International Journal of Biological and Biomedical Research

Agro-Industrial Wastes as Substrates for Solid-State Fermentation: Substrate Properties, Microbial Responses and Bioproduct Formation: A Review

Hassan Bashir Mukhtar [1*], Musab Muhammad Bello [2], Abubakar Yau Ibrahim [3], Auwal Abdullahi Zubairu [4], Safiyya Ibrahim BB Farouk [5]

[1-5] Department of Microbiology and Biotechnology, Swarrnim Startup and Innovation University, Gandhinagar, Gujarat, India

* Corresponding Author: **Hassan Bashir Mukhtar**

Article Info

E-ISSN: 3107-7137
Volume: 02
Issue: 04
Received: 14-06-2026
Accepted: 15-07-2026
Published: 17-08-2026
Page No: 98-107

Abstract

Agro-industrial residues could be used for solid-state fermentation (SSF) to produce enzymes, organic acids, biosurfactants, and microbial biomass; however, the results on the performance of the process have been varied and contradictory due to the differences in composition, physical structure, water relations and inhibitor content of the agro-industrial residues, and to the fact that this is seldom reported in comparable terms. The critical narrative review is organized around a single line of argument, which runs from substrate properties to microbial response, and from response to the formation of products, selectivity and reproducibility. A narrative analysis of publishers' records was conducted from August to October 2026 and sources were identified, with particular focus on those published between 2020 and 2026; a total of 28 sources were identified and retained that had confirmed bibliographic data. The evidence suggests that composition will determine what products are likely and the physical structure, water availability and sugar accessibility will determine whether the fermentation will reach them. Released nutrients in the process of pretreatment, but may remove the inducers, create inhibitors or cost; in one study, the reducing sugar increased threefold while the lipase activity decreased 11-fold. Units in the various studies vary, moisture basis and dry-matter basis are not always reported, strain identification is limited, and few pilot-scale, techno-economic and safety data were reported. It is proposed to build a qualitative framework that connects the three main product attributes (biochemicals, bioenergy, and bioextraction), the compatibility of the product with the targeted microorganism, the substrate, the conditioning, the process constraints, the safety, and the feasibility of scale-up, and the main gaps that can be expressed as research priorities to be tested. The framework is based on the published evidence and has not been tested in experiments.

DOI: https://doi.org/10.54660/IJBBR.2026.2.6.01-10

Keywords: Solid-state fermentation, Agro-industrial residues, Substrate characterization, water activity, Fungal enzymes, Bioproducts, scale-up

1. Introduction

Brans, husks, bagasse, pomaces, press cakes and spent grains are produced as a by-product of agro-industrial processing which cost little when compared to refined fermentation media. Cost of raw materials can account for more than 40% of bioprocess costs (Borkertas *et al*., 2025) [3] and substrate cost can be assigned as 30-40% of total production cost (Yazid *et al*., 2017) [28]. The functional performance of a residue is influenced by the polymers to be degraded, the amount of water available, the permeability of the product bed to respiration and inhibitory materials present at the surface of the particle(s).

Solid state fermentation (SSF) is the culture of microorganisms by growing on moist solid particles without a significant amount of free water. The main organisms are filamentous fungi due to the ability of hyphae to penetrate the particles, presence of enzymes and tolerance to low water activity (Borkertas *et al*., 2025) [3]. Lower capital cost (Borkertas *et al*., 2025) [3], need for less agitation and aeration equipment, unsterilized substrates and reduced wastewater (Yazid *et al*., 2017) [28] are some of the attractions reported. Enzymes, organic acids, biosurfactants, pigments, phenolic compounds and aroma compounds are different types of products produced (Soccol *et al*., 2017; Yazid *et al*., 2017) [24, 28].

It is still difficult to use the literature for making decisions, however. Most studies are conducted in a flask with one residue–strain combination, the pilot-scale testing is limited (typically 100–10,000 L), and there is not much data available for bioreactor scale testing (typically 0.5–10 L) (Astudillo *et al*., 2023) [2]. While it is often claimed that yield depends primarily on the composition of the wastes, the reported waste composition is given in mixed units and is not reported in conjunction with physical and water-related properties that determine whether even colonization occurs.

This review presents the evidence in a single chain: substrate properties affect the microbial responses, which in turn affect the product formation, selectivity, and reproducibility (Figure 1). Each link is considered as being modified by the pretreatment and process conditions. The objectives are: (1) to identify from experimental evidence which properties of the substrate are important; (2) to outline the available evidence to demonstrate consistency, conflict and the lack of evidence; and (3) to provide a qualitative framework for choosing and conditioning residues for a specific microbial system and product class. It is not a predictive model but rather reflects the synthesis of published work and any claim that it is the first is disclaimed.

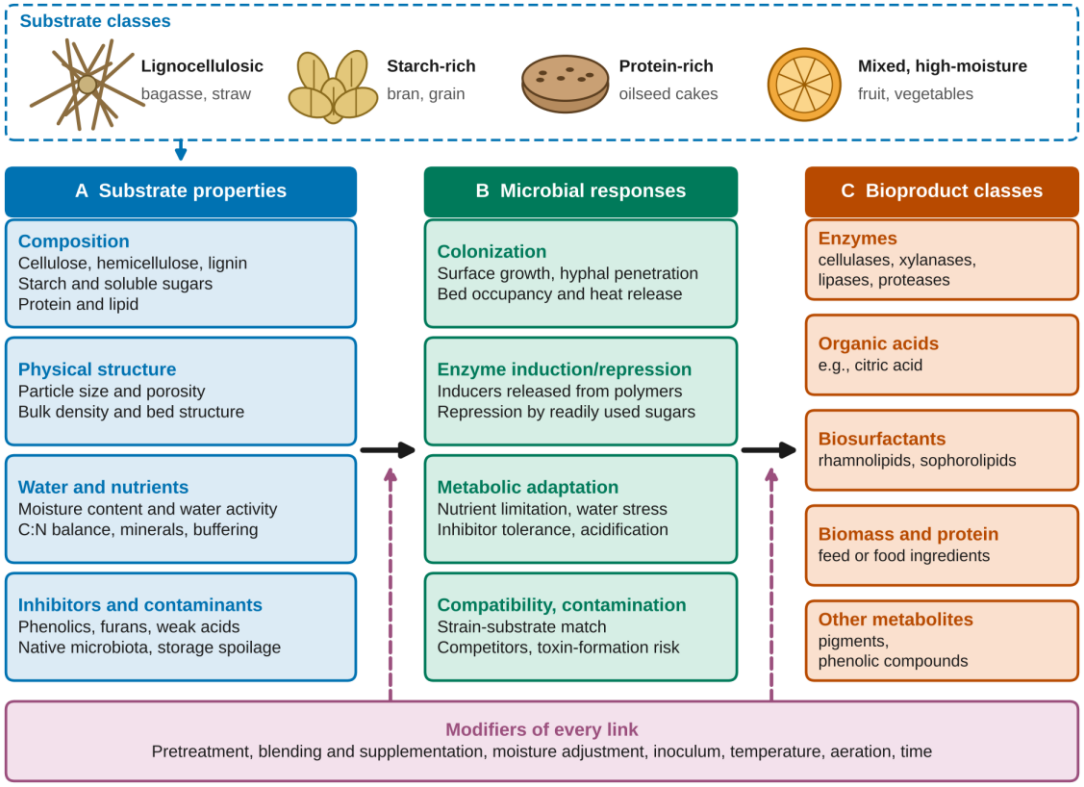


Fig 1: Conceptual framework linking substrate properties (A), microbial responses (B) and bioproduct classes (C) in solid-state fermentation. Solid arrows show the direction of influence synthesized in this review; dashed arrows show that pretreatment, blending, moisture adjustment, inoculum and process conditions modify each link. Substrate icons are schematic. Relationships are drawn from the studies discussed in Sections 3–6 (e.g., Astudillo *et al*., 2023; Borkertas *et al*., 2025; Costa *et al*., 2023; Nargotra *et al*., 2023) [2, 3, 7, 17] and represent qualitative associations, not quantified effects. C:N, carbon-to-nitrogen ratio.

