flavor compounds, pigments, antioxidants).

Data Extraction and Tabulation

To systematically analyze the selected papers, a structured table was developed ([table here](#)). The table was designed to capture different information from each study, namely the bioprocess employed, chemical composition of the resulting ferment, functional properties, and any additional analyses, allowing for cross-comparison between different fermentation features (SmF vs. SSF).

Each row in the table corresponds to a unique fermentation condition or treatment as reported in the source literature. The following features were extracted manually from the full texts:

- **Publication metadata:** Title, year, source, authors
- **Fermentation setup:** Fermentation type (SmF or SSF), microorganism used, specific strain of this microorganism and its taxonomic group (where specified), substrate(s) used, substrate category, substrate concentration (for SmF) and any enrichments added to the substrate (if applicable).
- **Bioprocess parameters:**
 - **Fermentation:** Fermentation time (in hours), temperature, vessel type, agitation (in rpm, for SmF), initial pH, and moisture content of the substrate.
 - **Downstream (DSP):** Descriptions of post-fermentation treatment, including filtration or drying methods, and biomass recovery techniques
 - **TPY:** Titer (defined as g/L for SmF or mg/g Substrate for SSF), productivity (defined as g/L/h, calculated only for SmF) and yield (g/biomass for SmF).
- **Nutritional composition:** Yield (with reported unit), Dry matter (%), Protein content (%), Protein analysis method, Fat content (%), Fat analysis method, Fiber content (%), Ash content (%), Total carbohydrates (g/100g), Total soluble sugars, Total soluble sugars (TSS) method, Total phenol content (with unit), and RNA content (mg/100 g DW).
- **Functional properties:** Water-holding capacity (%), Water absorption (mL water/g dry sample), Swelling capacity (mL/g), Emulsifying activity (with units), Emulsifying stability (%), Gelation capacity (%), Foaming capacity (%), Foam stability (%), Fat absorption capacity (%), *In vitro* protein digestibility (%), Pasting temperature (°C, 10% concentration), Viscosity at 90 °C (mPa·s, 10% concentration), Final viscosity at 25 °C (mPa·s), and Least gelation concentration (%).
- **Analytical screening:** Presence/absence of additional analyses performed, including Total amino acids profile (AA), Total fatty acids profile, Total digestible starch, antinutrient quantification, Antioxidant activity, and Polyphenols.
- **Additional notes:** Any relevant contextual information not captured in the predefined columns (for example, sensory evaluation, analytical chemistry analyses, growth kinetics)

Where multiple fermentation conditions or strains were tested within a single study, each condition was logged as a separate row to allow for granular comparison. When numeric data was missing or not applicable, cells were left blank to preserve the structure.

Results

Overview of Literature Evaluated

In this project, we analyzed 216 fermentation treatments reported across 40 scientific sources, covering both solid-state fermentation (SSF) and submerged fermentation (SmF). Each row in the [structured table](#) represents a unique treatment or experimental condition as

extracted from the literature. The data spans publications from 2011 to 2024, with a notable increase in research activity observed in the last five years.

Among the treatments, SmF was more prevalent, representing 161 treatments (74.5%), while SSF accounted for 55 treatments (25.5%). This distribution may reflect the dominance of SmF in the food biomass fermentation academic literature, although SSF is gaining attention due to its advantages in using low-moisture, agro-industrial side streams (as will be detailed later).

This report is divided into 3 main sections:

1. Identified gaps- Methodological limitations, missing measurements, and inconsistent reporting of values (different units, varying methods, or partial datasets).
2. Observed patterns- By examining the observed patterns in the data, we can establish a clear picture of the current state of biomass fermentation research. This includes identifying the microorganisms, substrates, fermentation types, and analytical methods most frequently reported, spotting emerging trends, and highlighting widely adopted practices.
3. Comparative Evaluation of SSF and SmF- Beyond patterns, we systematically compare outcomes reported for SSF and SmF across nutritional, functional, and process-related parameters. This allows us to move from description to interpretation, highlighting where each approach demonstrates clear advantages or limitations. The result is a set of insights that can guide future research and application by clarifying which fermentation mode best aligns with specific performance targets.

Identified Gaps

We first examined the completeness of reporting across the [dataset](#). To do this, we quantified the percentage of complete values for each measured feature, grouped into three categories: bioprocess parameters, nutritional properties of the biomass, and its functional properties. The resulting figures below provide a visual overview of where the literature is robust and where data gaps are most prevalent. This step is critical, as uneven reporting not only restricts the scope of meta-analyses but also skews our understanding of which features are considered essential or optional in biomass fermentation research. By highlighting areas with consistently low completeness, we can identify which types of measurements require standardization and greater emphasis in future studies.

Bioprocess Parameters

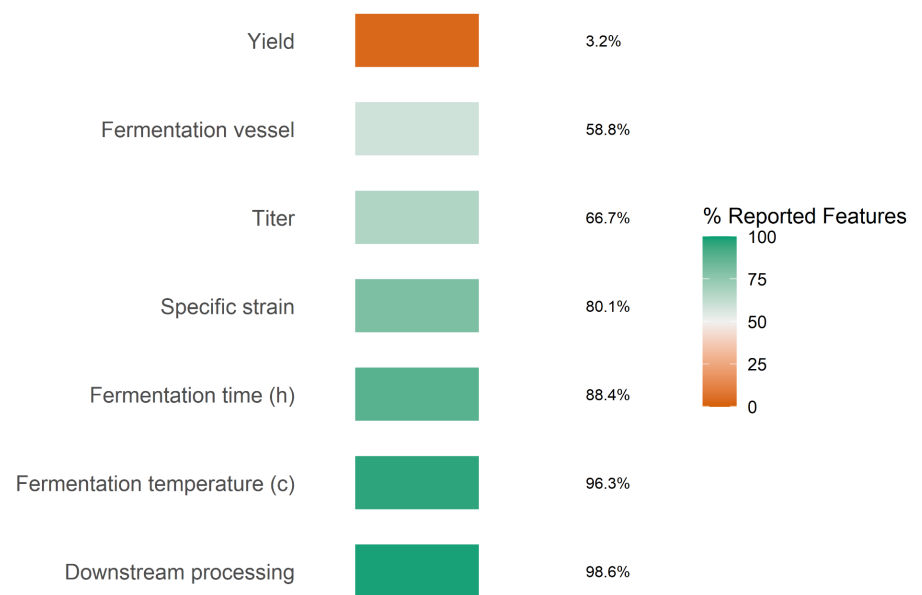
Bioprocess parameters were the most consistently documented, yet several important gaps were observed (**Figure 1**). Core process variables such as fermentation temperature (96% complete) and fermentation time (88% complete) were reported in nearly all treatments, as well as the downstream processes used with the resulting ferment (~99% complete), reflecting their central role in experimental design. In contrast, other parameters, such as fermentation vessel (like glass flasks, jars, bioreactors, ~60% complete) and titer (g/L for SmF, 66% complete) were less reliably recorded, suggesting areas where reporting could be better standardized. Beyond these variables, several missing parameters raise more

significant concerns.

