



Review

Reconversion of distillery vinasses using ligninolytic fungi: Sustainable strategies for processes and products

Blas Schettino-Salomón¹, Francisco Hernández-Rosas¹, Regulo Carlos Llarena Hernández²

¹ Colegio de Postgraduados, Campus Córdoba, Carretera Federal Córdoba -Veracruz km 348.5, Congregación Manuel León, Municipio Amatlán de los Reyes, Veracruz. C.P. 94946, México; schettino.blas@colpos.mx; fhrosas@colpos.mx

² Facultad de Ciencias Biológicas Agropecuarias. Universidad Veracruzana. Carretera Amatlán de los Reyes, Veracruz. C.P 94945, México; rllarena@uv.mx

* Correspondence: fhrosas@colpos.mx

Abstract

The industrial distillation of bioethanol generates massive volumes of sugarcane vinasse, a highly corrosive effluent characterized by low pH, elevated chemical oxygen demand (COD), and recalcitrant matrices of melanoidins and phenolic compounds. While field fertigation is conventionally proposed for nutrient circularization, its widespread implementation is constrained by logistical barriers, hyper-osmotic phytotoxicity to sugarcane (*Saccharum officinarum*) cuttings, and severe microbial imbalances. This review critically evaluates the deployment of ligninolytic and functional fungi as a multi-valent biotechnological platform for vinasse treatment, focusing on the enzymatic degradation of recalcitrant pollutants, bioactive compound production, and soil structure enhancement. Filamentous fungi—predominantly white-rot basidiomycetes (*Trametes*, *Pycnoporus*, *Ganoderma*) secrete high-redox extracellular enzymes (laccases, manganese peroxidases, and lignin peroxidases) that break down complex aromatic polymers through non-specific free-radical mechanisms. Concurrently, fungal bioprocessing generates high-protein biomass and mycelial networks that act as biological soil conditioners and aggregation precursors, enhancing soil porosity, water retention, and structural stability in degraded agricultural soils. Recent advances demonstrate that integrating fungal treatment with anaerobic digestion or physical pretreatments mitigates substrate toxicity and mass-transfer limitations in high-viscosity vinasse. Furthermore, biotransformation of vinasse yields valuable secondary metabolites, including industrial enzymes, biocides, and mycoproteins, provided that strict biosafety standards regarding heavy metal (Cd, Cu, Zn) accumulation and mycotoxin synthesis are met. This review synthesizes current mechanistic insights, compares operational configurations, and outlines strategic pathways for scaling hybrid fungal biorefineries.

Keywords: Sugarcane vinasse, Ligninolytic enzymes, Basidiomycetes, Biological soil conditioner, Bioactive synthesis, Hybrid biorefineries.



1. Introduction

Vinasses is a by-product generated at the bottom fraction of distillation columns within an ethanol refinery [1]. It is composed of non-volatile compounds, residual yeast, sugarcane protein fragments, cell debris, phenolic structures, and other complex molecules [2]. Production records indicate that 10–15 L of vinasse are generated per liter of ethanol produced [3]. A primary environmental concern regarding this effluent involves its conventional application in agricultural fertigation practices [4]. Various agricultural feedstocks generate vinasse globally; sugarcane vinasse (*Saccharum officinarum* L.) is primarily produced in ethanol-exporting nations such as Brazil, China, India, Pakistan, Thailand, Mexico, Colombia, Australia, South Africa, and Cuba [5,6]. In Europe, vinasse is mainly derived from sugar beet (*Beta vulgaris* ssp. *vulgaris* var. *altissima* Döll.) processing in Germany, France, Poland, and the UK [7], as well as from beverage distillation, such as mezcal (*Agave salmiana*, *A. angustifolia*, *A. potatorum*) [8] and tequila (*Agave tequilana* Weber var. *azul*) in Mexico [9]. Uncontrolled fertigation with raw sugarcane vinasse increases COD levels in groundwater due to ion leaching [10,11]. Furthermore, raw application to agricultural soil significantly stimulates nitrous oxide (N₂O) emissions from sugarcane fields [12].

1.1. Characterization and chemical composition of the vinasse

Accurately evaluating the environmental risks and valorization potential of vinasse requires defining its chemical matrix. Vinasse is an acidic (pH 3.5–5.5), high-temperature liquid waste with an exceptionally high organic load, presenting COD values ranging from 50,000 to over 100,000 mg/L [13]. Its characteristic dark brown color and high turbidity are primarily attributed to melanoidins—high-molecular-weight, nitrogenous, recalcitrant polymers formed via Maillard reactions during thermal sugar processing and distillation [13,14].

Furthermore, the raw matrix concentrates a massive fraction of dissolved inorganic ions predominantly potassium (K⁺), sulfates (SO₄²⁻), and chlorides (Cl⁻) alongside unrecovered yeast biomass debris, glycerol, volatile fatty acids and low-molecular-weight monomeric phenolic compounds (e.g., ferulic, p-coumaric, and vanillic acids) derived from the structural breakdown of the raw plant tissue [13].

1.2. Technical challenges for vinasse valorization

Transitioning vinasse from a hazardous waste liability into an industrial feedstock presents three primary technical barriers:

1. Microbial Inhibition and Phytotoxicity: The low-molecular-weight monomeric phenolics and derivatives present in the matrix act as potent antimicrobials [15].
2. Recalcitrance of Melanoidins: Due to their complex, heterogeneous, and non-defined polymeric structures bound by strong linkages, melanoidins are highly resistant to traditional biological oxidation and chemical precipitation, preventing effective effluent decolorization and environmental stabilization [16].
3. High Salinity and Osmotic Stress: The high concentration of dissolved inorganic salts, mainly potassium and sulfates, creates a severe osmotic microenvironment. This induces osmotic shock and cell lysis in non-adapted microorganisms, restricting steady, long-term continuous operation in industrial bioreactors [7,17].

1.3. Fungal ecology and ligninolytic adaptation in vinasse treatment

Ligninolytic fungi possess unique physiological adaptations for degrading high-density, recalcitrant organic waste [18]. As key decomposers of lignocellulosic biomass in natural ecosystems, they cycle carbon and energy through forest ecosystems [19,20]. Visually categorized into white-rot fungi (WRF), brown-rot fungi, and soft-rot fungi based on wood decay patterns [21,22], their ecological success stems from their ability to secrete non-specific extracellular enzymes that break down plant cell wall polymers (lignin, cellulose, and hemicellulose) [23,24].

Lignin is an amorphous polymer composed of phenylpropanoid subunits crosslinked with cellulose, imparting rigidity and hardness to plant cell walls [25,26]. Because natural lignin is bound by non-repeating, non-stereospecific linkages, WRF evolved an extracellular, non-stereoselective enzymatic cocktail rather than specific lock-and-key intracellular transport mechanisms [27]

This high-redox oxidative system is chiefly driven by Laccases (Lac), Manganese Peroxidases (MnP), and Lignin Peroxidases (LiP) [26,28,29].