2. Literature Search and Review Scope

This is a critical narrative review, not a systematic review as there is no screening counts reported nor the PRISMA flow. It searched on August to October 2026 using a general web search engine, then repeated several times with a limited search engine that only included those of the publishers (MDPI, Frontiers, BioMed Central, Springer and ScienceDirect) and opening candidate records on the publisher's article page. The retrieved set is not exhaustive, as no subscription indexes (as Scopus, Web of Science, PubMed, etc.) and DOI-registry services were able to be queried. The search words used were "solid-state fermentation" and "agro-industrial residue", "water activity", "moisture", "particle size", "packed-bed bioreactor", "pretreatment", "inhibitors", "oilseed cake", "lipase", "protease", "xylanase", "cellulase", "citric acid", "biosurfactant", "pigment", "mycotoxin" and "techno-economic".

Studies from 2020-2026 were considered more relevant, and a few older studies were retained, such as those that provided background information for water activity (Corona *et al*., 2005) [6] or general overview of the field (Soccol *et al*., 2017; Yazid *et al*., 2017) [24, 28]. The numbers given in the primary studies are used; numbers from reviews are marked as such. If the title, authors, journal, year and volume or article number could be read on the publisher page, then the source

was retained, 28 sources met this criterion. It is here where what has not been seen is not seen, and no pigments or other bioactive metabolites are seen. Comments on the strength of evidence are qualitative judgments.

3. Industrial Wastes and Their Substrate Properties

3.1. Substrate Classes and Composition

Residues are readily separated into the predominant polymer groups (Table 1). Lignocellulosic residues are mainly composed of cellulose, hemicellulose and lignin, with reported ranges ranging from 35–50%, 20–35% and 15–30% (Nargotra *et al*., 2023) [17] and 35–50%, 25–30% and 25–30% (Yazid *et al*., 2017) [28], which vary with species, plant part and method. The hydrolysis rate of polymers is strongly influenced by the quantity present as well as the accessibility; the amorphous cellulose is hydrolyzed 3–30 times faster than the crystalline cellulose, and the hydrolytic enzymes do not bind to the lignin in an effective and specific way (Nargotra *et al*., 2023) [17]. Bagasse is also of practical value that is not reflected in its composition: its fibrous texture contributes to aeration and heat removal since its matrix is composed of lignin, which resists degradation (Martins *et al*., 2026) [15].Residues rich in starch and sugar have different properties. The water content in fruit and vegetable residues ranges from 70 – 90% while the carbohydrate content is 20 – 30% in citrus pulp and up to 60 – 70% for potato peels in dry weight (Borkertas *et al*., 2025) [3]. The yields for citric acid were similar: fruit wastes yielded the highest, and cereal coproducts, the lowest (West, 2023) [27]. Wheat bran has been used as a source of hemicellulose (rich in arabinoxylan), protein, minerals and vitamins and also retains moisture (Martins *et al*., 2026) [15], which are advantageous for production of cellulase from the wheat for similar reasons (de Moura *et al*., 2026) [9].

The protein content of residues, mainly oilseed cakes and most meals, give preference to products requiring nitrogen and fats. Meals such as rapeseed meal and sunflower meal have a crude protein content of 38.3 and 37.0, respectively (Sentís-Moré *et al*., 2023) [20]; while soybean meal has been used as a rhamnolipid substrate (Dabaghi *et al*., 2023) [8]. These materials can contain anti-nutritional components like that gossypol in cottonseed meal and the glucosinolates in rapeseed meal (Wei *et al*., 2026) [26].

Table 1: Representative agro-industrial wastes and substrate characteristics relevant to solid-state fermentation. Values are as reported in the cited sources; ranges from reviews are labeled as such. dw, dry weight; dm, dry matter.

Substrate class	Representative waste	Relevant properties	Suitability considerations	Limitations	Sources
Lignocellulosic	Sugarcane bagasse, straw	Reported ranges: cellulose 35–50%, hemicellulose 20–35%, lignin 15–30%; fibrous, porous	Bulking agent; improves aeration and heat dissipation; source of hemicellulose for xylanases	Crystallinity and lignin limit access to polymers	Nargotra *et al*., 2023; Martins *et al*., 2026; Mitchell *et al*., 2023
Cereal bran	Wheat bran	Arabinoxylan-rich hemicellulose, protein, minerals, vitamins; retains moisture	Frequently a strong substrate for fungal hydrolases	Alone, packed beds heated by 7–16 °C (low porosity)	Martins *et al*., 2026; de Moura *et al*., 2026; Casciatori *et al*., 2024
Mixed polysaccharide–protein	Brewer's spent grain	>60% dw non-starch polysaccharides; 10–26% protein	Responds to thermal pretreatment (ohmic heating)	Spoils in storage if not cooled	Borkertas *et al*., 2025; Silva *et al*., 2025
Sugar-rich, high-moisture	Citrus pulp, potato peels, apple pomace	Water 70–90%; carbohydrate 20–30% dw (citrus pulp) to 60–70% dw (potato peels)	Highest citric acid yields were on fruit wastes	Moisture basis often unstated; cereal coproducts gave lower acid yields	Borkertas *et al*., 2025; West, 2023
Protein-rich	Rapeseed, sunflower and soybean meals	Crude protein 38.3 and 37.0 g/100 g dm (rapeseed, sunflower)	Substrate for proteases, lipases and biosurfactants	Anti-nutritional factors (e.g., gossypol, glucosinolates)	Sentís-Moré *et al*., 2023; Wei *et al*., 2026; Dabaghi *et al*., 2023
Amended or blended	Digestate + food waste + sawdust	Growth only when food waste was added; sawdust lowers humidity and raises porosity	Blending as a design variable	Growth varied among *Trichoderma* species	Alias *et al*., 2022

3.2. Physical Structure

Whether an advantageous composition can be utilized depends on the movement of air, water and heat through the bed which is dependent on particle size, porosity and packing. However, hemp and rice husk initial porosities have been reported at 0.48 and ~0.85, respectively, and a pilot bed of 50% wheat bran and 50% bagasse had an apparent dry bulk density of 90 kg m−3 (Mitchell *et al*., 2023) [16]. Myceliophthora thermophila was used to pack endoglucanase and xylanase, generating > 850 and > 3,850 U g−1 temperature rises, respectively, in the sugarcane bagasse fibers/wheat bran (7:3, w/w) pack, while heat rises above 7 °C were obtained in the pure wheat bran, and above 16 °C in power fibre bagasse packs; the lower bed porosity in the latter was responsible for the higher temperature rises. The units for the U g−1 values are not mentioned, which is again discussed in Section 7.

