Renewable and Sustainable Energy Reviews 213 (2025) 115456

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Fermentation of sorghum with *Aspergillus* strains: A promising and sustainable pathway to enzyme production- comprehensive review

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A B S T R A C T

The main objective of this article is to explore the utilization of sorghum as a potential substrate to produce valuable enzymes using Aspergillus strains. It focuses on two key aspects: (i) the environmental and economic sustainability of enzyme production from sorghum ii. enhancing enzymes and biofuel production through process and host cell optimization. A comparative study is conducted among sorghum, wheat, and corn to understand the current state of knowledge and research gap on large-scale enzyme production. Sorghum is an adaptable crop with all types of environments and is overall more sustainable than wheat and corn. With its rich composition of starch (60%-75%), lignin (11%-25%), hemicellulose (18%-25%), and cellulose (25%-45%), sorghum represents itself an excellent candidate for the enzyme, and also first and second-generation biofuel production. The advantages and associated challenges of the Aspergillus strains are then discussed for enzyme production. It highlights the development of an integrated process for enzyme and bioethanol production at a low cost without relying on external carbon and nitrogen sources through an eco-friendly and economically viable approach.

1. Introduction

Fungal fermentation has recently gained notable attention as an economical, eco-friendly, and sustainable method for manufacturing enzymes. The increasing demand for fuel and enzymes due to technological advancements and industrialization has become crucial in various industries. With the depletion of fossil fuels and the growing necessity of enzymes in industrial sectors such as pharmaceuticals, food, leather, textile, and biofuel, there is an urgent need to explore alternative, environmentally friendly, and cost-effective energy production methods. Enzymes are integral in energy production, requiring the research and development of low-cost enzyme production using sustainable technology. The contemporary approach to producing enzymes involves employing diverse microorganisms like fungal and bacterial strains, along with using multiple biomass sources such as aquatic plants, agricultural crops (sugarcane, wheat, rice, sorghum, rye, barley, etc.), forestry wastes, and their by-products, which are well-recognized as appropriate alternate resources to produce biofuels, biochemicals, and enzymes [1–3]. Enhancing fermentation's physical and chemical conditions and identifying the most suitable fungal and bacterial strains makes it feasible to augment enzyme production in contrast to conventional techniques. This review will mainly focus on enzyme production using *Aspergillus* strains and a comparative study of sorghum as a substrate for fungal fermentation with other substrates.

The novelty of this review is in the exploration of sorghum as a sustainable substrate for enzyme production, considering its genetic diversity, economic feasibility, and logistical challenges, which is a relatively new and emerging research area. In particular, this review narrows down to the specific combination of sorghum and *Aspergillus* strains, providing a focused and in-depth analysis and comparison with other cereals crops. This study also covers the critical aspects of fungal fermentation on sorghum, including advantages, challenges, research gaps, process optimization, host cell modification to enhance enzyme production ability, and the enzymatic potential of various *Aspergillus* species. In addition, this study highlights the development of an integrated method for low-cost enzyme and biofuel (first and second generation) production without relying on external carbon and nutrient sources through an eco-friendly and economically viable approach that aligns with sustainable development goals number seven (SDGs 7).

2. Methodological framework

This study is focused on enzyme production from sorghum using fungal fermentation in a sustainable and cost-effective method for industrial purposes. Two key aspects are considered: i. the agro-economic

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https://doi.org/10.1016/j.rser.2025.115456
Received 10 August 2024; Received in revised form 28 January 2025; Accepted 29 January 2025
Available online 9 February 2025

Abbreviations

SSF	Solid state fermentation
SmF	Submerged fermentation
FAN	Free amino nitrogen
TRS	Total reducing sugar
PSD	Particle size distribution
GRAS	Generally recognized as safe
SDG	Sustainable development goal
ANN	Artificial neural network
RSM	Response surface methodology
CRISPR	Clustered regularly interspaced short palindromic repeats

importance and suitability of sorghum for enzyme and biofuel production, and ii. enhancement of enzyme production through optimization processes using *Aspergillus* strains. To create the database, this study reviewed journals indexed by Scopus, Web of Science, and relevant patents. This approach led us to establish the following research objectives: (1) evaluate agro-industrial methods for enzyme production and (2) understand optimization and integrated processes for enzyme and biofuel production. We implemented a search approach based on Boolean logic, employing the subsequent keywords: Sorghum, *Aspergillus* and enzyme, wheat and enzyme, corn and enzyme, solid-state, liquid-state fermentation and *Aspergillus*, media optimization, and host cell modification to enhance enzyme production. The data analysis was conducted using the information obtained from each existing category and its respective subcategories.