When cultivated in vinasse, these fungi perceive recalcitrant melanoidins and toxic polyphenols as a pseudo-lignin structural network [30]. Through non-specific free-radical oxidative reactions, these extracellular enzymes cleave the aromatic rings of monomeric phenolics and break down melanoidin backbones without requiring direct cellular uptake [31,32]. Given the industrial demand for robust biocatalysts in the textile, paper, and biofuel sectors [27,33], as well as single-cell protein sources for animal feed [34], this review synthesizes current research on applying ligninolytic fungi to vinasse treatment, highlighting operational mechanisms, product diversification, and current technological gaps.

2. Literature Review

2.1. Methodology

To evaluate how vinasse can be utilized as a culture medium for ligninolytic fungi, a combined bibliometric network and qualitative content analysis was conducted. Primary literature extraction was executed using the Web of Science (WoS) and Scopus databases. Search parameters were restricted to full-text Open Access (OA) research articles published between 2021 and 2026 using Boolean terms:

(Title-ABS-KEY (vinasse) AND TITLE-ABS-KEY (fungi)) (Duplicates were removed using Zotero. Keyword co-occurrence network maps were constructed using VOSviewer. (Table 1). Duplicates were removed using Zotero. Keyword co-occurrence network maps were constructed using VOSviewer. (Table 1). Search inclusion

Table 1. inclusion criteria

Search	(TITLE-ABS-KEY (vinasse) AND TITLE-ABS-KEY (fungi))
Publication type	Peer-reviewed Open access (AO) articles
Period	2021-2026
Source	Web of Science (WOS), Scopus
Deduplication	Automated and manual removal via Zotero

3.1. Fungal biomass valorization for single-cell Protein and soil structure precursors

Cultivating filamentous fungi on vinasse transforms a liquid waste stream into protein-dense biomass suitable for animal feed and soil conditioning. Ascomycetous strains (*Aspergillus niger*, *Neurospora intermedia*, *Rhizopus delemar*) grown on diluted vinasse (50%–75%) show biomass yield increases of 160% to 235%, with harvested fungal biomass containing up to 50% crude protein and amino acid profiles comparable to commercial soybean or fish meal [35–37]. Supplementation with inorganic nitrogen (e.g., 2g/L urea) further optimizes protein accumulation and reduces overall matrix toxicity [37].

In addition to single-cell protein synthesis, fungal bioprocessing generates mycelial networks that act as biological soil conditioners and aggregation precursors[38]. Filamentous hyphae physically entangle soil particles, while exuded hydrophobic glycoproteins stabilize macroaggregates. This mitigates soil compaction, increases macroporosity, and improves water retention in degraded agricultural soils [38,39].

Co-culturing fungal strains (*Trichoderma harzianum*) with plant growth-promoting rhizobacteria (*Pseudomonas capeferrum*, *Rhizobium* sp.) produces bioinoculants that enhance the fertigation value of residual vinasse [38] (Table 2).

Table 2. Fungi utilized for biomass production and soil conditioning

Fungi species	Process configuration	Key yields & outcomes	Reference
<i>Aspergillus niger</i> sp. V2	50% vinasse + (NH ₄) ₂ H ₂ PO ₄	Balanced protein content for fish feed ingredient	[35]
<i>Aspergillus niger</i> , <i>A.oryzae</i> CBS 819.72, <i>Neurospora intermedia</i> CBS 131.92, <i>Rhizopus delemar</i> CBS 145,940	Diluted thin stillage (50%-75%)	Biomass yields increased 160%-235%; 50% crude protein.	[36]
<i>Trichoderma harzianum</i> MT2, <i>Pseudomonas capeferrum</i> WCS358, <i>Rhizobium</i> sp. N21.2.	Co-culture in residual vinasse	Enhanced biomass yield and improved fertigation properties	[38]
<i>Oudemansiella raphanipes</i>	Solid substrate (35% vinasse + agricultural residues)	Cultivation of high-value medicinal fruiting bodies.	[39]
<i>Neurospora intermedia</i> , <i>Aspergillus niger</i> , <i>A. oryzae</i> , <i>Rhizopus oryzae</i>	Beet vinasse + Glucose	High mineral accessibility and digestible protein fractions.	[40]
Mixed yeast/fungal consortia (<i>Candida</i> , <i>Aspergillus</i> , <i>Rhizopus</i>)	100% tequila vinasse liquid culture	63.5% TN, 86.8% P, and 46.2% COD removal.	[41]

3.2. Enzymatic degradation kinetics and decolorization of recalcitrant vinasse

White-rot basidiomycetes specialize in degrading persistent organic pollutants, polycyclic aromatic hydrocarbons (PAHs), synthetic dyes, and melanoidins [28,42,43]. In vinasse treatment, immobilized cultures of *Trametes* sp. T3 and *Pycnoporus* sp. P6 on polyurethane foam (PUF) achieve simultaneous COD reduction, phenolic abatement, and high laccase enzyme secretion [44,45]. Co-substrates such as glucose stimulate laccase production, driving effluent decolorization and reducing phytotoxicity in *Lactuca sativa* germination assays [44].

For pre-treated or anaerobically digested vinasse (digestate), persistent melanoidins remain resistant to bacterial degradation. Inoculating digestates with *Trametes hirsuta* or *Phanerochaete chrysosporium* achieves up to 68.8% decolorization, 65.5% total phenol removal, and peak laccase titers exceeding 3400 U/ml [46]. Furthermore, integrating fungal cartridges (*Trametes versicolor*, *Bjerkandera adusta*) into vertical-flow treatment wetlands significantly reduces total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), and COD under continuous flow [47,48]. (Table 3).

Table 3. Ligninolytic fungi deployed for vinasse bioremediation and decolorization

Fungi species	Process configuration	Degradation & Enzymatic Performance	Reference
<i>Trametes</i> sp. T3	PUF-immobilized in diluted vinasse	High laccase titers; reduced <i>Lactuca sativa</i> phytotoxicity.	[44]
<i>Trametes hirsuta</i> Bm-2, AHB-6, <i>Phanerochaete chrysosporium</i> Bm-4, <i>Cochliobolus lunatus</i> AHB-1 and <i>Athelia rolfsii</i> AT5, AT13 AT7.	Anaerobically digested vinasse medium	68.8% decolorization; 65.5% phenol removal; 3415 U/mL Laccase.	[46]
<i>Trametes versicolor</i> UAMH 8272, <i>Bjerkandera adusta</i> UAMH 4312, <i>Phanerochaete chrysosporium</i> UAMH 3642	Comparative evaluation for raw tequila vinasse treatment to assess feasibility for incorporating in vertical flow treatment wetlands	PVC columns designed inserted cartridges of polyester microfiber with fungi was feed it with raw vinasse	[47]
<i>Aspergillus niger niger</i> , <i>Aspergillus niger tubingensis</i>	Co-culture in vinasse + battery powder	Organic acid secretion driving heavy metal bioleaching.	[49]
<i>Saccharomyces cerevisiae</i> , <i>Scheffersomyces stipitis</i>	Detoxified bagasse hydrolysate + vinasse.	Fermentation efficiency increased (>80% degradation).	[50]
<i>Pycnoporus</i> . P6	Solid/submerged state on PUF	Phenol abatement and high laccase production.	[51]
<i>Ganoderma lucidum</i>	Delignification of brewer's grain & vinasse	Enhanced substrate suitability for biochemical conversion.	[52]