3.3. Water: Moisture Content and Water Activity

The moisture content (water per mass of substrate at a stated moisture content - either on a wet or dry basis) and water activity (aw, the thermodynamic availability of the water) are not the same amount, but they are related by a sorption isotherm that is dependent on the solid matrix. On wheat bran and starch, Gibberella fujikuroi required increasing moisture to maintain the same aw during fermentation with optimal growth and GAA production rate and yield around aw 0.99 (Corona *et al*., 2005) [6]. The authors noted that superabsorbent polymers provided additional water, which increased biomass, but not spore production, which was

thought to be due to water films limiting aeration with extra water, since microbes utilised mainly capillary water, which is lost in SSF.

The moisture content retained in primary studies ranges from 60% (Sentís-Moré *et al*., 2023) [20] to 85% (Rana *et al*., 2021) [19], with units being per dry matter (w/w) (Silva *et al*., 2025) [22] and per initial humidity (Dabaghi *et al*., 2023) [8]. However, fungi are reported to require 40 to 60% moisture, and its thermophilic fungus produced the maximum xylanase activity at 80% (Yazid *et al*., 2017) [28], while the bacteria require 60 to 85% moisture (Martins *et al*., 2026) [15]. Since the moisture optimum is different for each matrix, and neither remains stable throughout the run (Corona *et al*., 2005) [6], moisture optima can't be transferred from other matrices. For most of the retained primary studies, however, moisture content was not reported along with aw.

3.4. Nutrients, pH and Inhibitory Compounds

Product selectivity and growth are influenced by nutrient balance. The thermophilic fungus above achieved the maximum xylanase activity on water alone, as well as added nitrogen source from the bagasse–straw–wheat bran mixture, and added nitrogen source did not yield more enzyme activity than did the water alone, the authors conclude that this was because the mixture supplied sufficient nutrients (Martins *et al*., 2026) [15]. Contrary to this, in rhamnolipid production on soybean meal, glycerol had a high effect, humidity, temperature and inoculum size, and in citric acid production, methanol (usually 2-6%) and other supplements were used also (Dabaghi *et al*., 2023; West, 2023) [8, 27]. C:N ratio, minerals and buffering capacity were seldom studied as specific factors.

There are two sources of inhibitory compounds: inhibitory compounds from pretreatment and inhibitory compounds from lignin. Weak acids and furans degrade microorganisms by various mechanisms such as ATP depletion, cytoplasmic acidification and membrane damage; above a temperature of approximately 110 °C, lignin-derived aromatics such as furfural and 5-hydroxymethylfurfural degrade microorganisms (Sjulander & Kikas, 2020) [23]. However, this literature is based on bioethanol hydrolysates which is a complete contrast to SSF, where untreated residues are used which are not fully documented as to the extent to which these mechanisms are explored. Higher phenolics, protein and reducing sugars were solubilised from the Ohmic-heated brewer's grain, whereas no such effect was observed for the autoclaved grain and the phenolics production was higher which meant that these do not need to inhibit the production of xylanases and proteases for each other (Silva *et al*., 2025) [22], although the effect of solubilised phenolics was not separated.

3.5 Contamination, Variability and Storage

SSF is typically not completely sterilized (Yazid *et al*., 2017) [28], making native microbiota and storage spoilage potential issues, as with uncooled spent grain shows (Borkertas *et al*., 2025) [3]. However, there are costs associated with sterilization; steam or high pressure treatment can reduce protein content in oilseed meals, while in the case of non-sterile fermentation using local strains, it is avoided (Wei *et al*., 2026) [26]. No quantitative information on contamination levels or yields losses due to contamination was found in the retained sources. The variability of its feedstocks is also difficult to evaluate as composition tables combine different units (% dry basis, g kg−1, mg g−1, g per 100 g) that cannot directly be compared (Astudillo *et al*., 2023) [2].

4. Microbial Responses to Substrate Characteristics

4.1. Filamentous Fungi

The hybrid dominance in SSF is due to the filamentous fungi which penetrate the particles and have a more wide pH range, being pH 2–9 against 4–5 for yeasts, and a more or less wide range in the tolerance to low water activity, than the latter (Borkertas *et al*., 2025) [3]. Answers, however, are not uniform or consistent across genera and substrates. Aspergillus is the best studied genus for enzymes, and is the source of enzymes for most of the industrial cellulase platforms, except the industrial strain Trichoderma reesei which produces relatively little β-glucosidase, which can lead to the accumulation of cellobiose; Penicillium is a notable source of enzymes with high levels of β-glucosidase (de Moura *et al*., 2026) [9]. Mycelium yield estimated by qPCR varied greatly among related fungi: T. reesei RUT-C30 yielded 689.80 ± 80.53 mg g−1 on a digestate–food waste–sawdust mixture while T. harzianum T-22 yielded 174.26 ± 2.87 mg g−1 (Alias *et al*., 2022) [1].

4.2. Induction, Repression and Nutrient Accessibility

The role of available sugars is unclear. The effects of changing sugar availability are shown by experiments which induce or repress the cellulase genes in Trichoderma by changing conditions (de Moura *et al*., 2026) [9]. Among the results reported here, the enzymatic hydrolysis of an olive–sunflower cake mixture increased by three times the concentration of reducing sugars and nearly tripled the concentration of yeast cells, while decreasing the lipase activity by 11 times compared with the untreated control, associated with increased in sugar and protease (Costa *et al*., 2023) [7]. In the case of Aspergillus oryzae 2095, glucose was added to the fish flour media, with 5% favoring the production of neutral and alkaline proteases, while 10% and 15% inhibited the production of these enzymes, likely because of preferential use of a more easily assimilated carbon source (Londoño-Hernández *et al*., 2024) [13]. The conditions of product formation and growth can therefore not be related: a condition that optimizes growth may not optimize a product, so repressible products can be suppressed.

4.3. Yeasts and Bacteria

Low moisture content is less tolerable to bacteria, and for bacteria most of the reported SSF requires moisture content between 60–85% which is higher compared to 40–60% required for fungi (Yazid *et al*., 2017) [28]. Yarrowia lipolytica W29 had 99 ± 1 U g−1 in monoculture SSF using an olive–sunflower cake mixture which did not increase when grown co-cultured with Aspergillus niger (Costa *et al*., 2023) [7]. Bacillus subtilis is classified as a Generally Recognized Safe (GRAS) microorganism, as it secretes proteases, amylases and lipases. Although rhamnolipids have been produced by P. aeruginosa on soybean meal (Dabaghi *et al*., 2023) [8], bacterial surfactant producers are thought to be less safe to use in food products due to pathogenicity concerns, while yeasts like Y. lipolytica are thought to be safe (Vučurović *et al*., 2024) [25].