3. Agro-economic importance of sorghum for enzyme production

Exploring sorghum as an alternative, renewable, and low-cost substrate for fungal fermentation could be a promising approach to producing affordable, reliable, sustainable, and modern energy, aligning with sustainable development goal number 7 (SDG7) [4]. Sorghum (*Sorghum bicolour* L) is an ancient crop with a promising future as a multipurpose crop suited to the challenging growth conditions that climate change will bring. Sorghum, with a crop duration of 4 months, requires less water (8 ML/ha) and yields 45–65 t/ha, making it a highly efficient crop [5–7]. In comparison, sugarcane needs 12–13 months to grow, consumes 36 ML/ha of water, and yields 5–12 t/ha, and corn, has the shortest crop duration of 3–4 months like sorghum but a high water requirement (12 ML/ha) and yields 5–10 t/ha [6,7]. Regarding economic viability, sorghum emerges as a cost-effective and economically competitive feedstock option compared to corn, cassava, and sweet potato, with a lower total capital investment (TCI) of 89 USD, comparable annual operating cost (AOC) of 112 USD, and a total return (TR) of 142 USD, highlighting its favorable economic importance in the feedstock industry [8] (Table 1).

Due to its extensive genetic diversity and relatively recent domestication, sorghum has excellent potential for further improvement. This crop can serve as a model system for other grass species, particularly in abiotic and biotic stress responses, plant-microbiome interactions, and evolution [9]. Sorghum bicolour comprises five primary varieties—bicolour, kafir, guinea, caudatum, and durra—each contributing unique traits to sorghum breeding [10]. Bicolour is valued for its adaptability and grain quality, kafir for its high yield and environmental stress resistance, and guinea for its mold resistance and importance in forage production [11]. Caudatum excels in humid, lowland regions with high yield potential, while durra is notable for its drought and pest resistance, making it thrive in arid areas [12]. For enzyme and biofuel production, selecting sorghum varieties with high biomass and optimal amylose-to-amylopectin ratios can enhance enzyme production and boost second-generation biofuel yields for industrial applications [13]. Amylose enhances starch stability and retrogradation, supporting enzyme activity, while amylopectin's greater solubility and faster digestibility promote more efficient enzymatic breakdown [13]. Table 2 outlines the amylose and amylopectin percentages in different sorghum varieties from various global regions. The amylose content of sorghum starch varies based on the variety and grain color, with red and brown varieties typically having higher amylose content (up to 30 %) compared to white varieties (20 %–25 %) [14,15]. However, factors like sorghum genotype, growing conditions, and processing methods can cause significant variation, with some white sorghum genotypes reaching up to 35 % amylose [16]. High temperatures during grain filling and other environmental factors can also influence amylose levels across different sorghum varieties [17].

The widespread appeal of this crop stems from its i. versatility in various applications such as human consumption, animal feed, biofuel production, and forage, ii. high returns, iii. greater resistance to unfavorable environmental conditions compared to numerous other cereal crops and iv. ability to thrive even in regions with limited water and challenging temperature conditions, particularly in marginal areas [24–27]. Remarkably, sorghum was the second cereal crop to undergo sequencing and, combined with a robust germplasm collection, provided a wide range of genetic opportunities for developing sorghums tailored to various purposes and renewable uses [28]. By utilizing genetic engineering approaches such as gene editing (CRISPR), gene silencing (RNAi), and hybridization techniques (protoplast fusion, nucleic acid fusion), it is possible to target gene expression or suppression, regulate metabolic pathways and develop sorghum varieties with traits like drought resistance, increased grain yield, and improved starch digestibility, facilitating enzyme production and easier saccharification [29]. Mason and Botella [30] and Macelline et al. [31] reported that silencing the *GS3* gene, which encodes a G-protein gamma-subunit known as a negative regulator of grain size, resulted in transgenic sorghums with 79.3 % higher total amino acid concentration than commercial varieties. Additionally, Gao et al. [32] transformed sorghum with the *tlp* gene, encoding thaumatin-like protein, to impart resistance to fungal infections and improve drought tolerance. De Alencar Figueiredo et al. [33] reported that the *Wx* gene plays a crucial role in

Table 1
Economic and environmental sustainability comparison.

Feedstock	Economical Comparison			Environmental Sustainability				Reference
	TCI (USD)	AOC (USD)	TR (USD)	Carbon- dioxide emission (%)	Sulfuric acid contribution (AP, %)	Ammonia emission (AP, %)	Global warming potential[a]	
Sorghum	89	112	142	46.70	0	51.90	0.2099	[8]
Corn	94	103	144	50.89	0	55.89	0.2067	
Cassava	110	34	120	66.93	87.71	7.44	0.2452	
Sweet Potato	88	100	142	85.19	48.77	26.85	2.5261	

[Note: TCI: Total capital investment; AOC: Annual operating cost; TR: Total return; AP: Acidification potential].
[a] Global warming potential: overall greenhouse gas emissions per kg of ethanol production.