3.3. Production of High-value fungal bioactives, industrial enzymes and biopesticides

Beyond effluent remediation, fungal bioprocessing of vinasse yields high-value secondary metabolites, industrial enzymes (laccase, MnP, LiP, cellulases, xylanases), and organic platform acids [28,53]. Fungal co-cultures (*Aspergillus niger* and *Trichoderma reesei*) utilizing sugarcane bagasse soaked in vinasse under Solid-State Fermentation (SSF) hyper-produce citric acid in packed-bed bioreactors[54]. Similarly, sequential saccharification using *Earliella scabrosa* and *Candida tropicalis* on vinasse-impregnated bagasse yields high ethanol volumes without generating furfural or 5-hydroxymethylfurfural (5-HMF) inhibitors [15,55].

Raw vinasse fermented with specialized fungal strains also exhibits biocide and antifungal properties against agricultural pathogens such as *Fusarium circinatum* and *Fusarium* wilt in crops [56,57]. Optimization of culture media parameters (MgSO₄, FeSO₄, CuSO₄, and pH) using *Trametes villosa* or *Trametes sanguineus* maximizes laccase yield while achieving concurrent phenol and COD removal [58,59]. Immobilizing crude laccase in alginate-copper beads permits biocatalyst reuse across multiple operational cycles [60]. (Table 4)

Table 4. Bioactive compounds and industrial enzymes are synthesized from vinasse.

Fungi species	Target product	Operational parameters & performance	Reference
<i>Aspergillus niger niger</i> CCT4355, <i>Trichoderma reesei</i> CMAA1168	Citric acid	SSF packed-bed reactor bioreactor using impregnated bagasse	[54]
<i>Earliella scabrosa</i> , <i>Trametes hirsute</i> , <i>Aspergillus niger</i> (Fasp,Fe,Fh,Fk) <i>Trichoderma</i> , <i>Candida tropicalis</i> , <i>Pichia deserticola</i>	Cellulases, Laccase, 2G Ethanol	Simultaneous saccharification of bagasse + vinasse.	[55]
<i>Fusarium</i> , <i>Aspergillus niger</i> , <i>Penicilium</i>	Antifungal Biocide	Inhibits <i>Fusarium circinatum</i> growth in vitro.	[56]
<i>Trametes villosa</i>	Laccase enzyme	Media optimization (MgSO ₄ , CuSO ₄); COD and phenol removal	[58]
<i>Trametes sanguineus</i>	Laccase enzyme.	High tolerance to tequila vinasse; COD and color reduction.	[59]
<i>Trametes hirsute</i> Bm2	Reusable immobilized laccase.	Sodium alginate immobilization; phenol removal over 4 cycles.	[60]
<i>Fusarium solani</i>	Bioactive and biological extracts	Antioxidant and antibacterial activities from vinasse culture	[61]

3.4. Environmental and Agronomic Integration: Fertigation soil amendments

Despite environmental risks associated with raw vinasse fertigation—such as soil acidification, groundwater ion leaching, and elevated N₂O greenhouse gas emissions [4,11,62]—pre-treating vinasse with fungal consortia modifies its agronomic profile. Soil application of fungal-treated vinasse alters ammonia-oxidizing bacteria (AOB) and denitrifying fungal communities, restoring balanced nitrogen cycling [63].

Roots colonized by endophytic fungi like *Periconia macrospinosa* tolerate heavy metals (Cd, Cu, Zn) in vinasse while solubilizing calcium phosphate and synthesizing indoleacetic acid (IAA) phytohormones that promote root elongation [64]. Furthermore, *Agrocybe aegerita* cultured on vinasse secretes unspecific peroxygenases (UPO) alongside dye-decolorizing peroxidases [65]. Soil amendment experiments using *Aspergillus niger* sp. V2 pre-treated vinasse in wheat (*Triticum aestivum* L.) cultivation show enhanced soil enzyme activities (dehydrogenase, urease, catalase) and improved soil physicochemical health compared to raw vinasse applications [66]. (Table 5)

Table 5. Fungal application in soil fertigation, amendments and root interactions

Fungi species	Agronomic Product	Process & soil response	Reference
<i>Agrocybe aegerita</i>	Unspecific peroxidase (UPO)	Submerged fermentation in 4 liters bioreactor; proteomic expression.	[57]
Unclassified fungi	Denitrifying biomass	Shift in soil AOB and denitrifying community structures	[63]
<i>Periconia macrospinosa</i> (57 strains)	Indoleacetic acid hormone (IAA) & solubilized P	Solubilizes Ca ₃ (PO ₄) ₂ ; heavy metal tolerance (Cd, Cu, Zn)	[64]
<i>Aspergillus niger</i> sp. V2	Biological soil amendment	Pot fertigation on wheat; increased soil dehydrogenase and urease.	[66]

3.5. Materials, biofuels, and food processing.

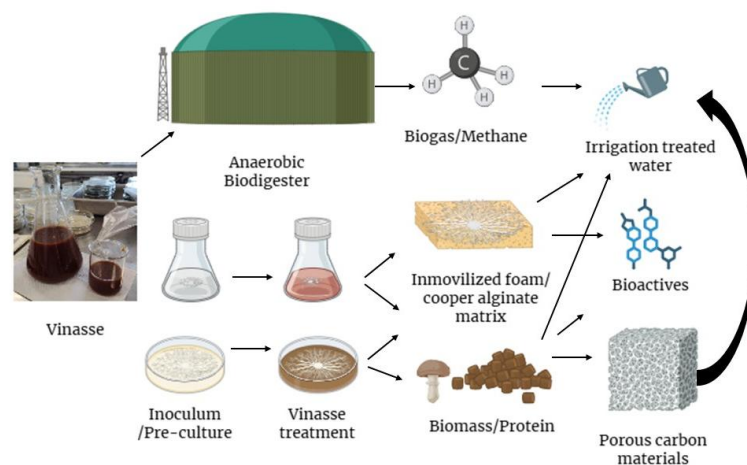
Recent innovations have expanded fungal-vinasse applications into advanced material synthesis and traditional food fermentations. Pyrolyzing residual fungal biomass cultivated on vinasse with inorganic activators (KOH, KHCO₃) yields hierarchical porous carbon materials with ultra-high surface areas suited for volatile organic compound (VOC) adsorption [67]. In food technology, vinasse is incorporated into traditional Asian fish fermentations (e.g., Zaoyu or hairtail processing), where fungal microbiota (*Saccharomyces*, *Rhizopus*) accelerates protein oxidation, flavor development, and amino acid degradation [68,69]. (Table 6)