4.4. Compatibility, Inhibition and Contamination

It is possible to have safety but not compositional compatibility.Safety does not imply compositional compatibility. The aflatoxigenic (Aflavus, Parasiticus) and ochratoxigenic (Ochraceus, Carbonarius) species of Aspergillus can contaminate feed and food chain (Pócsi *et al*., 2020) [18], and in the case of A. niger, there is a relatively low risk for mycotoxins and they can be reduced by selecting strains and managing fermentation conditions (Wei *et al*., 2026) [26]. These statements allow screening at the strain level, rather than blanket assurance. Overall, the retained evidence shows no organism is optimal in all substrates or all products, while matching between inducer availability and water tolerance, nutrient needs and formation mechanism of the product is possible.

5. Substrate–Microbe Relationships in Bioproduct Formation

Table 2 collects outcomes from the retained studies with units as published. Values are not ranked, because strains, substrates, moisture, assays and reporting bases differ and normalizing them would need data the studies do not give. Protease is reported per mL of extract by Londoño-Hernández *et al*. (2024) [13], Casciatori *et al*. (2024) [5] do not state the basis of their U g^{-1} values, and a review has set pectinase in U g^{-1} for SSF beside U mL^{-1} for static liquid culture (Sharma *et al*., 2022) [21].

Table 2: Literature-based substrate–microorganism–bioproduct examples, with units as published. Values are not ranked or normalized. †Values reported in a review and not checked against the original studies. CMCase, carboxymethyl cellulase; U, enzyme unit as defined in each source.

Substrate	Microorganism	Product	Conditions	Reported outcome	Source
Brewer's spent grain, ohmic-heated (145 °C, 20 min)	*A. niger* CECT 2088	Xylanase; protease	75% (w/w) moisture; 27 °C; 7 d	Xylanase 540 U g^{-1}; protease 49 U g^{-1} (dry substrate)	Silva *et al*., 2025
Olive cake + sunflower cake (50:50, dry basis)	*Y. lipolytica* W29	Lipase	75% moisture (wet basis); 27 °C; 2 d	99 ± 1 U g^{-1} (monoculture); 49 ± 7 and 8 ± 2 U g^{-1} in simultaneous and sequential co-culture with *A. niger*	Costa *et al*., 2023
Bagasse + straw + wheat bran (1:1:1)	*M. heterothallica*	Xylanase	75% moisture; 45 °C; 4 d; 5 g flasks	495.2 U g^{-1} dry substrate	Martins *et al*., 2026
Bagasse fibers + wheat bran (7:3, w/w), 0.1 g cm^{-3}	*M. thermophila*	Endoglucanase; xylanase	Packed bed; temperature rise <1 °C	>850 and >3,850 U g^{-1} (basis not stated)	Casciatori *et al*., 2024
Wheat bran + wheat straw; wheat bran + rice bran (1:1)	*F. oxysporum* MTCC 7229	CMCase; xylanase	85% (v/w) moisture; 28 ± 2 °C; 5 d	CMCase 6.84 U g^{-1} (bran–straw); xylanase 13.77 U g^{-1} (bran–rice bran); dry substrate	Rana *et al*., 2021
Fish flour + polyurethane foam (70:30, w/w)	*A. oryzae* 2095	Protease	Packed-bed tubes; 30 °C; 36 h; 0–5% glucose	20.0–20.5 U mL^{-1} extract (0% and 5% glucose; assignment not specified)	Londoño-Hernández *et al*., 2024
Soybean meal	*P. aeruginosa* PTCC 1074	Rhamnolipids	80% initial humidity; 34.5 °C; 10 d; 5% (v/v) glycerol	19.68 g kg^{-1} dry substrate	Dabaghi *et al*., 2023
Digestate + fruit waste + sawdust	*T. reesei* RUT-C30	Fungal biomass (qPCR)	26 °C; 6 d	689.80 ± 80.53 mg g^{-1} substrate (*T. harzianum* T-22: 174.26 ± 2.87)	Alias *et al*., 2022
Apple pomace + rice husk; orange-juice-processing waste	*A. niger* ATCC 12846; LPBBC	Citric acid	75% moisture, pH 3.4, 168 h, 30 °C; 65% moisture, pH 2.70, 96 h, 28 °C	Nearly 350 g kg^{-1} substrate; nearly 450 g kg^{-1} waste†	West, 2023

5.1. Enzymes

The best documented products of the hydrolysis are the ones produced on a hemicellulose-rich substrate (xylanase; Casciatori *et al*., 2024; Martins *et al*., 2026) [5, 15] and on a protein- or lipid-containing substrate (protease and lipase; Costa *et al*., 2023; Silva *et al*., 2025) [7, 22]. The substrate properties exert a strong influence through the amount and activity of the inducers supplemented (Section 4.2), by the physical characteristics of the substrate (limitation of heat and oxygen, Casciatori *et al*., 2024). It's important to note that formation is time dependent; protease activity was highest at 44 h decreasing to 72 h (Londoño-Hernández *et al*., 2024) [13] so comparing with a single end-point could be misleading.

5.2. Organic Acids

The sugar-availability-conditioning thing becomes apparent in citric acid. In a study where A. niger SSF was carried out using agricultural co-products, apple pomace (with rice husk) gave almost 350 g kg−1 whilst those of oranges-juice-processing waste gave nearly 450 g kg−1, and the cereal co-products gave the lowest yields, as described by West (2023) [27]. The result was very different in the case of one strain, 0.03 g g−1 starch on potato waste and 0.79 g g−1 starch on taro. The reported fold changes from the treatments are relative to one of several different controls, strains and scales and should not be pooled, and yields per kg of substrate moisture basis not stated indicate fold change in magnitude, not benchmark performance (West, 2023) [27].

5.3. Biosurfactants

Biosurfactants connect substrate to product via carbon and lipid supply, and are moisture and aeration sensitive. The main factors for the soybean meal were temperature, inoculum size and humidity with P. aeruginosa reaching 19.68 g kg−1 of dry substrate after 10 days at 34.5 °C and 100% of initial humidity with the addition of glycerol in the response surface model (Dabaghi *et al*., 2023) [8]. Optimal moistures were 70% rhamnolipids and 82% surfactin, and in the intermittent agitation experiment, the yield of sophorolipids increased by 31% (elsewhere yields were presented in g g−1 dry matter, mg g−1 dry substrate, and/or g kg−1 wet matter, so cannot be directly compared; Bustos-Vázquez *et al*., 2025) [4]. "Recovery matters – as much as formation" – in a techno-economic screening of the

production of sophorolipid from oil cake, recovery was the biggest contributor to the operating costs (Martínez *et al*., 2022) [14].

5.4. Biomass and Protein

In the case of feed or food biomass, the product is the organism itself and substrate effects are manifested as growth, protein quality and protein safety. The crude protein gains come in between about 2.38% with tea residue mixed with wheat bran to 132% with wheat straw and Inonotus obliquus; the residues of the anti-nutrients in the grain remain uncountable, and the agreed safe levels are not available, while another risk lies with the re-synthesis of mycotoxins during storage and accumulation of biogenic amines (He & Cui, 2025) [10].

5.5. Other Metabolites and Product Recovery

Pigments and Phenolic compounds are reported in SSF products (Soccol *et al*., 2017) [24]. There was not enough quantitative evidence to draw a cross-substrate conclusion for these classes. In one reference case cited by Yazid *et al*., 2017, up to 74% of the enzyme recovery was achieved after the extraction with ultrafiltration. The amount of water used per gram of substrate for extraction varies, for example 5 g water/80 mL in case of xylanase (Martins *et al*., 2026) [15] and 0.5% NaCl, with 0.5% Tween 80 for lipase (Sharma *et al*., 2022) [21], indicating that the reported activities are partially due to extraction efficiency.