Around 20% of the 40 studies reviewed did not report the specific microbial strain used, a serious limitation since even strains of the same species can have dramatic effects on fermentation performance, end-product characteristics, and regulatory classification.

For features specific to the fermentation type, the gaps were even more striking: in SSF, 44% of studies did not report the initial water content of the substrate, while in SmF, only about half reported the substrate concentration in the liquid medium. Finally, yield (reported as g/g substrate used) was missing in nearly all of the SmF studies, despite its importance for commercialization and technical-economic analyses ([TEAs](#)).

Figure 1 - Completeness % - Bioprocess



Taken together, these patterns show that while bioprocess parameters are generally the most complete portion of the dataset, critical gaps in strain identification, substrate characteristics, and yield reporting significantly limit reproducibility and cross-study comparisons.

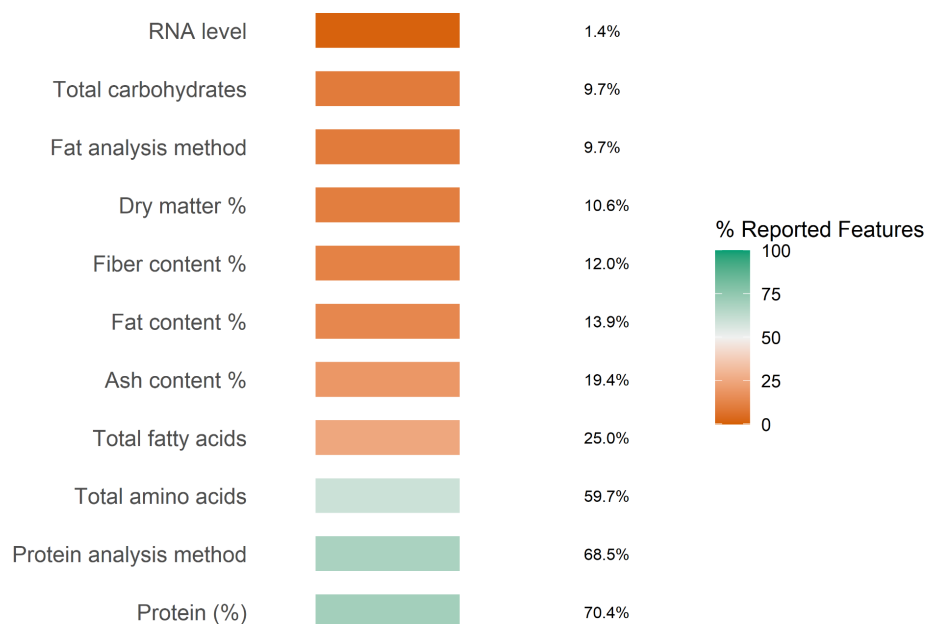
Nutritional Parameters

Nutritional properties showed a mixed level of reporting (**Figure 2**). Protein-related features were the most complete within this category, with protein content and analysis method documented in the majority of studies (~70% complete in both cases). However, this still means that almost one-third of papers did not report biomass protein content at all, despite it being one of the most critical features for the alternative protein industry. Even among the papers that did report protein content, there were methodological differences: most followed an [AOAC-approved method](#) for protein quantification (Kjeldahl or Dumas), but over 20% used non-AOAC assays such as Lowry or BCA, which measure soluble protein fractions rather than total protein. This lack of consistency complicates direct comparisons, as values generated by Lowry or BCA are not equivalent to total protein determinations.

Total amino acid profiles were somewhat less consistently captured (60% complete), but still better reported than most other nutritional measures. Reporting other macronutrients was much weaker. Fat content (~14% complete) and its associated analysis method (~10% complete) were rarely provided. This is a major limitation, since fats are essential for mimicking the sensory and nutritional attributes of animal-based products, especially meat analogues. Carbohydrates and fibers were also largely absent, with total carbohydrate and

fiber content missing in most studies. Similarly, information on ash (only 20% complete) was very limited. Reporting on RNA content was particularly poor (~2% complete), which is highly concerning given its regulatory and safety implications. Excess RNA intake can elevate uric acid levels and lead to health risks, which is why the WHO recommends that nucleic acid content in microbial protein products be reduced to [below 2% w/w](#).

Figure 2 - Completeness % - Nutritional properties



Industrial mycoprotein processes, such as the Quorn™ or ENOUGH ABUNDA™ production system, address this by applying a [heat-shock step](#) (for example, >68 °C for 30–45 min) to disrupt ribosomes and activate RNases, thereby degrading RNA to nucleotides and lowering its content to safe levels. The lack of RNA reporting in most studies represents a critical blind spot, as heat treatment not only improves safety but can also impact product yield and quality. Without systematic data on nucleic acid levels, it is difficult to assess safety, align with regulatory expectations, or explore alternative RNA-reduction strategies that could inform the design of more efficient production and downstream processing pipelines.

Overall, nutritional reporting was skewed toward protein, but still incomplete even for protein itself. The inconsistent use of methods, together with the lack of data on fats, carbohydrates, and nucleic acids, leaves major gaps in assessing the nutritional value of fermentation products and their potential to replace or mimic conventional meat.

Functional and bioactive parameters

The functional properties of the resulting samples were the least reported category in the [table](#) (**Figure 3**). Almost all functional features were missing in the large majority of studies.

Critical functional traits that determine how fermentation products can be applied in foods, such as emulsifying activity (12.5% complete), emulsifying stability (~3% complete), swelling capacity (~4% complete), and fat absorption capacity (~4% complete), were only sporadically reported. Water-holding capacity, a key parameter for formulation, appeared in <5% of studies. Additional features not shown in Figure 3 (e.g., pasting at different temperatures) were each measured only once. These isolated measurements highlight not just the rarity of functional reporting but also the lack of standardization: different papers use different features, units, or protocols for the same attribute, making “apples-to-apples” comparison difficult. The mycoprotein field can overcome this by adopting the

[well-established plant-protein pipelines and assays](#), for instance, [Pearce & Kinsella's](#) for emulsification, and [Beuchat-style](#) water and oil-holding capacities, to generate comparable, decision-ready functionality data.