Table 6. Specialized application of fungal-vinasse bioprocessing in materials and foods

Fungi/residue source	Advance product	Manufacturing/ Fermentation process	Reference
<i>Trichoderma reesei</i> CMAA 1168, <i>Aspergillus niger niger</i> CCT, <i>Saccharomyces cerevisiae</i> PE-2	2G Ethanol production	SSF og bagasse impregnated with vinasse; high sugar yield.	[15]
Pyrolyzed fungal residues + vinasse	Porous carbon materials	Pyrolysis with KOH/KHCO ₃ ; VOC adsorption material.	[67]
<i>Saccharomyces cerevisiae</i>	Fermented hairtail seafood	Accelerates protein degradation and flavor enrichment.	[68]
<i>Rhizopus</i> , <i>Saccharomycopsis</i> , <i>Saccharomyces</i>	Traditional Zaoyu fish	Starters culture fermentation with glutinous rice vinasse	[69]

3.6. Technological bottlenecks in fungal biorefineries

Comparing submerged fermentation (SmF) and solid-state fermentation (SSF) reveals distinct operational trade-offs. SmF using basidiomycetes achieves rapid liquid-phase decolorization and high laccase titers but faces mechanical shear stress and biomass clogging [44,46]. Conversely, SSF using ascomycetes efficiently processes vinasse-impregnated agricultural solids (bagasse) into organic acids and soil conditioners, though mass-transfer limitations impede continuous operation [15,54] (**Error! Reference source not found.**). Scaling these processes introduces four major technological bottlenecks:

**Figure 2.** Current process and products develop from vinasse using fungi

3.6.1. The Immobilization Engineering Bottleneck

Immobilizing filamentous strains (*Trametes* sp. T3, *Pycnoporus* sp. P6) in PUF or alginate matrices protects biomass from oxidative shock in raw vinasse[44,45]. However, high Total Suspended Solids (TSS) and elevated viscosity in raw vinasse cause physical surface fouling, pore clogging, and mass-transfer limitations. In continuous industrial bioreactors, fluid shear forces trigger matrix erosion and premature enzyme denaturation, restricting high laccase titers (>3400 U/mL) to short batch cycles [46].

3.6.2. The Exogenous Carbon Subsidy Paradox

Maximum decolorization and phenolic degradation by white-rot fungi often require supplementing vinasse with external co-substrates, such as glucose [44,55]. Because high-redox ligninolytic enzymes are produced during secondary metabolism, fungi require an easily assimilable carbon source to build baseline biomass before degrading recalcitrant melanoidins. Relying on commercial glucose to treat low-value industrial waste compromises the economic viability of industrial-scale operations[44].

3.6.3. Ecotoxicological Shifts and Nitrogen Accumulation in Fertigation

While fungal-treated vinasse supplies bio-available nutrients and phytohormones [64,66], improper soil loading alters the native soil microbiome. Applying partially treated vinasse stimulates ammonia-oxidizing bacteria (AOB) and fungal denitrifiers, accelerating soil nitrogen turnover and increasing greenhouse gas (N₂O) emission rates [63].

3.6.4. The Fragmented Biorefinery Architecture

Current literature primarily evaluates isolated endpoints—such as laccase production, single-cell protein generation, or porous carbon synthesis—rather than integrated processing streams [56,67]. Achieving circularity requires multi-stage biorefineries where raw vinasse undergoes initial fungal detoxification, harvested enzymes are extracted, and residual nutrient slurry is funneled into anaerobic digestion or biological soil conditioning.

4. Discussion

4.1. Mechanistic role of ligninolytic enzymes in vinasse detoxification

The ability of white-rot basidiomycetes to remediate vinasse relies on an extracellular, non-specific oxidative enzymatic battery comprising Laccases (Lac, EC 1.10.3.2), Manganese Peroxidases (MnP, EC 1.11.1.13), and Lignin Peroxidases (LiP, EC 1.11.1.14) [15,28,46]. Because melanoidins and polyphenols possess non-repeating aromatic backbones that cannot cross fungal cell membranes, oxidation must occur extracellularly via free-radical cascades [16,19].

- Laccases (Lac): Multi-copper oxidases that catalyze the four-electron reduction of molecular oxygen (O₂) to water (H₂O) while extracting electrons from phenolic substrates. Laccases cleave monomeric phenolics (ferulic, vanillic, and p-coumaric acids), reducing immediate phytotoxicity [44,58,60]. However, their redox potential is insufficient to oxidize non-phenolic melanoidin backbones directly unless low-molecular-weight redox mediators (e.g., ABTS, vanillin) are present [46].
- Manganese Peroxidases (MnP) and Lignin Peroxidases (LiP): Heme peroxidases that require hydrogen peroxide (H₂O₂) as an electron acceptor. MnP oxidizes Mn²⁺ to Mn³⁺, which, chelated

by fungal organic acids (e.g., oxalate), acts as a diffusible mediator that penetrates dense melanoidin polymers [46,55]. LiP possesses a high redox potential capable of directly oxidizing non-phenolic aromatic rings, driving chromophore cleavage and effluent decolorization [28,53].

4.2. Genus specific advantages and operational scenarios

Distinct fungal genera offer specific operational strengths:

- Basidiomycetes (*Trametes*, *Pycnoporus*, *Phanerochaete*): Possess complete ligninolytic enzymatic machinery. *Trametes versicolor* and *Pycnoporus* sp. achieve peak laccase titers (3000 U/mL) and superior decolorization (>65%) in raw and digestate vinasses [44–46]. They are suited for liquid-phase detoxification and high-redox enzyme harvesting. However, their slower growth rates and sensitivity to high osmotic pressure require cell immobilization to maintain operational stability [44,60].
- Ascomycetes (*Aspergillus*, *Neurospora*, *Trichoderma*): Lack high-redox LiP enzymes but exhibit high tolerance to raw vinasse acidity (pH 3.5–4.5), osmotic stress, and high salt concentrations [35,36,54]. Their remediation mechanism relies on organic acid secretion, bioleaching, and biosorption rather than complete enzymatic mineralization. Consequently, *Aspergillus niger* and *Trichoderma reesei* excel in producing single-cell proteins and soil conditioning biomass [38,49,66].

4.3. Soil conditioning mechanism: Mycelium as a structural aggregation precursor

Applying harvested fungal biomass or fungal-inoculated vinasse digestate to degraded agricultural land improves physical soil architecture [38,64]. Filamentous hyphae physically weave through soil particles, forming structural networks that promote macroaggregate formation (>250µm). Simultaneously, fungal exudates, including hydrophobic glycoproteins and extracellular polymeric substances (EPS), bind clay and silt particles into water-stable aggregates [64,66]. This increases soil macroporosity, enhances hydraulic conductivity, and reduces surface crusting caused by raw vinasse fertigation [63,66].