6. Pretreatment, Process Optimization and Scale-Up

Figure 2 summarizes the SSF process chain used in this review, from feedstock preparation to residue management, and the four variables (moisture, oxygen supply, temperature and bed structure) through which operating conditions interact with substrate properties.

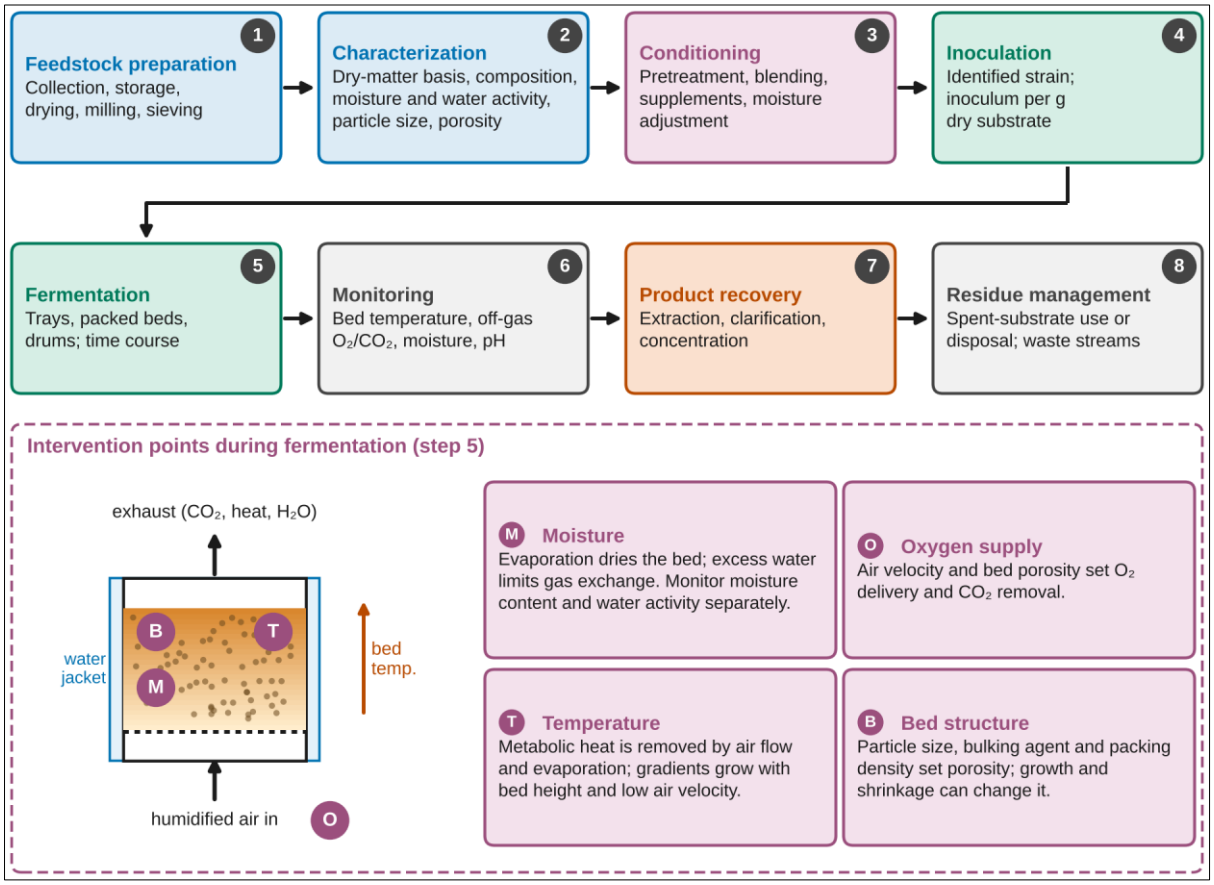


Fig 2: Solid-state fermentation process (steps 1–8) and intervention points during fermentation: M, moisture; O, oxygen supply; T, temperature; B, bed structure. The packed-bed drawing is schematic, with forced aeration and a water jacket; the shading indicates that bed temperature can rise along the airflow path when heat removal is inadequate (Mitchell *et al*., 2023; Sentís-Moré *et al*., 2023). O_2, oxygen; CO_2, carbon dioxide.

6.1. Conditioning: Pretreatment, Blending, Supplementation and Moisture Adjustment

Pretreatment affects particle structure, free sugars, phenolics, and accessibility which in turn affects product formation, depending on what the product requires. Ohmic processing of brewer's spent grain at 145 °C for 20 min released the highest phenolics, protein and reducing sugars with A. niger CECT 2088 (540 U g−1 xylanase activity and 49 U g−1 protease activity) compared to autoclaved spent grain (Silva *et al*., 2025, 121 °C for 15 min) [22]. In a yeast system, the ultrasound treatment had a significant effect on the lipase only in semi-solid system and the enzymatic hydrolysis had significant reduction of lipase (Costa *et al*., 2023) [7]. The acid-treated materials resulted in 2- to 2.3-fold greater yield of citric acid than the untreated materials (West, 2023) [27]. This can consequently benefit, cause no benefit, or cause harm depending on the organism, product, system, and general rules regarding pretreatment.

Few SSF studies report the costs of pretreatment. Mechanical treatment is energy consuming and can generate furfural and hydroxymethylfurfural (Kucharska *et al*., 2018) [12] which represent up to 40% of total costs in biomass processing.

Detoxification steps added: Overliming reduce furans, but may lose up to 70% of sugars at pH 12 (Sjulander & Kikas, 2020) [23]. Comparisons of this sort are, therefore, essential to the interpretation of the results, and chemical inputs, energy values and water and waste streams should be recorded in addition to yield.

Levers that are cheaper to manipulate are blending, supplementation and moisture control. The two residue mixtures (bagasse–straw and wheat bran–straw) were significantly superior to the individual residues in respect of xylanase (Martins *et al*., 2026) [15], and temperature control (Casciatori *et al*., 2024) [5] in the low-density packed bed of bagasse–wheat bran. There are also ways of moisture management that can be active: Superabsorbent polymers (SAP) and negative-pressure irrigation (NPI) both increased cellulase activity by 2.47-fold. When combined together, cellulase activity increased to 2.80-fold, and with increased moisture, temperature gradients lower than 0.4 °C cm−1 produced better heat transfer, but no improvement for sporulation (He *et al*., 2019) [11].

6.2. Temperature, Aeration, Inoculum and Time

Bed structure interacts with temperature, aerobic conditions, inoculum, pH and time. In forced aeration, oxygen transfer and the removal of metabolic heat is of the main concern and thus, superficial air velocity is basically central. Good temperature control was observed in many packed-bed studies with velocities below 1 cm s−1 and even in some, velocities of 6 and 10 cm s−1 maintained the bed temperature within 1 °C of the inlet air temperature (Mitchell *et al*., 2023) [16]. The inoculum should be reported per gram of dry substrate and the identity of the strain since, the use of an inoculum (size, temperature and humidity) are important factors for rhamnolipid production (Dabaghi *et al*., 2023) [8].