Table 2
Composition of amylose, amylopectin, and protein in different sorghum varieties.

Sorghum variety	Region	Amylopectin (%)	Amylose (%)	Protein (%)	References
Liaoza 19	China	68.12	22.12	0.89	Haziman et al. [18]
Jiza 127	China	63.08	26.90	0.87	Htet et al. [19]
Jianxian	China	75.95	20.47	0.80	Htet et al. [19]
Jinnuo 3	China	76.66	8.60	1.20	Htet et al. [19]
KD-4	Indonesia	50.5–51.44	26.62–28.16	8.60–9.31	Haziman et al. [18]
Genjah	Indonesia	81.38	18.62	3.06	Sitanggang et al. [20]
Numbu	Indonesia	77.52	22.48	4.21	Haziman et al. [18], Sitanggang et al. [20]
Waxy2	Mexico	59.87	9.62	12.50	Chuck-Hernández et al. [21]
RR1	Mexico	33.93	32.69	10.90	Chuck-Hernández et al. [21]
WR1	Mexico	40.66	25.33	14.50	Chuck-Hernández et al. [21]
PAN 606	South Africa	75.6	5.6	11.4	Mezgebe et al. [22]
Yaga 2	Africa	44.93	19.76	5.87	Bazié et al. [23]

amylose synthesis, and genetic modifications targeting the PAMP-triggered immunity (PTI) pathway could be used to produce sorghum starch with desired amylose content, improving enzyme production. Moreover, the brown midrib (*bmr*) mutation, which decreases lignin content and alters lignin composition, has proven valuable for enhancing biomass processing [34]. *Sh2* and *Bt2* are two essential genes in sorghum, influencing grain yield and composition, while a mutation in the starch synthases *(SSSIIa)* gene decreases starch gelatinization temperature by 10 °C , facilitating starch breakdown [13,33].

The most challenging aspect of sorghum-based biorefineries is the hydrolysis of starch and lignocellulosic materials to convert them into simple sugars for enzyme and biofuel production, often requiring costly commercial enzymes, acids, and heat treatments, which increase production costs. Using genetically modified or transgenic varieties that overexpress the *SSSIII* and *GBSSI* genes can increase the proportion of long starch chains, enhancing retrogradation and digestibility. These genetically modified varieties are easily digestible through fungal fermentation, eliminating the need for commercial enzymes, heat, and chemical treatments for saccharification, thereby reducing costs and making the process more environmentally friendly.

3.1. Economic feasibility study of sorghum and enzyme

There is a substantial market demand for sorghum and enzymes across various regions worldwide, driven by their applications in sustainable agriculture and industrial processes. This demand highlights significant growth opportunities for sorghum-based enzyme production, especially in regions prioritizing eco-friendly and cost-effective solutions. Table 3 presents an analysis of the economic feasibility, yield, and global market dynamics of sorghum and enzyme production utilizing

Table 3
Economic feasibility of sorghum and enzyme production.

a. Sorghum yield and profit estimation among different regions of the world

Raw materials	Country	Yield (2024–25) kg/ha	TPC USD/ha	TPC per kg (USD/kg)[a]	Price (USD/kg)	Profit per kg (USD/kg)[b]	Profit per ha (USD/ha)[c]	References
Sorghum grain	Australia	4000	314.34	0.078	0.23	0.152	608.2	www.dpi.nsw.gov.au
	USA	3600	201.23	0.06	0.189	0.129	464.4	www.ipad.fas.usda.gov; USDA*
	India	1300	299.18	0.23	0.272	0.042	54.6	[36,37]
	Brazil	3100	162.29	0.053	0.30	0.247	765.7	[38], USDA*
	China	6750	1337.69	0.198	0.25	0.052	351	USDA*
	Nigeria	1230	103.96	0.085	0.77	0.685	842.55	[39]
	Mexico	3400	200.0	0.06	1.75	1.69	5746	USDA*
	Hungary	5050	504.0	0.19	0.34	0.15	757.5	[40], USDA*

b. Profit estimation of enzymes using sorghum as a substrate

Enzyme	**Fungal strain**	**Max yield (U/g)**	**Enzyme production (U/kg)**	**Production cost (based on 0.24 $/1000U) (USD/kg)**	**selling price (USD/kg)**	**Estimated profit USD per 1 kg Batch (USD/kg)[d]**	**References**
β-glucosidase	*A. niger*	54.9	54,900	13.176	281,420	281405.1	[4]
β-xylosidase	*A. niger*	64.88	64,880	15.58	253,950	253,932.67	[39]
Xylanase	*T. lanuginosus*	10145	10,145,000	2434.8	10,900	8463.45	[4,35]
α-Amylase	*Trichoderma* sp.	258	258,000	61.92	5500	5436.3	[35,41]
Glucoamylase	*Trichoderma* sp.	84	84,000	20.16	95,000	94978.1	[35,41]

Note: The price of sorghum grain is estimated as per the data of October 2024 among different regions of the world, USDA: U.S. Department of Agriculture; TPC: total production cost; kg: kilogram; ha: hectare.