4.4. Biosafety constraints, heavy metal profiling and agronomic realities

Integrating fungal bioprocess into commercial agricultural systems requires addressing safety and socio-economics constraints

Biosafety and Heavy Metal Profiling: Filamentous fungi (especially *Aspergillus* species) biosorb heavy metals (Cd^{2+} , Cu^{2+} , Zn^{2+}) present in vinasse due to industrial equipment corrosion and agrochemical runoff [49,64]. Furthermore, nutritional stress during culture fluctuations can trigger mycotoxin synthesis (e.g., aflatoxins, ochratoxins) [35,37]. Comprehensive chromatographic profiling (HPLC-MS) is mandatory to ensure that harvested mycoproteins and soil conditioners meet food and feed safety standards [35,65].

Agronomic and Logistical Constraints in Mexico: In Mexico, the fragmented distribution of farmland (ejidos and smallholder plots) creates severe logistical bottlenecks for liquid vinasse fertigation. Transporting corrosive, high-temperature liquid waste across non-contiguous plots is economically unviable for local producers. Furthermore, applying raw, hyper-osmotic vinasse causes chemical scorching on freshly planted sugarcane (*Saccharum officinarum*) cuttings, inhibiting germination.

The By-Product Market Paradox: Unlike sugarcane bagasse—which is burned in mill boilers for energy—or molasses, which commands a stable market for yeast fermentation, vinasse is treated as an

economic liability. Permissive environmental legislation has historically allowed raw disposal into natural aquatic streams during peak crushing seasons, underscoring the need for mandatory closed-loop treatment standards.

5. Conclusion

Deploying ligninolytic fungi for distillery vinasse treatment offers a sustainable pathway for converting a high-COD, corrosive effluent into industrial enzymes, single-cell protein, and biological soil conditioners. White-rot basidiomycetes (*Trametes*, *Pycnoporus*) degrade recalcitrant melanoidins and phenolic matrices via high-redox extracellular enzymes, while ascomycetes (*Aspergillus*, *Trichoderma*) convert organic carbon into mycoprotein biomass that stabilizes soil macroaggregates.

However, scaling these biological systems requires resolving key operational challenges: mitigating mass-transfer resistance in high-viscosity media, eliminating reliance on expensive carbon co-substrates, and enforcing strict heavy metal and mycotoxin biosafety controls.

Overcoming the logistical and agronomic limitations of raw land disposal requires moving away from single-endpoint treatments. Industrial adoption depends on establishing hybrid biorefineries that couple physical or anaerobic pretreatments with downstream fungal bioprocessing, ensuring complete waste circularization within the bioethanol industry.

Author Contribution

Conceptualization (B.S.S), **Data curation** (B.S.S), **Formal analysis** (B.S.S, F.H.R), **Funding acquisition** (B.S.S), **Investigation** (B.S.S), **Methodology** (B.S.S), **Project administration** (F.H.R), **Resources** (B.S.S), **Software** (B.S.S), **Supervision** (F.H.R), **Validation** (F.H.R), **Visualization** (B.S.S), **Writing – original draft** (B.S.S), **Writing – review & editing** (B.S.S, F.H.R, R.C.L.H).

Funding

The research was supported thanks to Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for the scholarship 62431005 of the student Blas Schettino Salomón to continue doctoral studies at the Innovación Agroalimentaria Sustentable Program at Colegio de Postgraduados Campus Córdoba, Veracruz.

Abbreviations

2G – Second Generation (Bioethanol)
 5-HMF– 5- Hydroxymethylfurfural
 ABTS – 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
 AO – Open Access
 AOB – Ammonia-Oxidizing bacteria
 APC – Article Processing Charges
 BOD – Biological Oxygen Demand
 COD – Chemical Oxygen demand
 DO – Dissolved Oxygen
 EC – Electrical Conductivity
 EPS – Extracellular Polymeric Substances
 HPLC – High- Performance Liquid Chromatography
 HPLC-MS – High-Performance Liquid Chromatography-Mass spectrometry
 IAA – Indoleacetic acid
 JCR – Journal Citation Reports
 LiP. –. Lignin peroxidase (EC 1.11.1.14)

MnP. –. Manganese peroxidase (EC 1.11.1.13)
 OP – Open Access
 PAH. –. Polycyclic aromatic hydrocarbons
 PCB. –. Polychlorinated biphenyls
 PGP – Plant Growth Promoter
 PUF – Polyurethane foam
 ROS – Reactive Oxygen Species
 SCP – Single-Cell Protein
 SmF – Submerged Fermentation
 SSF – Solid-State Fermentation
 TCMR–Traditional Chinese Medicine Residue
 TN – Total Nitrogen
 TOC – Total Organic Carbon
 TP – Total phosphorus
 TSS – Total Dissolved Solids
 UPO – Unspecific peroxigenasa
 VOC – Volatile Organic Compound
 VOS – Visualization of Similarities
 WOS– Web of Science
 WRF – White-Rot-Fungi

References

- [1] Reis CER, Hu B. Vinasse from sugarcane ethanol production: Better treatment or better utilization? *Front Energy Res* 2017;5:260276. <https://doi.org/10.3389/FENRG.2017.00007/BIBTEX>.
- [2] España-Gamboa E, Vicent T, Font X, Dominguez-Maldonado J, Canto-Canché B, Alzate-Gaviria L. Pretreatment of vinasse from the sugar refinery industry under non-sterile conditions by *Trametes versicolor* in a fluidized bed bioreactor and its effect when coupled to an UASB reactor. *J Biol Eng* 2017;11:1–11. <https://doi.org/10.1186/S13036-016-0042-3/FIGURES/4>.
- [3] Santos F, Eichler P, Machado G, De Mattia J, De Souza G. By-products of the sugarcane industry. *Sugarcane Biorefinery, Technology and Perspectives* 2020:21–48. <https://doi.org/10.1016/B978-0-12-814236-3.00002-0>.
- [4] Christofoletti CA, Escher JP, Correia JE, Marinho JFU, Fontanetti CS. Sugarcane vinasse: Environmental implications of its use. *Waste Management* 2013;33:2752–61. <https://doi.org/10.1016/J.WASMAN.2013.09.005>.
- [5] Montiel-Rosales A, Montalvo-Romero N, García-Santamaría LE, Sandoval-Herazo LC, Bautista-Santos H, Fernández-Lambert G. Post-Industrial Use of Sugarcane Ethanol Vinasse: A Systematic Review. *Sustainability* 2022, Vol 14, Page 11635 2022;14:11635. <https://doi.org/10.3390/SU141811635>.
- [6] España-Gamboa E, Mijangos-Cortes J, Barahona-Perez L, Dominguez-Maldonado J, Hernández-Zarate G, Alzate-Gaviria L. Vinasses: Characterization and treatments. *Waste Management and Research* 2011;29:1235–50. <https://doi.org/10.1177/0734242X10387313;WEBSITE:WEBSITE:SAGE;REQUESTEDJOURNAL:JOURNAL:WMRA;WGROU:STRING:PUBLICATION>.
- [7] Satyawali Y, Balakrishnan M. Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *J Environ Manage* 2008;86:481–97.