6.3. Scale-Up

In laboratory SSF, a usual amount of substrate in a flask is between 5–10 g of substrate, and heat and gas exchange are the factors rarely constrained.In a laboratory SSF, usually the amount of substrate in flasks ranges from 5–10 g of substrate, with heat and gas exchange being the factors not limiting. In large solid beds, this is the opposite of the rule: it is difficult to maintain uniform temperature, gas exchange and moisture, and localized overheating, anoxia and desiccation result (Borkertas *et al*., 2025) [3]. Mitchell *et al*. (2023) [16] predict that for packed beds the height shall be limited to approximately 1 m and diameter be increased for the same bed with superficial velocities up to 10 cm s−1 for reasonable temperature control. Compared to columns and packed beds, which could be operated with forced aeration, tray reactors are always operated by natural diffusion (Bustos-Vázquez *et al*., 2025) [4].

Little evidence is there above the bench. Studies on biosurfactant production in reactors with sizes of 100-10,000 L are few (Astudillo *et al*., 2023) [2] and the reactors with 22 L (3 kg wet matter) and 100 L (10.5 kg wet matter) were a biomass reactor and a biosurfactant reactor, respectively (Bustos-Vázquez *et al*., 2025) [4]. The try techno-economic screening of the sophorolipid production assumed a 15% production loss with scale-up and identified no existing commercial packed-bed bioreactor with the assumed capacity of 5 m3 (Martínez *et al*., 2022) [14]. Current monitoring consists mainly of temperature measures, proxies for off-gas and moisture, while low cost sensor arrays have been able to map tray gradients (Sentís-Moré *et al*., 2023) [20] and biomass is difficult to measure in solids (Yazid *et al*., 2017) [28].

6.4. Safety, Economics and Sustainability

Safety is dependent on both the organism and the substrate and application. In addition to the Aspergillus toxins (Section 4.4), there are no consensus safe post-fermentation levels for anti-nutrients as gossypol (Zhou *et al*., 2010), and the risk of re-synthesis of mycotoxins in fermented food stored for an extended period of time is a pathogenicity issue (He & Cui, 2025) [10]; some bacterial producers are not safe to use in food (Vučurović *et al*., 2024) [25].

The benefits of the economy and the environment are only suggested. The cellulosic enzyme unit cost for SSF and submerged fermentation was USD 15.67 kg−1 and USD 40.36 kg−1, respectively, with SSF economic studies being nonexistent (Yazid *et al*., 2017) [28]. A screening of the sophorolipid production costs estimated USD 5.1, 5.7 and 6.9 kg−1 for production on wheat straw, rice husk and coconut fiber supports, respectively, but it is only a preliminary estimation whose Monte Carlo net present value ranged from −2.1 to 10.9 million USD and that did not consider the transport cost and several other costs (Martínez *et al*., 2022) [14]. Thus, the use of a waste does not imply the sustainability of a process: the benefits of pretreatment, drying, transport, sterilization, extraction and waste treatment are not measurable and so any benefits should be limited to what has been measured.

7. Integrated Substrate Selection Framework and Research Priorities

7.1 Selection Framework

The evidence suggests a progression of questions, rather than a prediction formula (Figure 3). The moisture retention capability is the most important parameter for lipase and biosurfactants (Costa *et al*., 2023; Dabaghi *et al*., 2023) [7, 8], while the hemicellulose content is relevant for xylanase (Casciatori *et al*., 2024; Martins *et al*., 2026) [5, 15] and the availability of sugar for citric acid (West, 2023) [27]. The organism class is then correlated by water tolerance, enzyme repertoire and documented safety (Section 4) and the substrate is characterized on dry-matter basis, such as polymers, free sugars, nitrogen, inhibitors, moisture, aw, and particle size, or porosity. When the match is not good enough, conditioning is assessed based on energy and waste streams, chemical use, and the characteristics of the conditioned substrate is recharacterized, because the more accessible the sugar, the less of this product that is formed (Costa *et al*., 2023) [7]. The process constraints, safety and quality, and scale-up feasibility are then discussed, one by one.

There is not uniform support for such measures. Its strongest strength lies within its performance as a bed structure and heat removal in packed beds (Casciatori *et al*., 2024; Mitchell *et al*., 2023; Sentís-Moré *et al*., 2023) [5, 16, 20] and is reasonably consistent for the product-specific effects of accessible sugar (Costa *et al*., 2023; de Moura *et al*., 2026; Londoño-Hernández *et al*., 2024) [7, 9, 13]. It is moderate for matching product class to substrate (where studies are conducted in incompatible units); and lowest for inhibitors (SSF), contamination, safety, pilot-scale performance and economics.

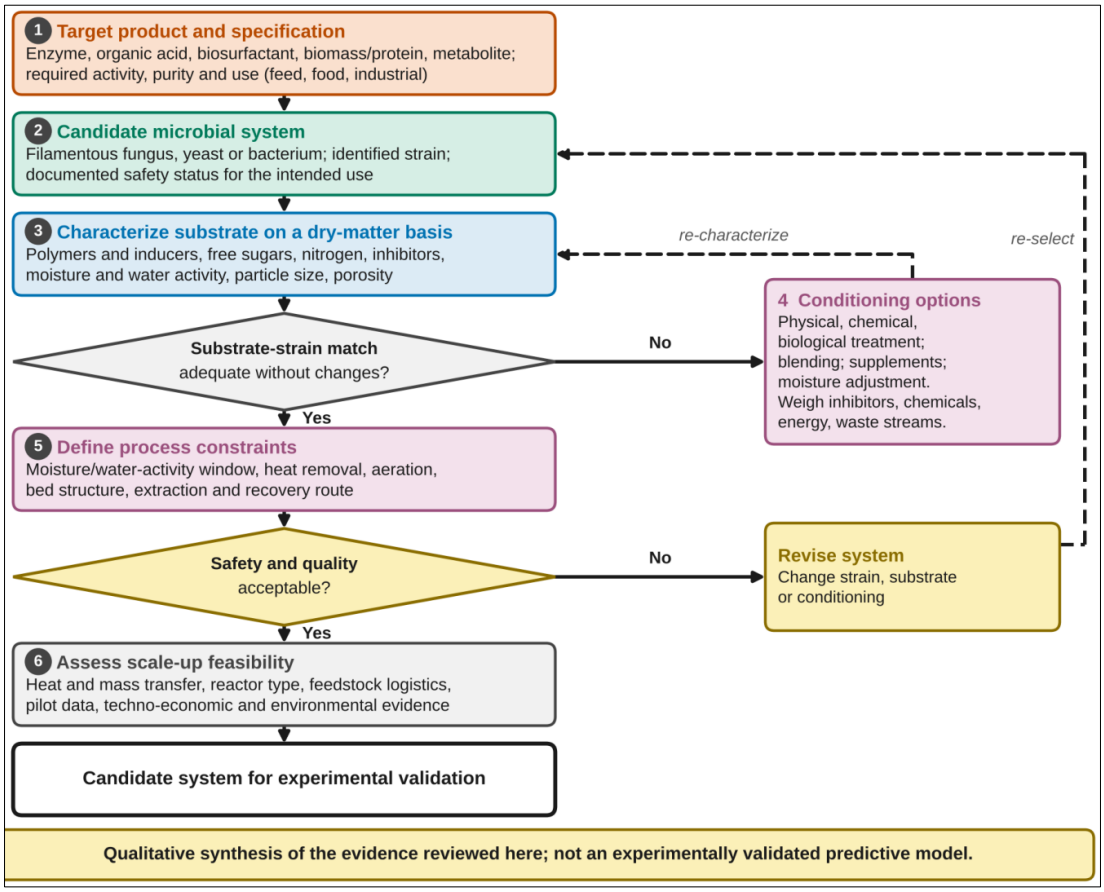


Fig 3: Evidence-informed framework for selecting and conditioning agro-industrial residues for solid-state fermentation. Numbered boxes are steps, diamonds are decisions, and dashed arrows are return loops. The scheme is a qualitative synthesis of the studies discussed in Sections 3–6; it has not been validated experimentally and is not a predictive model.