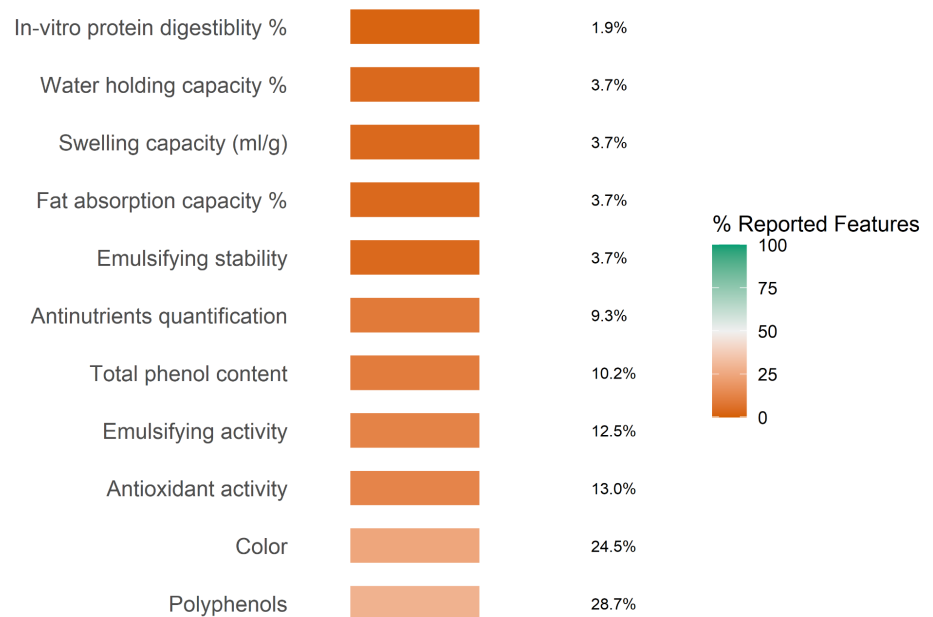
A particularly critical gap concerns flavor and sensory attributes. Only ~30% of the studies reported the color of the resulting samples, despite color's role in product formulation and target applications. In addition, while flavor is one of the critical features in food

development and in the success of alternative proteins, only a single study across the [entire dataset](#) explicitly examined the flavor profile of the ferment or final product ([Zhang et al. 2024](#)), and only one performed a product development test with the resulting ferments ([Gamarra-Castillo et al. 2022](#)). This is highly concerning: while texture and nutrition are essential, consumer acceptance is overwhelmingly driven by taste. The absence of a flavor evaluation step, even a minimal one, not only limits our understanding of how microbial biomass can replicate or complement animal-based foods, but also creates a blind spot for industry translation, where flavor is often the make-or-break factor.

Interestingly, when comparing fermentation types, [SSF papers](#) more often reported on bioactive properties such as polyphenol content and antioxidant activity. This may reflect the fact that SSF typically uses plant-based solid substrates, and authors highlight these bioactive compounds as added value for consumption.

Overall, the minimal and inconsistent reporting of functional properties is a major gap, especially given their importance for determining texture, stability, bioactivity, and processing behavior in food applications. Without more standardized and comprehensive reporting, functional evaluation remains the weakest link in the current fermentation literature.

Figure 3 - Completeness % - Functional properties

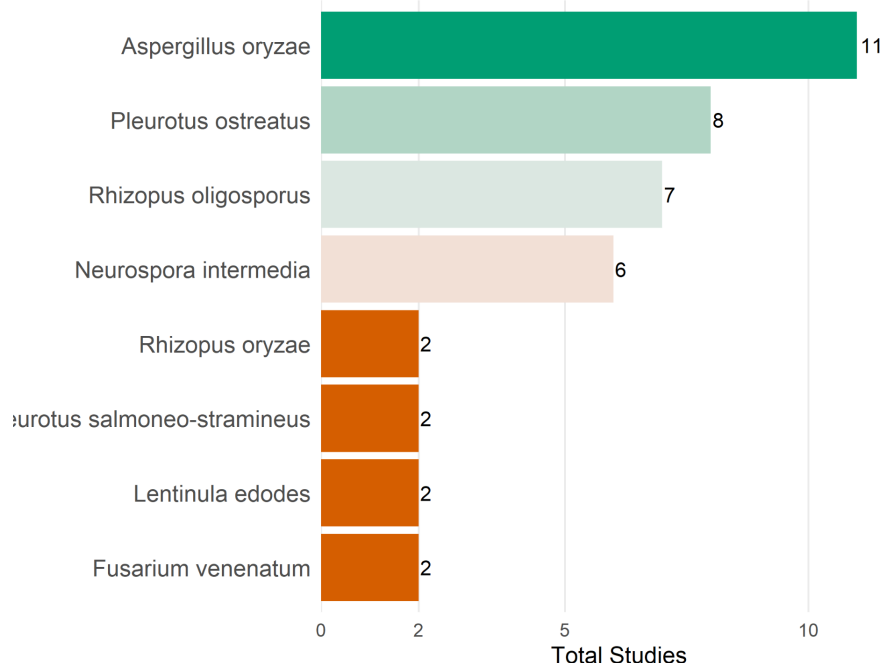


Observed Patterns

Microorganism Usage Patterns

Across the [evaluated sources](#), we identified **34** unique microbial species used in biomass fermentation experiments. However, their representation was far from uniform. Out of microorganisms appearing in more than one source, *Aspergillus oryzae*, *Pleurotus ostreatus*, *Rhizopus oligosporus*, and *Neurospora intermedia* alone accounted for >80% of all papers (**Figure 4**). This result may reflect their broad applicability, industrial relevance, and possibly the comfortable regulatory status they hold across geographies (for example, GRAS status and clear history of use in the food space).

Figure 4- Microorganisms use by Study

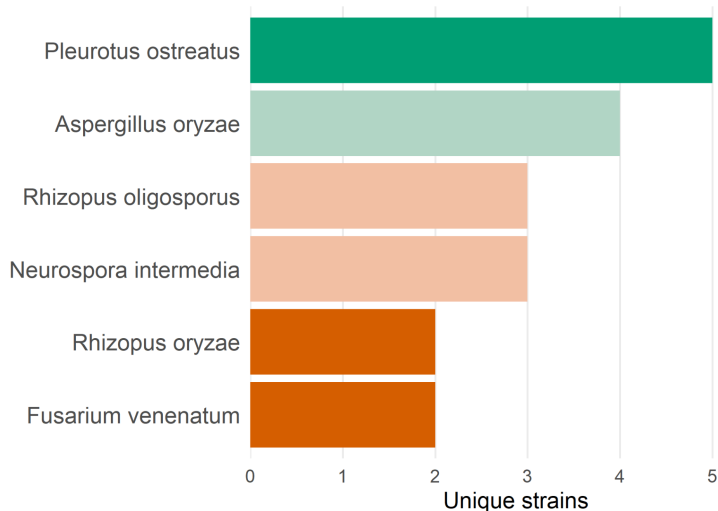


In contrast, 26 other microorganisms were examined in only a single paper, often in a narrow context. These include a diverse range of organisms such as yeasts (*Saccharomyces cerevisiae*), bacteria (like *Lactobacillus plantarum*), and various filamentous fungi from different genera, including several *Penicillium* spp. species, a few *Agaricus* spp. species and *Monascus purpureus*. In addition, one paper used two different consortia treatments, composed of a mix of filamentous fungi.