- <https://doi.org/10.1016/j.jenvman.2006.12.024>.
- [8] Robles-González V, Galíndez-Mayer J, Rinderknecht-Seijas N, Poggi-Varaldo HM. Treatment of mezcal vinasses: A review. *J Biotechnol* 2012;157:524–46. <https://doi.org/10.1016/J.JBIOTEC.2011.09.006>.
- [9] Govea-Paz Y, Arellano-García L, Aguilar-Juárez Ó. Simultaneous hydrogen and methane production by tequila vinasses dark fermentation in series with anaerobic digestion. *Bioresour Technol Rep* 2024;25:101779. <https://doi.org/10.1016/J.BITEB.2024.101779>.
- [10] Moraes BS, Junqueira TL, Pavanello LG, Cavalett O, Mantelatto PE, Bonomi A, Zaiat M. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: Profit or expense? *Appl Energy* 2014;113:825–35. <https://doi.org/10.1016/j.apenergy.2013.07.018>.
- [11] Fraga PD, de Mello Gabriel GV, do Carmo JB, Espindola ELG, Pinto TJ da S. Sugarcane vinasse provokes acute and chronic responses and bioaccumulation of metals in benthic macroinvertebrates. *Environ Sci Pollut Res Int* 2024;31:4067–79. <https://doi.org/10.1007/S11356-023-31446-Z/TABLES/4>.
- [12] Galdos M V., Soares JR, Lourenço KS, Harris P, Zeri M, Cunha-Zeri G, Vargas VP, Degaspari EAM. Multi-experiment assessment of soil nitrous oxide emissions in sugarcane. *Nutr Cycl Agroecosyst* 2023;127:375–92. <https://doi.org/10.1007/s10705-023-10321-w>.
- [13] Fuess LT, Garcia ML. Implications of stillage land disposal: A critical review on the impacts of fertigation. *J Environ Manage* 2014;145:210–29. <https://doi.org/10.1016/J.JENVMAN.2014.07.003>.
- [14] Olguín EJ, Sánchez-Galván G, González-Portela RE, López-Vela M. Constructed wetland mesocosms for the treatment of diluted sugarcane molasses stillage from ethanol production using *Pontederia sagittata*. *Water Res* 2008;42:3659–66. <https://doi.org/10.1016/J.WATRES.2008.05.015>.
- [15] Brito Codato C, Gaspar Bastos R, Ceccato-Antonini SR. Sequential process of solid-state cultivation with fungal consortium and ethanol fermentation by *Saccharomyces cerevisiae* from sugarcane bagasse. *Bioprocess Biosyst Eng* 2021;44:1–8. <https://doi.org/10.1007/s00449-021-02588-6>.
- [16] Li Y, Zhang Q, Xiao S, Yang Q, Wang L, Hao J. Review of Melanoidins as By-Product from Thermal Hydrolysis of Sludge: Properties, Hazards, and Removal. *Processes* 2024, Vol 12, Page 135 2024;12:135. <https://doi.org/10.3390/PR12010135>.
- [17] Fuess LT, Rodrigues IJ, Garcia ML. Fertirrigation with sugarcane vinasse: Foreseeing potential impacts on soil and water resources through vinasse characterization. *Journal of Environmental Science and Health, Part A* 2017;52:1063–72. <https://doi.org/10.1080/10934529.2017.1338892>.
- [18] de Godoi LAG, Camiloti PR, Bernardes AN, Sanchez BLS, Torres APR, da Conceição Gomes A, Bottas L. Seasonal variation of the organic and inorganic composition of sugarcane vinasse: main implications for its environmental uses. *Environmental Science and Pollution Research* 2019;26:29267–82. <https://doi.org/10.1007/S11356-019-06019-8/FIGURES/3>.
- [19] Bödeker ITM, Clemmensen KE, de Boer W, Martin F, Olson Å, Lindahl BD. Ectomycorrhizal Cortinari species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist* 2014;203:245–56. <https://doi.org/10.1111/NPH.12791>.
- [20] Harley JL. Fungi in Ecosystems. *J Appl Ecol* 1971;8:627. <https://doi.org/10.2307/2402673>.
- [21] Li T, Cui L, Song X, Cui X, Wei Y, Tang L, Yanhong M, Zhihong X. Wood decay fungi: an analysis of worldwide research. *J Soils Sediments* 2022;22:1688–702.

- <https://doi.org/10.1007/S11368-022-03225-9/TABLES/4>.
- [22] Alshammari N, Ameen F, Alkahtani MDF, Stephenson S. Characterizing the assemblage of wood-decay fungi in the forests of northwest Arkansas. *Journal of Fungi* 2021;7:309. <https://doi.org/10.3390/JOF7040309/S1>.
- [23] Procópio L, Barreto C. The soil microbiomes of the Brazilian Cerrado. *J Soils Sediments* 2021;21:2327–42. <https://doi.org/10.1007/S11368-021-02936-9/TABLES/1>.
- [24] Blanchette RA. Delignification by wood-decay fungi. *Annual Review of Phytopathology* Vol 29 1991;29:381–98. <https://doi.org/10.1146/ANNUREV.PY.29.090191.002121/CITE/REFWORKS>.
- [25] Conesa A, Punt PJ, Van Den Hondel CAMJJ. Fungal peroxidases: Molecular aspects and applications. *J Biotechnol* 2002;93:143–58. [https://doi.org/10.1016/S0168-1656\(01\)00394-7](https://doi.org/10.1016/S0168-1656(01)00394-7).
- [26] Datta R, Kelkar A, Baraniya D, Molaei A, Moulick A, Meena RS, Formanek P. Enzymatic Degradation of Lignin in Soil: A Review. *Sustainability* 2017, Vol 9, Page 1163 2017;9:1163. <https://doi.org/10.3390/SU9071163>.
- [27] Saldarriaga-Hernández S, Velasco-Ayala C, Leal-Isla Flores P, de Jesús Rostro-Alanis M, Parra-Saldivar R, Iqbal HMN, Carrillo-Nieves D . Biotransformation of lignocellulosic biomass into industrially relevant products with the aid of fungi-derived lignocellulolytic enzymes. *Int J Biol Macromol* 2020;161:1099–116. <https://doi.org/10.1016/J.IJBIOMAC.2020.06.047>.
- [28] Ningombam L, Mana T, Pradhan S, Apum G, Singh YD. Fungal bioremediation in environmental pollution and recent strategies. *Discover Environment* 2025 3:1 2025;3:100-. <https://doi.org/10.1007/S44274-025-00267-X>.
- [29] Kinnunen A, Majjala P, Jarvinen P, Hatakka A. Improved Efficiency in Screening for Lignin-Modifying Peroxidases and Laccases of Basidiomycetes. *Curr Biotechnol* 2017;6:105–15. <https://doi.org/10.2174/2211550105666160330205138>.
- [30] Rajhans G, Sen SK, Barik A, Raut S. Ligninolytic enzyme system of white-rot fungi: a natural approach to bioremediation and detoxification of azo dyes in textile wastewater. *Environ Eng Manag J* 2020;19:1983–2002. <https://doi.org/10.30638/eemj.2020.188>.
- [31] Zhuo R, Fan F. A comprehensive insight into the application of white rot fungi and their lignocellulolytic enzymes in the removal of organic pollutants. *Science of The Total Environment* 2021;778:146132. <https://doi.org/10.1016/J.SCITOTENV.2021.146132>.
- [32] Fayeulle A, Veignie E, Schroll R, Munch JC, Rafin C. PAH biodegradation by telluric saprotrophic fungi isolated from aged PAH-contaminated soils in mineral medium and historically contaminated soil microcosms. *J Soils Sediments* 2019;19:3056–67. <https://doi.org/10.1007/S11368-019-02312-8/FIGURES/3>.
- [33] Chapman J, Ismail AE, Dinu CZ. Industrial Applications of Enzymes: Recent Advances, Techniques, and Outlooks. *Catalysts* 2018, Vol 8, Page 238 2018;8:238. <https://doi.org/10.3390/CATAL8060238>.
- [34] Gaudhaman A, Karimi S, Lundh T, Øverland M, Taherzadeh MJ, Langeland M, Baruah K, Vidakovic A. Fungal Protein from Non-Food Bioresources in Diets for Rainbow Trout (*Oncorhynchus mykiss*). *Fishes* 2025;10:149. <https://doi.org/10.3390/FISHES10040149/S1>.
- [35] Rulli MM, Villegas LB, Barcia CS, Colin VL. Bioconversion of sugarcane vinasse into fungal biomass protein and its potential use in fish farming. *J Environ Chem Eng* 2021;9:106136. <https://doi.org/10.1016/j.jece.2021.106136>.
- [36] Karimi S, Agnihotri S, Ferreira JA, Taherzadeh MJ. Evaluating three fungal biomasses grown on diluted thin stillage as potential fish feed ingredients. *Bioresour Technol Rep* 2023;24:101677.