[a] Total Production Cost in USD/kg: $\frac{\text{TPC}\left[\frac{\text{USD}}{\text{ha}}\right]}{\text{Yield}\left[\frac{\text{kg}}{\text{ha}}\right]}$

[b] Profit per kg: Price per kg- TPC per kg.

[c] Profit per hectare: Profit per kg X Yield (kg/ha).

[d] Estimated profit of enzyme in USD/kg: Selling price (USD/kg) – (Production cost (USD/kg) + raw sorghum wholesale price (USD/kg)). Raw sorghum wholesale price was assumed to be 1.75 USD/kg.

Table 4
Comparison of polyphenols content from various cereal grains.

Cereals (Total polyphenols)	[50] (mg/100g dw)	[51] (mgGAE[a]/g)	[52] (mg/100g)	[49] (mg/100g)	[53][1] [54][2] [55][3] (mg GAE[a]/g)
Sorghum	10.26–170	1.0	175.75	43.1	18.22[1]
Wheat	22–40	3.9 (Bran)	164.49	20.5	3.9[2]
Barley	1.2–1.5	0.5	–	16.4	0.5[2]
Rice	8.6	0.2	–	2.51	0.5[3]
Maize	30.9	1.7	226.6	2.91	3.4[3]

[a] Expressed as mg equivalent of gallic acid/g.

sorghum as a substrate through fungal fermentation. This table encompasses various regional and global markets, providing insights into the potential profitability and market opportunities associated with sorghum-based enzyme production. The wholesale price of sorghum grain was estimated using data from October 2024 (www.selinawamucii.com) and yield information from 2024 to 2025 across different regions of the world, based on information from the U.S. Department of Agriculture (USDA). Based on Table 3a, India experiences the lowest profitability (54.6 USD/ha) from sorghum grain, while China (351.0 USD/ha) and the USA (464.4 USD/ha) also show lower benefits compared to Australia (608.2 USD/ha), Nigeria (842.55 USD/ha) and Mexico (5746 USD/ha). These regions are projected to experience significant benefits from using sorghum grain, bran, and biomass for enzyme production, especially if they develop industrial-scale enzyme production facilities. This would not only be beneficial from an industrial perspective but would also contribute to sustainable waste management.

The estimated cost of enzyme production in this study is based on the work of Gupta et al. [35], who estimated the total cost of culture medium, equipment, and operations. According to their study, the net cost of crude enzymes was calculated as $0.47, $0.21, and $0.04 per 1000 units of FPase, CMCase, and β-glucosidase, respectively, from wheat bran using *Aspergillus niger* under solid-state fermentation. However, due to the lack of studies specifically focused on cost estimation for enzyme production from sorghum grain and bran, this study adopts an average production cost of $0.24 per 1000 units for various enzymes, including β-glucosidase, β-xylosidase, xylanase, α-amylase, and glucoamylase, using sorghum as the substrate.

The yield data of enzymes are derived from the studies of Dias et al. [4] and Pacheco-Chávez et al. [41], as presented in Table 9. All units have been converted into U/g, and enzyme prices have been obtained based on supplier quotations (Sigma-Aldrich and Novozymes). Table 3b shows an estimated profit ranging from 5436.3 USD/kg for α-amylase and 253,932.67 USD/kg for β-xylosidase.

The world sorghum production increased from 55.8 million tons to about 59.35 million tons in 2018 [42].

Fig. 1 represents the trend analysis of sorghum production among the world's top sorghum-producing countries. It shows that sorghum production has increased gradually all over the world, as per information from FAOSTAT. According to the Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES), in 2018, Australia produced 1.161 million tons of sorghum grain, and production has increased by more than doubled by 2.62 million tons in 2022 (Fig. 2). As stated by the Food and Agriculture Organization (FAO) projections, an upward trajectory in sorghum cultivation is expected by 2027. This growth will predominantly originate from developing nations, contributing 37 % of the global sorghum production. By 2017, their share is anticipated to rise to 42 % [43].