Strain Differences

As noted above, not all papers reported the specific strain used, but even when strains were reported, the level of diversity varied considerably across the [dataset](#) (**Figure 5**). Widely studied species such as *Rhizopus oligosporus*, *Aspergillus oryzae*, *Pleurotus ostreatus*, and *Neurospora intermedia* were each represented by 3–5 distinct strains, while most other microorganisms appeared with only one or two. This highlights a double-edged challenge: on the one

Figure 5- Unique strains by species



hand, greater strain diversity is valuable, as it broadens the exploration of metabolic capacities and potential applications; on the other hand, it makes direct comparisons across studies difficult, since grouping results under a single species name, for example, “*A. oryzae*”, can mask important strain-level differences. Recognizing and consistently reporting strain information is therefore essential both for expanding diversity and for enabling reproducible, meaningful comparisons.

Fermentation Setting Distribution

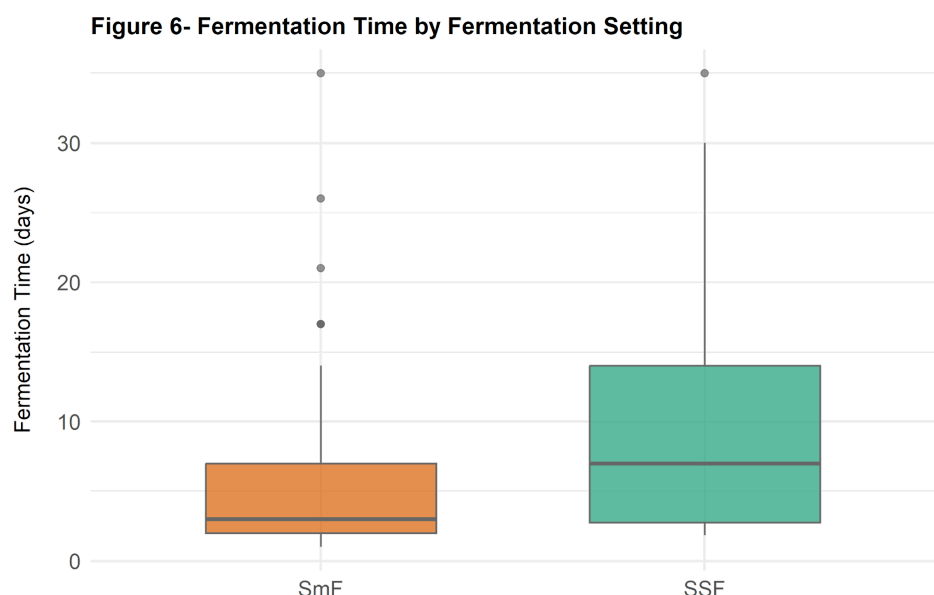
The distribution of microorganisms across SSF and SmF was highly uneven (**Table S1, Appendix A below**). Only four species (*A. oryzae*, *P. ostreatus*, *R. oligosporus*, and *N. intermedia*) were reported in both fermentation types. These can be considered the “core workhorses” of the field, as they are repeatedly used across contexts and represent the main bridge between SSF and SmF research. Even within this group, the balance was not uniform. *R. oligosporus* was evenly distributed (4 SSF vs. 4 SmF), while *A. oryzae* was more common in SmF (7 vs. 4), and *P. ostreatus* was more common in SSF (5 vs. 3).

Outside of these four bridge species, most other organisms were restricted to a single fermentation mode. Several fungi of industrial or nutritional interest, such as the widely used *Fusarium venenatum*, *Lentinula edodes*, *Pleurotus salmoneo-stramineus*, and *Rhizopus oryzae*, appeared exclusively in SmF. The case of *F. venenatum* is particularly notable, as it is the microorganism behind Quorn™ mycoprotein, as well as other companies, and has been developed specifically for large-scale SmF processes. Conversely, a smaller set (including *Agaricus blazei*, *Auricularia fuscusuccinea*, *Cordyceps militaris*, *Flammulina velutipes*, and *Hericium erinaceus*) were reported only in SSF. This is not surprising, as these fungi are best known in the context of their fruiting bodies, which are naturally cultivated on solid substrates. Extending this tradition, researchers appear to use SSF as the logical platform for growing their mycelia as well.

Overall, [the data](#) show a strong reliance on a few versatile fungi that dominate both SSF and SmF research, while the majority of species remain confined to one fermentation type and appear only sporadically. This low evenness suggests that comparisons between SSF and SmF are currently driven by a narrow set of shared species, limiting broader insights into how microbial diversity might influence outcomes across fermentation systems.

Fermentation Time

Fermentation duration differed substantially between SSF and SmF (**Figure 6**). SmF experiments were typically shorter, with most runs lasting less than one week. In contrast, SSF covered a broader range, spanning rapid fermentations of only a few days up to prolonged processes extending over



three to four weeks. This wider spread reflects both the ability of SSF to accommodate fast-growing filamentous fungi, such as *Aspergillus* and *Rhizopus*, and slower colonizers like *Pleurotus*. The substrate context also plays a role, as dense or lignocellulosic matrices can extend colonization times compared to simpler substrates.

These contrasting patterns suggest that fermentation type is closely tied to microbial growth dynamics and substrate characteristics. However, direct comparative studies between fungal species under both conditions remain limited. Expanding such comparisons will be important to understand whether observed differences stem from fermentation mode itself or from the choice of organism and substrate combination.