- <https://doi.org/10.1016/j.biteb.2023.101677>.
- [37] Del Gobbo LM, Iglesias JMP, Almeida CA, Villegas LB, Colin VL. Conversion of sugarcane vinasse into biomass of *Aspergillus* sp. V1 and its potential application as a fish feed ingredient. *Process Safety and Environmental Protection* 2023;177:391–9. <https://doi.org/10.1016/j.psep.2023.06.086>.
- [38] Torres MA, Valdez AL, Angelicola MV, Raimondo EE, Pajot HF, Nieto-Peñalver CG. Vinasse as a substrate for inoculant culture and soil fertigation: Advancing the circular and green economy. *Science of The Total Environment* 2023;887:164014. <https://doi.org/10.1016/J.SCITOTENV.2023.164014>.
- [39] Du P, Yin Y, Zhou H, Zhao H, Yang H, Cao T. Domesticated cultivation, nutrient composition and antioxidant activities of *Oudemansiella raphanipes*. *Mycosystema* 2022;41:1471–82. <https://doi.org/10.13346/j.mycosystema.220003>.
- [40] Hoxha L, Wang R, Taherzadeh MJ, Undeland I. In vitro protein digestion and mineral accessibility of edible filamentous fungi cultivated on winery and distillery by-products. *Food Biosci* 2025;73:107711. <https://doi.org/10.1016/j.fbio.2025.107711>.
- [41] Ramos-Reyes MF, Padilla-Alatorre F, Díaz-Vazquez D, González-López ME, Barajas-Álvarez P, Tuesta-Popolizio DA, Musatto S, Gradilla-Hernández M. Yeast-filamentous fungi co-culture: A circular bioeconomy approach for distillation stillage treatment and revalorization. *Sustain Chem Pharm* 2025;46:102053. <https://doi.org/10.1016/j.scp.2025.102053>.
- [42] Pointing SB. Feasibility of bioremediation by white-rot fungi. *Appl Microbiol Biotechnol* 2001;57:20–33. <https://doi.org/10.1007/S002530100745>.
- [43] Torres-Farradá G, Thijs S, Rineau F, Guerra G, Vangronsveld J. White Rot Fungi as Tools for the Bioremediation of Xenobiotics: A Review. *Journal of Fungi* 2024, Vol 10, Page 167 2024;10:167. <https://doi.org/10.3390/JOF10030167>.
- [44] Ahmed PM, Nieto-Peñalver CG, de Figueroa LIC, Pajot HF. Vinasse odyssey: sugarcane vinasse remediation and laccase production by *Trametes* sp. immobilized in polyurethane foam. *Biodegradation* 2022;33:333–48. <https://doi.org/10.1007/S10532-022-09985-Y/TABLES/4>.
- [45] Farfán C, Lozano VL, Borja CN, Alvarez Dalinger F, Muñoz C, Moraña L. Utilization of the Fungus *Pycnoporus* sp. for Remediation of a Sugarcane Industry Effluent. *Sustainability* 2024;16:7020. <https://doi.org/10.3390/su16167020>.
- [46] Whitaker S, España-Gamboa E, Chablé-Villacis R, Alzate-Gaviria ; L, Dominguez-Maldonado ; J, Leal-Baustista ; R M, Soberanis-Monforte G, Tapia-Tussell R. Native fungal strains from Yucatan, an option for treatment of biometanated vinasse Cepas fúngicas nativas de Yucatán, una opción para el tratamiento de vinazas biometanada. *Rev Mex Ing Quim* 2021;20:607–20. <https://doi.org/10.24275/rmiq/IA2063>.
- [47] Ramírez-Ramírez AA, Sulbarán-Rangel BC, Jáuregui-Rincón J, Lozano-Álvarez JA, la Torre JAF, Zurita-Martínez F. Preliminary Evaluation of Three Species of Ligninolytic Fungi for Their Possible Incorporation in Vertical Flow Treatment Wetlands for the Treatment of Tequila Vinasse. *Water Air Soil Pollut* 2021;232:456. <https://doi.org/10.1007/s11270-021-05412-9>.
- [48] Ramírez-Ramírez AA, Lozano-Álvarez JA, Gutiérrez-Lomelí M, Zurita F. Tequila Vinasse Treatment in Two Types of Vertical Downflow Treatment Wetlands (with Emergent Vegetation and Ligninolytic Fungi). *Water (Basel)* 2024;16:1778. <https://doi.org/10.3390/w16131778>.
- [49] Alavi N, Partovi K, Majlessi M, Rashidi M, Alimohammadi M. Bioleaching of metals from cellphones batteries by a co-fungus medium in presence of carbon materials. *Bioresour Technol Rep* 2021;15:100768. <https://doi.org/10.1016/j.biteb.2021.100768>.