However, research and utilization of sorghum for enzyme and biofuel production are still lagging behind compared to wheat, corn, and rice. According to the data of Science Direct, in 2023, only 2217 research articles related to sorghum and enzymes were published, whereas 10,716 and 12,427 research articles related to enzyme production from wheat and corn, respectively. Fig. 3 depicts the last five years' scenario of enzyme and cereal crop-related research (Sorghum, Rice, Wheat, and

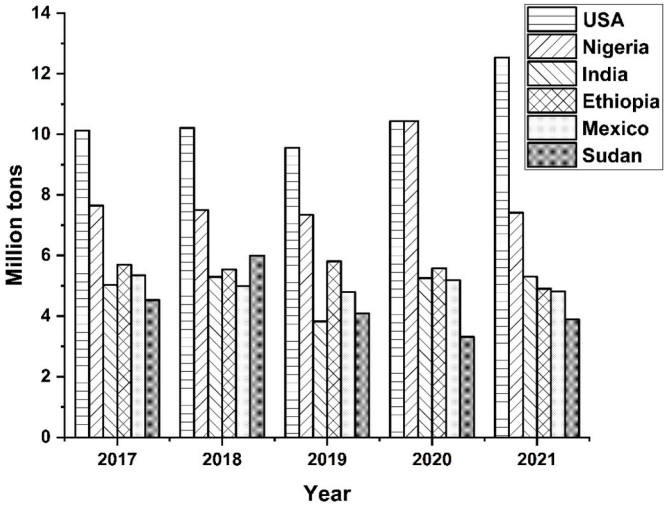

Fig. 1. Sorghum production trend analysis among the world's top sorghum-producing countries.

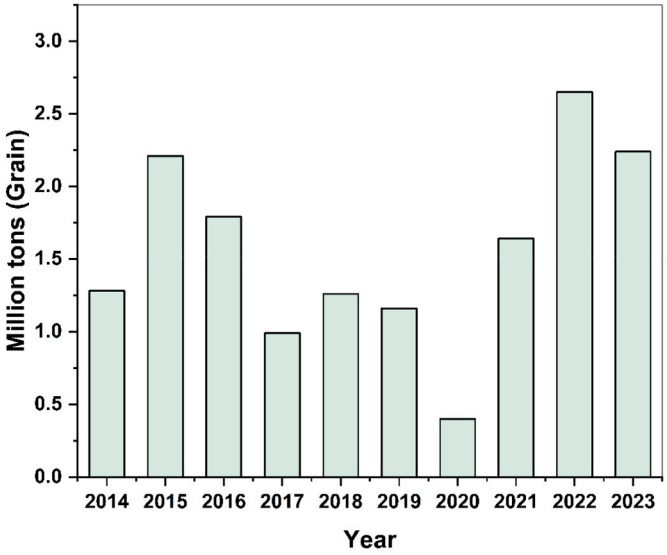

Fig. 2. Trend of last ten years of sorghum grain production in Australia.

Corn).

This observation underscores the need for a more thorough examination of sorghum's potential in enzyme production, particularly in comparison to other commonly studied crops. With proper attention and consideration, sorghum could emerge as a next-generation energy crop, offering diverse advantages across various sectors.

3.2. *Sorghum as a viable and sustainable substrate*

Sorghum has the potential to become a significant contender in the

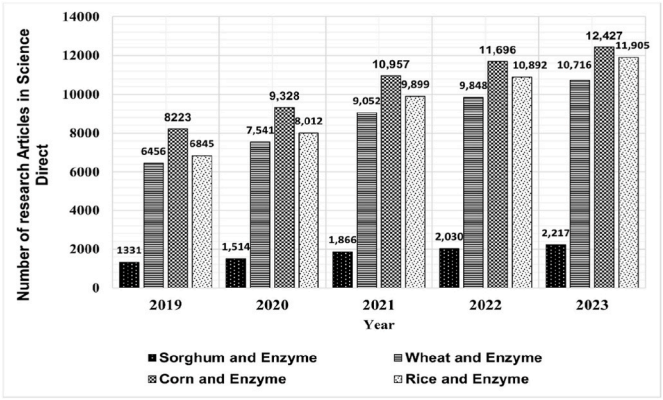

Fig. 3. Comparison of research articles on Sorghum, Wheat, Corn, and Rice (Source: www.Sciencedirect.com).

renewable feedstock sector due to its ability to produce high yields with limited water and inputs. Researchers are currently exploring its potential in various renewable applications, including both cellulosic and lignocellulosic contexts [44]. Sorghum shows immense promise as a lignocellulosic source for enzyme production. Its rapid growth and high productivity, yielding over 50 tons per hectare of dry biomass, make it an ideal candidate for enzyme production [4]. Sorghum and other agro-industrial wastes have been used for extracellular amylase and cellulase production [45,46]. Additionally, Pennells et al. [47] reported that sorghum can be used as an innovative source of biomass for the eco-friendly production of cellulose nanofiber. Therefore, fungal fermentation of sorghum can be a potential strategy to enhance its quality and functionality by degrading lignocellulosic compounds and releasing bioactive molecules. Its abundance, low cost, and minimal competition with food production make it an attractive candidate for enzyme production [44].