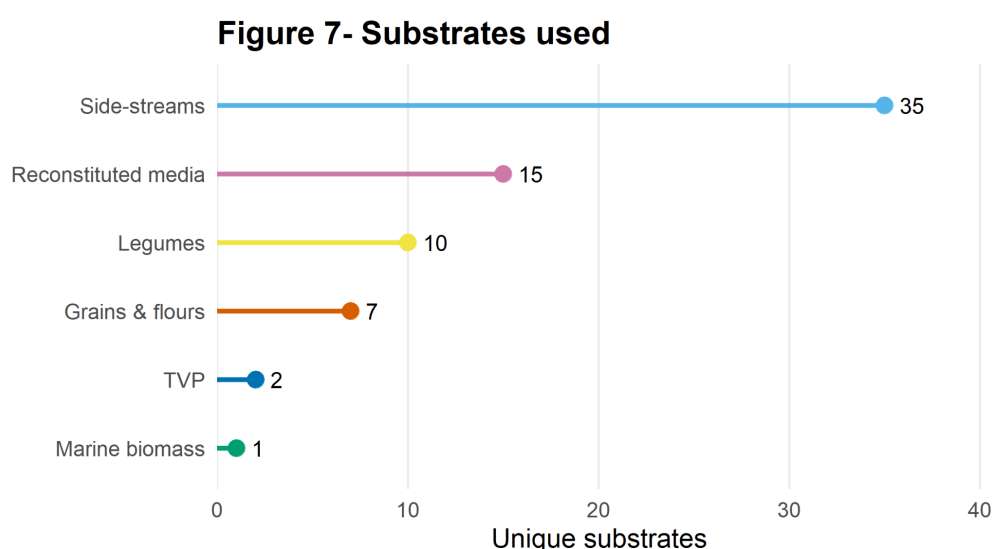
Substrate Use

Substrate Diversity

The range of substrates used across [the reviewed studies](#) was highly diverse, reflecting both the creativity of researchers and the opportunities for upcycling. In total, 70 unique feedstocks were identified, spanning agro-industrial side-streams (like fruit pomace, brewer's spent grain, okara, pistachio hulls, molasses), legumes and grains from different geographies (chickpeas, lentils, black-eyed peas, quinoa, oats), reconstituted sugar mixtures with varying glucose-to-xylose ratios, as well as more specialized sources such as marine biomass (*Durvillaea* seaweed, *Sargassum*) and textured vegetable protein (TVP). This richness highlights not only the wide scope of possible feedstocks for biomass fermentation but also the field's growing alignment with circular bioeconomy principles.

However, when these substrates are grouped into broader categories (**Figure 7**), the pattern becomes clearer.

Side-streams dominated the literature, with 35 unique examples, accounting for the majority of substrate diversity. This was followed by reconstituted media (15), used largely for controlled laboratory studies using SmF, and then legumes (10) and grains and flours (7), which represent more conventional food and feed crops. TVP (2) and marine biomass (1) appeared rarely.



These results highlight both the importance and the complexity of substrate choice in fermentation research. On the one hand, the wide range of materials demonstrates how strongly upcycling has become a central theme, with both SSF and SmF emerging as powerful tools to convert diverse side-streams into biomass, ingredients, and other

value-added applications. On the other hand, this diversity makes it difficult to compare samples or functional properties across studies, as each substrate differs in composition, origin, and required pre-treatment. Moreover, a publication bias likely shapes the picture: negative or failed trials are rarely reported, even though many substrates require extensive optimization and processing before they can support microbial growth or yield meaningful results. Thus, while the literature provides a strong foundation for sustainability through waste valorization, it also underscores the need for systematic exploration of underrepresented substrate classes and more transparent reporting practices to enable broader applicability and fairer cross-study comparisons.

Substrate Use by Fermentation Type

When comparing substrate use between SSF and SmF, clear patterns emerged (**below table**). Agro-industrial side-streams were the most versatile category- SSF relied on 12 of them, SmF accounted for 19, with 4 used in both. Notably, many of the SmF studies involved pre-processing inherently solid wastes (for example, fruit peels, pomaces, husks) into liquid extracts or hydrolysates before fermentation- an approach useful for lab-scale control but one that may not be economically viable for real-life industrial scenarios at scale. Legumes and pulses (10 total) were applied exclusively in SSF, consistent with their traditional role in solid fermentations. Grains and flours showed a more balanced distribution (3 SSF, 4 SmF). By contrast, reconstituted media were overwhelmingly used in SmF (13 out of 15), typically comprising simple sugars such as glucose, sucrose, xylose, or defined glucose-xylose blends. More specialized categories followed expected patterns: TVP appeared once in each fermentation mode, and marine biomass (1 unique case) was tested only in SmF. Together, these patterns indicate that SmF favors soluble or defined substrates (sometimes at the cost of heavy pre-processing), whereas SSF is selected for complex, minimally processed plant materials such as legumes and side-streams.

Category	Total unique substrates	Used in SSF	Used in SmF	Used in both
Side-streams	35	12	19	4
Legumes & pulses	10	10	0	0
Grains & flours	7	3	4	0
Reconstituted media	15	2	13	0
TVP	2	1	1	0
Marine biomass	1	0	1	0

Fermentation Effects

Downstream Processing (DSP)

The downstream processing workflows observed in the [dataset](#) differ between SmF and SSF, reflecting the inherent properties of each fermentation mode.

In SmF, DSP almost always begins with a separation step to recover mycelial biomass from the liquid medium. Two main routes were common:

- Centrifugation → Washing → Drying (oven or freeze-drying)
- Filtration/Sieving → Washing → Pressing → Oven or Freeze-drying → Milling

Variants of these flows included additional treatments such as heat shock or repeated washing, but the principle remained the same: separation of biomass from broth, stabilization by drying, and homogenization into powder. This separation-first logic explains why SmF yields can be measured in g/L (as will be presented below) as the mycelium is recovered independently from the medium.

In SSF, DSP flows were simpler and matrix-oriented, since the biomass is embedded in the solid substrate. The dominant route was:

- Whole-matrix drying (oven or freeze-drying) → Milling

In most cases, the ferment was processed as-is, without separating mycelium from substrate. This approach preserves the entire matrix, but also makes it difficult to quantify yields in absolute units. Although SSF can in some cases produce aerial mycelium that is harvested separately, such instances were not observed in the sources reviewed here.