- [50] Candido JP, Almeida ÉC, de Oliveira Leite DN, Brienzo M, de Franceschi de Angelis D. Vinasse from sugarcane bagasse (hemicellulose) acid hydrolysate and molasses supplemented: biodegradability and toxicity. *Ecotoxicology* 2021;30:818–27. <https://doi.org/10.1007/s10646-021-02401-w>.
- [51] Farfán C, Lozano VL, Borja CN, Alvarez Dalinger F, Muñoz C, Moraña L. Utilization of the Fungus *Pycnoporus* sp. for Remediation of a Sugarcane Industry Effluent. *Sustainability (Switzerland)* 2024;16:7020. <https://doi.org/10.3390/su16167020>.
- [52] Saldarriaga-Hernandez S, García-Béjar J, Esparza-Vasquez A, González-Díaz RL, López-Torres EJ, López-Velázquez JC, Amaya-Delgado L, García-Cayuela T, Choudhary H, Simmons B, Carrillo-Nieves D. Integrated Biorefinery of Brewer's Spent Grain for Second-Generation Ethanol, Mycoprotein, and Bioactive Vinasse Production. *Fermentation* 2025;11:627. <https://doi.org/10.3390/fermentation11110627>.
- [53] Kumar A, Chandra R. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon* 2020;6:e03170. <https://doi.org/10.1016/J.HELİYON.2020.E03170>.
- [54] Rocha LM, Campanhol BS, Bastos RG. Solid-State Cultivation of *Aspergillus niger*–*Trichoderma reesei* from Sugarcane Bagasse with Vinasse in Bench Packed-Bed Column Bioreactor. *Appl Biochem Biotechnol* 2021;193:2983–92. <https://doi.org/10.1007/s12010-021-03579-9>.
- [55] Jayasekara SK, Madusanka TGY, Rupasinghe CP, Weerasinghe HAS, Abayasekara CL, Seneweera S, Ratnaye R. Bagasse and vinasse, factory wastes from sugarcane industry as potential substrates for bioethanol production. *J Natl Sci Found* 2021;49:169. <https://doi.org/10.4038/jnsfsr.v49i2.9776>.
- [56] Bailón-Salas AM, Ordaz-Díaz LA, Domínguez-Calleros PA. *Agave durangensis* vinasse as a biocide for forest pest control. *Bioresources* 2022;17:1285–300. <https://doi.org/10.15376/biores.17.1.1285-1300>.
- [57] Li N, Yang X, Liu J, Liu Y, Chen Q, Wu F, et al. Effect of raw material and application rate of biogas slurry on Cucumber growth, *Fusarium* wilt suppression, and soil properties. *Environ Technol Innov* 2023;32:103396. <https://doi.org/10.1016/j.eti.2023.103396>.
- [58] Caballero RE, Jiménez V, Miranda M, Rovira D, Branda G, de Perez JRC. An Optimal Culture Medium for Laccase Production and Sugar Cane Vinasse Biotreatment with *Trametes villosa* Using Plackett-Burman and Central Composite Designs. *Applied Environmental Research* 2024;46. <https://doi.org/10.35762/AER.2024001>.
- [59] Teymennet-Ramírez KV, García-Morales S, Hernández-Fernández O, Barrera-Martínez I. Detoxification of tequila vinasse by *Trametes sanguineus*: a biotechnological approach to laccase production and water reuse in seedling growth. *Environmental Science and Pollution Research* 2025;32:30087–98. <https://doi.org/10.1007/s11356-025-36377-5>.
- [60] Hernández-Calderón C, Tapia-Tussell R, Lizama-Uc G, Rivera-Muñoz G, Magaña-Ortiz D, Solís-Pereira S. Immobilization of a crude extract of laccase from *T. hirsute* Bm2 on copper alginate for environmental vinasse remediation. *Rev Mex Ing Quim* 2023;22:1–13. <https://doi.org/10.24275/rmiq/Bio3037>.
- [61] Yousef R, Basuiony M, Ahmed S. Valorization of sugarcane by-products for valuable compounds production using *Fusarium solani*. *Egypt J Chem* 2024;0:0–0. <https://doi.org/10.21608/ejchem.2024.272306.9382>.
- [62] Galdos M V., Soares JR, Lourenço KS, Harris P, Zeri M, Cunha-Zeri G, Vargas V, Degaspari I,

- Cantarella H. Multi-experiment assessment of soil nitrous oxide emissions in sugarcane. *Nutr Cycl Agroecosyst* 2023;127:375–92. <https://doi.org/10.1007/s10705-023-10321-w>.
- [63] Lourenço KS, Costa OY de A, Cantarella H, Kuramae EE. Ammonia-oxidizing bacteria and fungal denitrifier diversity are associated with N₂O production in tropical soils. *Soil Biol Biochem* 2022;166:108563. <https://doi.org/10.1016/j.soilbio.2022.108563>.
- [64] Silva LC, Saggin-Júnior OJ, Carneiro Mac, Silva EMR Da, Zilli JE, Berbara Rll. Characterization of dark septate endophytic fungi *Periconia macrospinosa* isolated from roots of sugarcane in São Paulo, Brazil. *An Acad Bras Cienc* 2024;96:e20231367. <https://doi.org/10.1590/0001-3765202420231367>.
- [65] González-Rodríguez S, Trueba-Santiso A, Lu-Chau TA, Moreira MT, Eibes G. Valorization of bioethanol by-products to produce unspecific peroxygenase with *Agrocybe aegerita*: Technological and proteomic perspectives. *N Biotechnol* 2023;76:63–71. <https://doi.org/10.1016/j.nbt.2023.05.001>.
- [66] Rulli MM, Del Gobbo LM, Aparicio JD, Colin VL. Short-term effect of biologically treated sugarcane vinasse on the growth of *Triticum aestivum* L. (wheat) and soil properties. *J Biotechnol* 2025;404:1–8. <https://doi.org/10.1016/j.jbiotec.2025.03.020>.
- [67] Yu H, Lin F, Li K, Wang W, Yan B, Song Y, Cheng G. Triple combination of natural microbial action, etching, and gas foaming to synthesize hierarchical porous carbon for efficient adsorption of VOCs. *Environ Res* 2021;202:111687. <https://doi.org/10.1016/j.envres.2021.111687>.
- [68] Zhang Y, Tu C, Lin H, Hu Y, Jia J, Shui S, Wang J, Hu Y, Zhang B. Changes in Physicochemical Characteristics and Microbial Diversity of Traditional Fermented Vinasse Hairtail. *Fermentation* 2023;9:173. <https://doi.org/10.3390/fermentation9020173>.
- [69] Nie J, Zhu S, Zhang X, Wu D, Li X, Huang Q. Effects of starters on the quality of fermented fish (Zaoyu): Key microorganisms for coloring, softening, and improving flavor. *Food Chem* 2025;465:142087. <https://doi.org/10.1016/j.foodchem.2024.142087>.