7.2. Research Gaps and Priorities

Table 3 converts the key gaps into specific enhancements and four key priorities are measurable from current approaches. First, the effect of increasing the severity of pretreatment should be explored in a graded manner across one substrate–strain pair to see if there is an optimum point for sugar availability for product formation as suggested by Costa *et al.* (2023) [7] and Londoño-Hernández *et al.* (2024) [13]. Second, the moisture content and aw should be varied separately and the sorption isotherms obtained before and after the fermentation process (Corona *et al.*, 2005) [6]. Third, packed-bed trials should be carried out with a fixed packing blend and the bed height, packing density, air velocity and temperature profiles should be reported in order to be able to separate the porosity effects from the composition effects (Casciatori *et al.*, 2024) [5]. Fourth, the strain identity, the dry-substrate basis, the definition of the assay and the ratio of the extract need to be reported for any study to allow for the possibility of combining results in forthcoming quantitative syntheses.

Table 3: Critical research gaps and practical research priorities. a_w, water activity; TEA, techno-economic analysis; LCA, life-cycle assessment.

Gap	Consequence	Proposed improvement	Evidence basis
Incomplete substrate characterization	Differences in output cannot be attributed to composition, structure or water	Report dry matter, cellulose, hemicellulose, lignin, sugars, starch, protein, lipid, ash, moisture (stated basis), a_w, particle size, bulk density and porosity	Astudillo *et al.*, 2023; Nargotra *et al.*, 2023; Yazid *et al.*, 2017
Nonstandard units and reporting bases	Yields cannot be compared across studies	Report U g^{-1} dry substrate with assay definition and extraction ratio; state the basis of g kg^{-1} values	Table 2; Bustos-Vázquez *et al.*, 2025; Sharma *et al.*, 2022
Moisture reported without a_w	Moisture optima do not transfer between substrates	Measure a_w and sorption isotherms before and after fermentation; vary moisture and a_w separately	Corona *et al.*, 2005; He *et al.*, 2019
Inadequate strain identification and safety assessment	Toxin risk; poor reproducibility	Report species, strain and collection number; screen products for relevant toxins	Pócsi *et al.*, 2020; Wei *et al.*, 2026; He & Cui, 2025
Pretreatment judged without full accounting	Benefits contradictory; costs, inhibitors and waste omitted	Compare with an untreated control on the same dry basis; record sugars, phenolics, chemicals, energy and waste	Costa *et al.*, 2023; Silva *et al.*, 2025; Kucharska *et al.*, 2018
Limited pilot-scale and heat and mass transfer data	Scale-up uncertain; flask results may not transfer	Report bed height, packing density, air velocity, temperature and moisture profiles	Mitchell *et al.*, 2023; Astudillo *et al.*, 2023; Bustos-Vázquez *et al.*, 2025
Missing recovery and techno-economic data	Cost and sustainability unquantified	Report extraction efficiency; add TEA with sensitivity analysis and LCA	Martínez *et al.*, 2022; Yazid *et al.*, 2017; He & Cui, 2025

8. Conclusion

In the retained evidence, composition determines what products are possible, whereas physical structure, water relations and sugar accessibility will dictate if a fermentation will reach them. No organism or pretreatment gave an absolute best performance under all substrates and products and the same pretreatment appeared to increase, keep the same level or decrease the output in different systems. A variety of factors have constrained those conclusions: characterization of the substrates was inconsistent; the units and moisture bases were inconsistent; the strains were not fully identified; there was limited pilot-scale and safety data, and there was not an exhaustive search. The selection framework is designed to guide the next experiments and not a validated predictor. The best way to get the field from case reports into rules that could be transferred is through standardized reporting, and pilot size studies to measure recovery, safety and costs.

Conflict of Interest

The authors declare that no conflict of interest exists.

Funding

This work did not receive any external funding.

Ethical Approval

Ethical approval was not required for this review, as it did not involve human participants, animals, or the collection of primary data.

Data Availability

No new datasets were generated or analyzed in this study. All sources consulted are cited in the References section.

Author Contributions

Hassan Bashir Mukhtar was responsible for conceptualizing the review, designing the study, and writing the Introduction and oversaw the manuscript design. Musab Muhammad Bello, Abubakar Yau Ibrahim, Auwal Abdullahi Zubairu, and Safiyya Ibrahim BB-Farouk contributed to the remaining sections of the manuscript. All authors reviewed the final version and gave their approval.