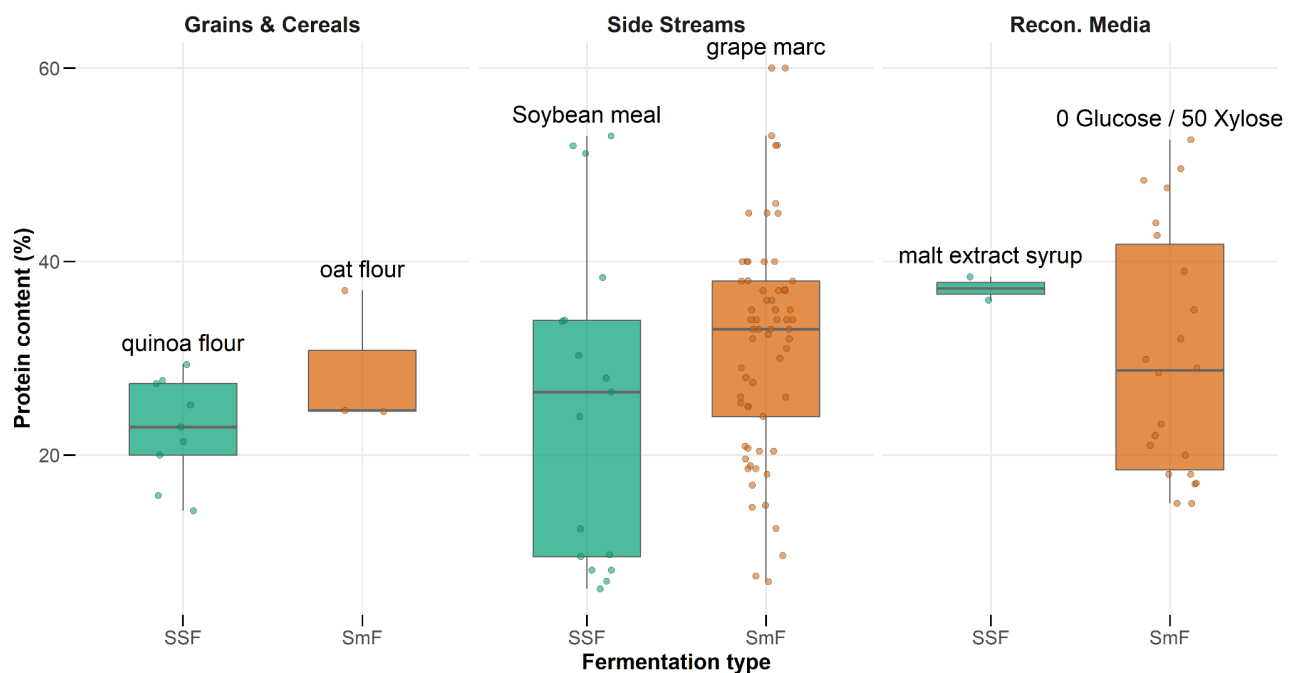
Taken together, SmF DSP is characterized by separation and purification of biomass, while SSF DSP emphasizes stabilization of the whole fermented matrix. These methodological differences not only influence the physical form of the final ingredient but also shape how data such as yield, nutritional and chemical composition, and functionality can be reported and compared across fermentation modes.

Protein Content

Figure 8 compares protein content across SSF and SmF for the three substrate categories where at least two treatments were available and analyzed by standard methods (Dumas/Kjeldahl): grains and flours, industrial side streams, and reconstituted media. In grains, protein contents clustered in the 20–35% range, with quinoa flour (SSF) and oat flour (SmF) reaching the highest values. Industrial side streams displayed the widest spread, from ~15% to over 50%, with soybean meal under SSF producing the highest value, while grape marc defined the upper end for SmF. Reconstituted media showed the opposite trend, with SmF on glucose–xylose blends approaching 50% protein, whereas malt extract syrup under SSF was substantially lower.

Across all three categories, the spread of values was substantial, often exceeding the average difference between SSF and SmF. This highlights that substrate identity and microbial strain both have a greater influence on protein outcomes than fermentation mode alone. It should also be noted that SmF values generally reflect only the mycelial biomass, while SSF values represent the entire fermented matrix, including residual substrate- an important methodological distinction when interpreting protein contents.

Figure 8- Protein content by fermentation type



To allow a more direct comparison between SSF and SmF, we filtered the [dataset](#) for cases where the same substrate was tested in both fermentation modes, with protein content measured using standardized methods (Dumas or Kjeldahl). After applying these restrictions, only a single substrate remained: malt extract syrup. For malt extract syrup, SSF consistently produced slightly higher protein contents (~37–39%) than SmF (~32–35%). Although the difference is modest, it suggests that even on a uniform substrate, fermentation mode can shift protein levels. However, the real takeaway from this analysis is not the performance of malt extract syrup itself, but rather the severe lack of comparable data. The fact that only one substrate fulfilled the criteria underscores the difficulty of making robust, head-to-head assessments between SSF and SmF. Without more systematic studies applying standardized analytical methods across identical conditions, conclusions will remain tentative and context-specific.

Fat Content

When examining fat content, the [dataset](#) becomes even more fragmented. To ensure consistency, we only included studies that reported fat content using the [AOAC-approved](#) Soxhlet extraction method, which remains the standard for the determination of total lipids. After applying this filter, very few comparable cases remained (as noted above, most papers did not measure fat content at all), underscoring the scarcity of systematic data on lipid dynamics in biomass fermentation.

Within this narrow dataset, a clear difference in substrate type emerges between SSF and SmF. SSF samples cluster tightly at ~13–14% fat (narrow spread), whereas SmF shows a much lower median ~3–4% with a broader dispersion (~2–6%) and an occasional high value around ~10–11% (fermented with sugarcane bagasse). Overall, SSF yields ~3–4× higher fat content than SmF for these co-tested side-streams, with SSF also exhibiting less variability. The high fat content of the SSF-based samples may be explained by the substrate used in

these studies- these primarily relied on substrates naturally rich in lipids, such as seeds, legumes or other fat-containing agro-industrial residues. By contrast, the SmF studies were based on fibrous, low-lipid side streams such as peels or sugarcane bagasse, resulting in lower values of around 2-6%. In fact, many lipid-rich materials are inherently solid in nature, like oilseed cakes, cereal brans, or seeds, making them more compatible with SSF, whereas SmF has historically been applied to soluble or aqueous sugar-rich feedstocks. As a result, comparisons of fat content across fermentation modes must be interpreted cautiously.

Fiber Content

Fiber is an important component in fermented biomass, not only for its nutritional and health benefits but also for its functional role in meat alternatives. Dietary fibers improve digestive health and add nutritional value, while in meat analogues, they are directly linked to texture, juiciness, and mouthfeel. Insoluble fibers in particular contribute to bite, firmness, and water-holding capacity, all of which are essential for consumer acceptance.

Despite its relevance, fiber content was reported [in only a small subset of studies](#), and methods were often inconsistent. In grains and flours, SSF generally produced higher fiber values, with quinoa seed fermentation exceeding 20%, while SmF on oat flour showed lower levels (~12–13%). Legumes and pulses under SSF displayed a broad spread, from 4–5% to over 20% in fava bean flour, with no comparable SmF cases. Industrial side streams showed the strongest divergence: SSF consistently retained fiber levels above 55–60%, with some reaching ~80% (grape byproducts), whereas SmF on similar residues was lower and more variable, between 10–40%. As with protein, it is essential to note a methodological distinction: SSF values reflect the entire fermented matrix, including the residual solid substrate, whereas SmF values usually measure only the harvested mycelial biomass after separation from the medium. This methodological difference partly explains the consistently higher fiber contents reported for SSF.