Sorghum straw has also been identified as a cheap and readily available carbon source for xylanase under solid-state fermentation [48]. The chemical composition of sorghum includes a higher content of polyphenols, and its grain contains 43.1 mg/100g polyphenol, whereas wheat and maize contain 20.5 mg/100g and 2.91 mg/100g, respectively [49] compared with other cereals (Table 4).

According to Ganzle's research, polyphenol content has an impact on enzyme production [56]. It can influence various biological processes, including enzyme activity [57]. Polyphenols in the fermentation medium may act as antioxidants and protect fungal cells from oxidative stress, thereby promoting their growth and enzyme production; on the other hand, high concentrations of polyphenols can potentially inhibit enzyme activity by interfering with enzyme-substrate interactions or denature enzymes, leading to reduced enzyme yields or activity [58]. Polyphenolic inhibition of starch hydrolysis and enzyme activity can be controlled and optimized through parameters such as temperature, incubation time, liquid-feed ratio, and inoculum volume [59]. de Jong et al. [60] reported minimal polyphenolic inhibition at 20 % grain sorghum slurry concentration; however, as the slurry concentration increases to 28 % or higher, the polyphenolic inhibition became more noticeable. Dhankher and Chauhan [61] found that phytic acid and polyphenol content decreased with increased fermentation time, and maximum reduction after 9 h at 30–50 °C in the case of pearl millet, and Zhang et al. [59] also demonstrated that polyphenol content significantly decreased at temperatures above 33 °C.

So, optimizing the fermentation process is essential to harness any potential benefits of polyphenols while minimizing their adverse effects on enzyme production. Sorghum grain is also gluten-free, high in resistant starch, a good source of minerals, and a variety of bioactive phenolic compounds [62]. Moreover, it has played a significant role in maintaining an eco-friendly environment compared to other crops in terms of water requirement, soil integration, carbon footprint, environmental sustainability, and denitrification process (Details in Table 5). The carbon footprint refers to the total greenhouse gas (GHG) emissions associated with a product or service [63]. Due to the lack of concrete data on the carbon footprint of enzyme production from sorghum, this discussion instead focuses on the carbon footprint of bioethanol production. As enzyme production is an integral part of the bioethanol process, the carbon footprint estimates for both enzyme and biofuel production from sorghum would likely be similar. The life cycle of sorghum-based enzymes and bioethanol includes four stages: cultivation, production, transportation, and consumption [64]. Shi et al. [64] stated that the carbon footprint of pure bioethanol (E100) production from sweet sorghum was reduced by 33.42 %–49.94 % compared to gasoline. Additionally, for E10 (a blend of 10 % bioethanol and 90 % gasoline), the reduction ranged from 2.68 % to 4.49 %.

Hossain et al. [74] mentioned that approximately half of the introduced nitrogen is lost through denitrification, runoff, and soil leaching mechanisms, but sorghum can help to reduce nitrogen loss with its enhanced nitrogen absorption and utilization capability, which in turn lowers crop production costs, minimizes groundwater contamination, and preserves soil structure. This makes sorghum crucial for achieving more sustainable and efficient agricultural practices, while also reducing greenhouse gas emissions and environmental degradation [74].

3.3. *Composition of sorghum and its relevance in enzyme production*

There has been an increasing interest in utilizing sorghum as a substrate for producing hemicellulases and cellulases by solid-state fermentation (SSF) using *Aspergillus* strains [4]. Table 6 demonstrates that sorghum is a sustainable and renewable source of carbon (cellulose, hemicellulose, lignin, and starch) like Wheat, Corn, Rice, Sugarcane, Barley, Rye, and Oats, etc. Moreover, Sorghum as a substrate for enzyme production has several advantages over other crops; for example, it is a low-input crop that requires minimal fertilizers (30 USD/acre) and production costs (Table 5), making it a cost-effective and environmentally friendly crop [75]. Sorghum grain has a starch content ranging from 68 % to 74 %, while wheat and corn have starch contents of 64%–72 % and 62%–74 % respectively (Table 6). Table 7 also provides information on other chemical components of sorghum, including starch. Additionally, it contains 9.28%–14.86 % protein [45], 5%–7% insoluble fiber, 1.5%–8% soluble fiber, and 2%–5% lipid (shown in Table 7). Based on available data from Table 8, it can be inferred that the protein content of sorghum, which is within the range of 8%–15 %, is comparable to that of wheat (10.55 %) and corn (9 %) [46,47].