References

1. Alias, C., Bulgari, D., & Gobbi, E. (2022). It works! Organic-waste-assisted Trichoderma spp. solid-state fermentation on agricultural digestate. Microorganisms, 10(1), Article 164. https://doi.org/10.3390/microorganisms10010164
2. Astudillo, Á., Rubilar, O., Briceño, G., Diez, M. C., & Schalchli, H. (2023). Advances in agroindustrial waste as a substrate for obtaining eco-friendly microbial products. Sustainability, 15(4), Article 3467. https://doi.org/10.3390/su15043467
3. Borkertas, S., Viskelis, J., Viskelis, P., Streimikyte, P., Gasiunaite, U., & Urbonaviciene, D. (2025). Fungal biomass fermentation: Valorizing the food industry's waste. Fermentation, 11(6), Article 351. https://doi.org/10.3390/fermentation11060351
4. Bustos-Vázquez, M. G., Rodríguez-Durán, L. V., Pichardo-Sánchez, M. A., Rodríguez-Durán, N. R., Rodríguez-Durán, N. A., Trujillo-Ramírez, D., & Torres-de los Santos, R. (2025). Recent advances in biosurfactant production in solid-state fermentation. Fermentation, 11(10), Article 592. https://doi.org/10.3390/fermentation11100592
5. Casciatori FP, Mafra NSC, Rodrigues NA, Sales EH, Maia MMS, Dias ACP, *et al*. Flow configuration and packing density of sugarcane bagasse and wheat bran affecting enzymes production by solid-state fermentation. BioEnergy Research. 2024;17(1):187-197. doi:10.1007/s12155-023-10675-7
6. Corona A, Sáez D, Agosin E. Effect of water activity on gibberellic acid production by Gibberella fujikuroi under solid-state fermentation conditions. Process Biochemistry. 2005;40(8):2655-2658.
7. Costa, A. R., Fernandes, H., Salgado, J. M., & Belo, I. (2023). Solid state and semi-solid fermentations of olive and sunflower cakes with Yarrowia lipolytica: Impact of biological and physical pretreatments. Fermentation, 9(8), Article 734. https://doi.org/10.3390/fermentation9080734
8. Dabaghi, S., Ataei, S. A., & Taheri, A. (2023). Production of rhamnolipid biosurfactants in solid-state fermentation: Process optimization and characterization studies. BMC Biotechnology, 23, Article 2. https://doi.org/10.1186/s12896-022-00772-4
9. de Moura, I. V. L., Araujo, S. C., Sampaio, I. C. F., da Silva, E. G. P., Franco, M., & de Oliveira, J. R. (2026). Production of cellulases by Trichoderma, Aspergillus, and Penicillium: Optimization strategies, biomass valorization, and industrial perspectives. Biomass, 6(4), Article 64. https://doi.org/10.3390/biomass6040064
10. He, D., & Cui, C. (2025). Fermentation of organic wastes for feed protein production: Focus on agricultural residues and industrial by-products tied to agriculture. Fermentation, 11(9), Article 528. https://doi.org/10.3390/fermentation11090528
11. He, Q., Peng, H., Sheng, M., Hu, S., Qiu, J., & Gu, J. (2019). Humidity control strategies for solid-state fermentation: Capillary water supply by water-retention materials and negative-pressure auto-controlled irrigation. Frontiers in Bioengineering and Biotechnology, 7, Article 263. https://doi.org/10.3389/fbioe.2019.00263
12. Kucharska, K., Rybarczyk, P., Hołowacz, I., Łukajtis, R., Glinka, M., & Kamiński, M. (2018). Pretreatment of lignocellulosic materials as substrates for fermentation processes. Molecules, 23(11), Article 2937. https://doi.org/10.3390/molecules23112937
13. Londoño-Hernández, L., García-Gómez, M. J., Huerta-Ochoa, S., Polanía-Rivera, A. M., Aguilar, C. N., & Prado-Barragán, L. A. (2024). Effect of glucose concentration on the production of proteolytic extract by different strains of Aspergillus under solid-state fermentation. Fermentation, 10(2), Article 97. https://doi.org/10.3390/fermentation10020097
14. Martínez, M., Rodríguez, A., Gea, T., & Font, X. (2022). A simplified techno-economic analysis for sophorolipid production in a solid-state fermentation process. Energies, 15(11), Article 4077. https://doi.org/10.3390/en15114077
15. Martins, E. da S., Poço, D. M. L. A., & Martins, H. L. (2026). Production of thermostable xylanase by Myceliophthora heterothallica in solid-state culture using agro-industrial residues. Journal of Fungi, 12(7), Article 461. https://doi.org/10.3390/jof12070461

16. Mitchell, D. A., Ruiz, H. A., & Krieger, N. (2023). A critical evaluation of recent studies on packed-bed bioreactors for solid-state fermentation. Processes, 11(3), Article 872. https://doi.org/10.3390/pr11030872
17. Nargotra, P., Sharma, V., Lee, Y.-C., Tsai, Y.-H., Liu, Y.-C., Shieh, C.-J., Tsai, M.-L., Dong, C.-D., & Kuo, C.-H. (2023). Microbial lignocellulolytic enzymes for the effective valorization of lignocellulosic biomass: A review. Catalysts, 13(1), Article 83. https://doi.org/10.3390/catal13010083
18. Pócsi, I., Giacometti, F., Ambrus, Á., & Logrieco, A. F. (2020). Editorial: Aspergillus-derived mycotoxins in the feed and food chain. Frontiers in Microbiology, 11, Article 606108. https://doi.org/10.3389/fmicb.2020.606108
19. Rana, P., Inbaraj, B. S., Gurumayum, S., & Sridhar, K. (2021). Sustainable production of lignocellulolytic enzymes in solid-state fermentation of agro-industrial waste: Application in pumpkin (Cucurbita maxima) juice clarification. Agronomy, 11(12), Article 2379. https://doi.org/10.3390/agronomy11122379
20. Sentís-Moré, P., Romero-Fabregat, M.-P., Rodríguez-Marca, C., Guerra-Sánchez, A.-J., & Ortega-Olivé, N. (2023). Design optimization of a tray bioreactor for solid-state fermentation: Study of process parameters through protein modification of by-products. Fermentation, 9(10), Article 921. https://doi.org/10.3390/fermentation9100921
21. Sharma, V., Tsai, M.-L., Nargotra, P., Chen, C.-W., Kuo, C.-H., Sun, P.-P., & Dong, C.-D. (2022). Agro-industrial food waste as a low-cost substrate for sustainable production of industrial enzymes: A critical review. Catalysts, 12(11), Article 1373. https://doi.org/10.3390/catal12111373
22. Silva, B. F., Machado, L., Fernandes, A. M., Pereira, R. N., & Belo, I. (2025). Effect of ohmic heating pretreatment on enzyme production by solid-state fermentation of brewer's spent grain. Fermentation, 11(8), Article 421. https://doi.org/10.3390/fermentation11080421
23. Sjulander, N., & Kikas, T. (2020). Origin, impact and control of lignocellulosic inhibitors in bioethanol production—A review. Energies, 13(18), Article 4751. https://doi.org/10.3390/en13184751
24. Soccol CR, da Costa ESF, Letti LAJ, Karp SG, Woiciechowski AL, Vandenberghe, *et al.* Recent developments and innovations in solid state fermentation. Biotechnology Research and Innovation; 2017. doi:10.1016/j.biori.2017.01.002
25. Vučurović, D., Bajić, B., Trivunović, Z., Dodić, J., Zeljko, M., Jevtić-Mučibabić, R., & Dodić, S. (2024). Biotechnological utilization of agro-industrial residues and by-products—Sustainable production of biosurfactants. Foods, 13(5), Article 711. https://doi.org/10.3390/foods13050711
26. Wei, J., Yao, C., Akram, M., Wang, X., Rasheed, S., Duan, Y., Chen, D., Hu, K., Li, W., & Zhang, H. (2026). Advances in solid-state fermentation technology for oilseed meal: Strain selection, fermentation strategies, and high-value applications. Foods, 15(18), Article 3177. https://doi.org/10.3390/foods15183177
27. West TP. Citric acid production by Aspergillus niger using solid-state fermentation of agricultural processing coproducts. Applied Biosciences. 2023;2(1):1-13. doi:10.3390/applbiosci2010001
28. Yazid, N. A., Barrena, R., Komilis, D., & Sánchez, A. (2017). Solid-state fermentation as a novel paradigm for organic waste valorization: A review. Sustainability, 9(2), Article 224. https://doi.org/10.3390/su9020224

How to Cite This Article
Mukhtar HB, Bello MM, Ibrahim AY, Zubairu AA, Farouk SIB. Agro-Industrial wastes as substrates for Solid-State fermentation: substrate properties, microbial responses and bioproduct formation: a review. Int J Biol Biomed Res. 2026;2(4):98-107. doi:10.54660/IJBBR.2026.2.6.01-10.

Creative Commons (CC) License
This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution NonCommercial-ShareAlike 4.0 International (CC BY-NCSA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.