Taken together, the limited [dataset](#) suggests that SSF tends to preserve higher fiber fractions across diverse substrates, both because of the solid, fiber-rich feedstocks typically used and because the substrate matrix itself remains part of the final product. However, the small number of comparable SSF–SmF pairs highlights the need for more systematic studies with standardized analytical methods to fully understand how fermentation mode shapes fiber outcomes.

Titer and Productivity

Unlike protein, fat, or fiber contents, production values (either titer, yield, or productivity) were reported sporadically and were reported using different indices. To ease comparability, we coded titer as g/L for SmF and g/g substrate for SSF and productivity as titer divided by fermentation time in hours (g/L·h). Yield was measured using different methods- as g/mg dry or weight substrate, mg ergosterol/g substrate and mg glucosamine/g dry mass.

Given the sharp differences between SSF and SmF in terms of mycelia growth, separation options and intended applications, we only compared SmF titer and productivity for different substrates and strains.

Figures 9 and 10 summarize SmF titer and productivity across the three substrate categories with sufficient data: grains & flours, reconstituted media, and industrial side streams. For titer, grains & flours show the highest typical values, with a tight distribution centered at ~19–20 g/L (n=3). Reconstituted media, composed of refined sugars, exhibit low titers overall (generally ~0–6 g/L; median ~2–3 g/L). Industrial side streams have a low central tendency (most observations ~0–5 g/L) but a pronounced upper tail, with several runs >20 g/L. These contrasts highlight the strong effect of substrate class on attainable titer: refined formulations yield predictable but low values in this dataset, side streams are more variable yet can occasionally perform well (which can be promising given their low price in most cases), and grains & flours yield consistently high titers within the limited sample available. The productivity patterns mirror

Figure 9- Titer (g/L) by substrate category

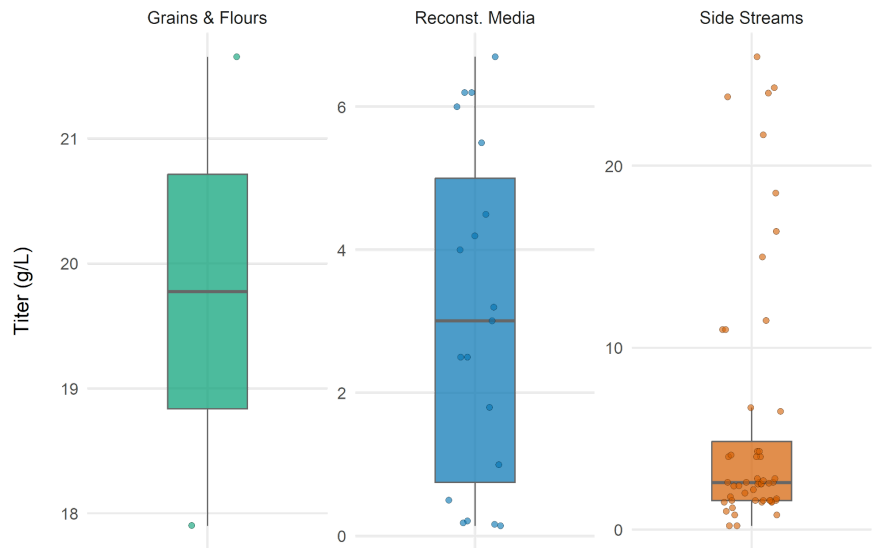
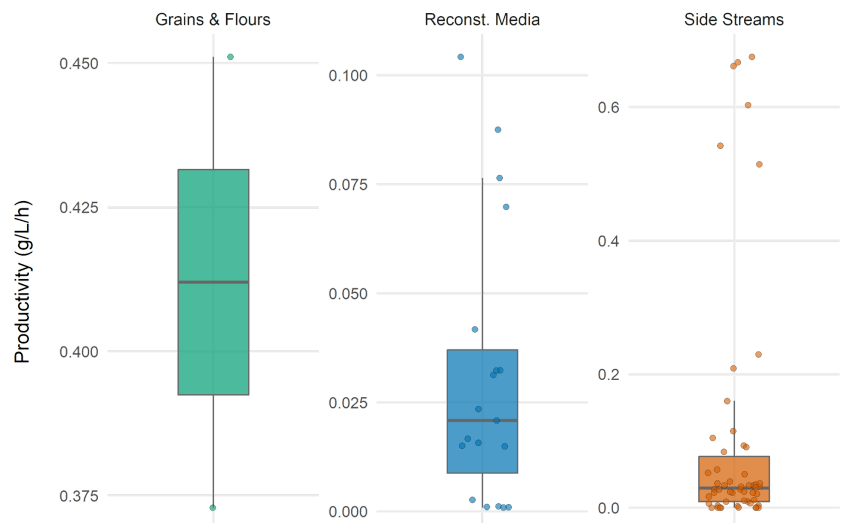


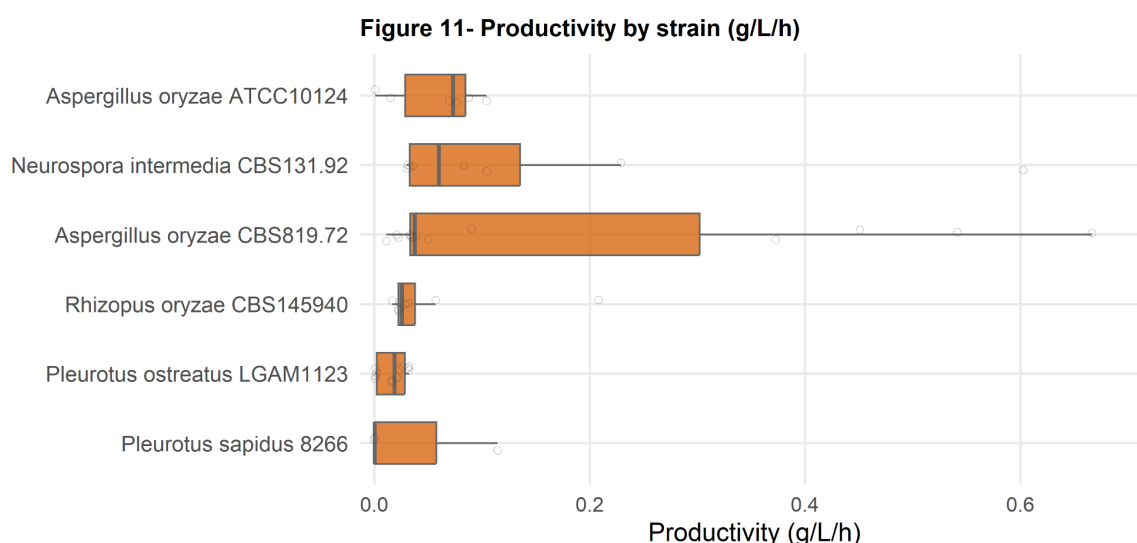
Figure 10- Productivity (g/L·h) by substrate category



titer. Grains & flours cluster at substantially higher rates (median ~0.41–0.43 g/L·h with a compact interquartile range). Reconstituted media are generally modest (most values ~0.01–0.04 g/L·h). Industrial side streams again show a low central tendency (<0.05 g/L·h for most runs) alongside a small number of extreme cases approaching ~0.5–0.7 g/L·h.