Table 9 compares the production and yield of essential enzymes, including β-glucosidase, β-xylosidase, xylanase, α-amylase, and glucoamylase, using sorghum, wheat, and corn as substrates. It provides comprehensive details, such as pH, fermentation type (SSF and SmF), temperature, inoculum size, substrate, fermentation time, and agitation speed, based on the optimum fermentation conditions for each enzyme. Table 9 also shows that the yield of enzyme production using sorghum is equal to or higher than that of wheat and corn. The yield of xylanase production from sorghum straw is significantly higher(10,145 ± 141 U/g) compared to wheat (2191 ± 73 U/g) and corn (2480 ± 87 U/g) [103]. Additionally, the production yields of β-glucosidase, β-xylosidase, and β-xylanase are also higher when sorghum is used as a substrate in fungal solid-state fermentation (SSF) compared to wheat and corn, as indicated in Table 9. From Table 9, the highest enzyme yields were achieved under SSF conditions at pH 6, temperature 30 °C, and an inoculum size of 10^7 spores/mL, except for xylanase, for which 50 °C was optimum. Kaur, Rishi [59] reported that xylanase production peaked at 35 °C with a yield of 41.14 ± 1.04 IU, while β-glucosidase (3.62 ± 0.028 IU), pectinase (14.55 ± 0.15 IU), glucoamylase (42.31 ± 2.56 IU), and amylase (2774.0 ± 2.67 IU) achieved maximum yields at 30 °C, within a pH range of 6–7, after six days of incubation using *Aspergillus niger* in solid-state fermentation (SSF). Increasing or decreasing the incubation

Table 5
Environmental and economic potentiality comparison among Sorghum, Wheat, and Corn.

Parameter	Sorghum	Corn	Wheat	References
Production Cost (USD/Acre)	497	712	575	[65]
Yield (Tone/Hectare)	6.4 (Residue)	8.9 (Residue)	5.9 (Residue)	[66]
Yield (Tone/Hectare) NSW, Australia	1.90 (Grain)	1.20 (Grain)	1.05 (Grain)	[67]
Number of Geographical regions\Crop (World)	128	245	503	[66]
Water requirement (mm)	250–300	400–600	400–450	[68]
Fertilizer cost (per acre/USD)	30	97	38	[69]
Optimum Temperature for growth (°C)	32–35	32–35	25	[70]
Environmental Impact (Carbon Footprint)	Low	High	High	[71]
Sustainability	• Minimizing soil disturbance • Low water requirement • environmentally friendly	• Responsible for land degradation • Increase water scarcity • Responsible for climate change	• Responsible for land degradation • Increase water scarcity • Responsible for climate change	[72,73]

Table 6
Comparison of carbohydrate sources of sorghum and other cereal crops.

Name of crops	Cellulose (%)				Hemicellulose (%)				Lignin (%)				Starch (%)			
	[76,77]	[78]	[79]	[80]	[76,77]	[81]	[79]	[82]	[76]	[77,83]	[84]	[78]	[76]	[85, 86]	[87]	[49]
Sorghum crop	27–44.6	NR	27	NR	25–27.1	NR	25	18–27	11–24.7	18	14–21	NR	NR	NR	NR	NR
Sorghum grain	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	70.8	74.1	68	73.8
Sorghum Straw	32–35	31	32	26.93 ± 1.2	24–27	30	24	NR	NR	15–21	NR	11	NR	NR	NR	NR
Wheat straw	44.2	30	33–38	43.4	27.3	50	26–32	26–32	NR	16.7	17–19	15	NR	NR	NR	NR
Wheat Grain	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	71.9	62.1	65	64
Maize	35–45.0	45	39–47	42.21	28.0	35	26–31	35–39	15–21	16.6	7–19	15	73.4	70–75	75	62.3
Rice grain	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	80.8	NR	77.2
Rice Straw	34.4–44	33	28–36	37.8 ± 0.2	28	26	23–28	23–28	1–12	15.38	12–14	7	75.8	NR	80	NR
Sugarcane	41.6	44 (B)	32-48 (B)	46.1 ± 0.7	19–25.1	23	19–24	19–25 (B)	20.3–32	NR	20–42 (B)	20	NR	NR	NR	NR
Barley	36–43 (S)	NR	31-45 (S)	NR	24–33 (S)	NR	27-28 (S)	27–38	NR	6.3–9.0 (S)	14–19 (S)	NR	73.5	50–56	55	58.5
Sugar Beets	52.0	NR	NR	NR	32.0	NR	NR	NR	NR	16	NR	NR	NR	NR	NR	NR
Rye	NR	NR	NR	36.5 ± 0.1 (S)	NR	NR	NR	27–30 (S)	NR	NR	16-19 (S)	NR	71.9	40–44	NR	68.3
Oats	31–35 (S)	NR	NR	35 (S)	20-26 (S)	NR	NR	27–38 (S)	NR	10–15 (S)	16–19 (S)	NR	55.5	NR	60	52.8