Figure 11 collapses across substrates and examines productivity by strain for SmF (strains with ≥ 3 observations). Clear intra-species differences are apparent: *Aspergillus oryzae* CBS819.72 shows the highest central tendency and the broadest dynamic range, with several observations reaching ~0.6–0.7 g/L·h, whereas *A. oryzae* ATCC10124 performs at intermediate levels (on the order of a few hundredths to a tenth g/L·h). *Neurospora intermedia* CBS131.92 is likewise intermediate, while *Pleurotus ostreatus* LGAM1123, *Pleurotus sapidus* 8266, and *Rhizopus oryzae* CBS145940 occupy the lower end of the spectrum. These results indicate that strain identity, even within the same species, has a profound effect on productivity, and it underscore the need for broader strain diversity in

future studies, together with consistent, strain-resolved reporting (species name and strain code) to enable robust cross-study comparisons.



Functional Properties

Functional properties such as water absorption, foaming capacity, emulsifying stability, and gelation are central to the performance of fungal-derived ingredients in food applications and in meat analogs. However, the [table](#) reveals a major limitation: not only are these properties reported inconsistently across studies, but even when the same parameter is measured, units and methodologies vary widely.

For example, hydration-related traits may be expressed as *water-holding capacity (%)*, *water absorption (mL/g)*, *swelling capacity (mL/g)*, or even *g water/g dry biomass*. Similarly, emulsifying activity is variously reported as *emulsion capacity (%)*, *surface area (m²/g at a given pH)*, or *separation rate (%/s)*. These discrepancies make direct comparison extremely challenging, and in many cases prevent meaningful aggregation of values across studies.

To address this, we filtered for properties with at least three data points per fermentation type. Only water absorption capacity and foaming capacity met this threshold (**Table S2**). Water absorption was slightly higher in SmF (mean ~3.6 mL/g) than in SSF (mean ~2.7 mL/g), while foaming capacity was nearly identical between the two (SSF ~13.4%, SmF ~12.8%). The broader point is that methodological inconsistency is the biggest barrier in comparing functional properties across SSF and SmF. While compositional metrics such as protein or fat can at least be normalized by weight, functional assays depend heavily on protocol details (sample preparation, concentration, pH, temperature, units). Without standardized methods, results remain fragmented and cannot be reliably used to benchmark processes or guide industrial formulation.

Conclusion

This review underscores both the promise and the fragmentation of biomass fermentation research. SSF and SmF each bring distinct strengths: SmF allows for standardized yield

measurement and purified biomass recovery, while SSF is optimized on fiber-rich side streams and produces ingredients with strong hydration and textural properties. Yet across both modes, comparisons remain constrained by inconsistent reporting, heterogeneous methods, and gaps in nutritional and functional data.

To move beyond fragmented case studies, the field urgently needs a standardized reporting framework. This should include a core set of attributes (for example, protein, fat, fiber, yield, and key functional properties like water-holding, emulsifying, foaming, digestibility), each measured using validated, reproducible methods (AOAC-approved assays) and reported in consistent units with clear methodological notes (like device type, sample preparation, concentration and pH, among others). Such harmonization would enable true “apples-to-apples” comparisons across studies and reveal valid performance trends.

A recent study by [Gautheron et al. \(2024\)](#), which was also part of the reviewed studies, provides a useful reference point for what this can look like in practice: explicit bioprocess parameters; standard compositional analyses; functional readouts (e.g., WHC/OHC, emulsifying/foaming or texture where relevant); and complementary product-relevant evaluations such as color and olfactory/sensory characterization. While no single paper is exhaustive, this style of methodical, strain- and process-resolved reporting is the closest to the common language the field needs.

Establishing and adopting such a standard is not merely an academic exercise: it is foundational for streamlining innovation, de-risking industrial adoption, and accelerating commercialization. Converging on shared protocols will let researchers and companies benchmark outcomes reliably, identify promising strain-substrate-process combinations more efficiently, and translate laboratory findings into real-world applications with greater confidence. The opportunity is clear: biomass fermentation can convert diverse feedstocks into functional, nutritional, and sustainable proteins. Realizing that potential requires not just more studies, but studies conducted and reported under a common, rigorous framework.

Appendix A

Table S1

Microorganism	SSF Papers	SmF Papers
aspergillus oryzae	4	7
Pleurotus ostreatus	5	3
rhizopus oligosporus	4	4
neurospora intermedia	2	4
fusarium venenatum	0	2
lentinula edodes	0	2
pleurotus salmoneo-stramineus	0	2
rhizopus oryzae	0	2
agaricus bisporus	0	1
agaricus blazei	1	0
agrocybe aegerita	0	1
aspergillus allahabadii	0	1
aspergillus awamori	0	1
aspergillus niger	0	1
auricularia fuscusuccinea	1	0
bacillus subtilis	1	0
consortia 1 (fungi)	0	1
consortia 2 (fungi)	0	1
cordyceps militaris	1	0
flammulina velutipes	1	0
hericium erinaceus	1	0
hydnum repandum	0	1
kluyveromyces marxianus	1	0
lentinus sajor-caju	0	1
monascus purpureus	0	1
morchella crassipes	0	1
penicillium chermesinum	0	1
penicillium citrinum	0	1
penicillium crustosum	0	1
pleurotus albidus	1	0
pleurotus sapidus	0	1

saccharomyces cerevisiae	0	1
stropharia rugosoannulata	0	1
wolfiporia cocos	0	1

Table S2

Parameter	Fermentation type	N	Value
Water Absorption	SSF	7	2.73
Water Absorption	SMF	4	3.63
Foaming Capacity (%)	SSF	13	13.4
Foaming Capacity (%)	SMF	3	12.8