B: Bagasse; S: Straw; NR: Not Reported.

Table 7
Chemical composition of Sorghum grain.

Chemical Composition of sorghum	[88] *Sorghum bicolour (L.)*	[89] (White Sorghum)	[89] (Red Sorghum	[90] (Ungerminated sorghum flour)	[91] *Sorghum bicolour (L.)*	[92] *Sorghum bicolour (L.)*	[93] *Sorghum bicolour (L.)*	[94] *Sorghum bicolour (L.)*	[95] *rabi* sorghum variety	[96] *Sorghum bicolour (L.)*
Starch (%)	70–72	66.38 ± 0.31	65.28 ± 0.19	65.2 ± 0.23	72	64.3–73.8	65.15–76.28 (C)	55.60–75.20	75.1	71.80–85.20 (C)
Protein (%)	8.90–11.02	12.27 ± 0.18	12.59 ± 0.03	7.25 ± 0.27	10.4	8.19–14.02	6.23–13.81	4.40–21.10	8.65	9.28–14.86
Fiber (%)	1.40–2.70	5.52 ± 0.23 (IS)	6.21 ± 0.57 (IS)	3.59 ± 0.24	1.6	1.41–2.55	1.65–7.94	1.00–3.40	3.94	1.47–2.45
Lipid (%)	2.30–2.80	4.37 ± 0.09	4.21 ± 0.02	NR	1.9 (Fat)	2.28–4.98	5.12–10.54 (Fat)	1.30–3.30 (Fat)	1.52	1.38–4.50 (Fat)
Ash (%)	0.92–1.75	1.61 ± 0.09	1.47 ± 0.03	1.79 ± 0.12	NR	1.46–2.32	1.12–1.68	1.30–3.30	2.86	0.90–1.52
Moisture (%)	8.10–9.99	9.81 ± 0.01	11.32 ± 0.07	9.9 ± 0.27	11.9	14	1.39–19.02	NR	14	NR

IS: Insoluble; C: Carbohydrate; NR: Not reported.

temperature and pH beyond these conditions resulted in a decrease in enzyme yields [59].

It is rich in essential nutrients, including protein, carbohydrates, vitamins, polyphenols, and starch (Tables 7 and 8), and has biochemical diversity with different varieties. Utilizing sorghum grain and biomass in enzyme production not only taps into its natural biochemical resources but also aligns with the growing emphasis on sustainable and environmentally friendly biotechnological processes.

Table 8
Comparison of nutritional value among valued agro-industrial crops with sorghum.

Agro-Industry Crops	Carbohydrate/Starch	Ash	Protein	Lipid/Fat	Fiber	Moisture	References
Sorghum (%)	70-72(S)	0.92–1.75	8.90–11.02	2.30–2.80 (L)	1.40–2.70	8.10–9.99	[88]
Rice Grain (%) (Brown)	22.5 ± 0.5 (C)	2.5 ± 0.3	12.1 ± 0.3	5.1 ± 0.1(L)	48.5 ± 0.6	9.3 ± 0.4	[97]
Wheat Flour (%)	74.88 ± 0.508(C)	0.94 ± 0.010	10.55 ± 0.032	0.94 ± 0.006(F)	0.36 ± 0.010	12.67 ± 0.025	[98]
Corn (%)	74.5	1.1	9.0	3.4	1.0	NR	[99]
Rye grain (%)	55–65 (S)	2	10–15	2–3(F)	19–22	NR	[100]
Sugarcane Bagasse (%)	NR	5	2.65	NR	NR	9.1	[101]
Barley (%)	75.9 (C)	3.1	12.2	1.9(F)	6.8	NR	[102]

S: starch; C: carbohydrate; L: Lipid; F: Fat; NR: Not reported.

3.4. Factors affecting sorghum's suitability for fungal fermentation

Fungal fermentation is a complex process involving fungi's growth and metabolism to produce enzymes or other valuable products. So, factor screening and optimization are important considerations for the fermentation process. The growth and metabolism of fungi are affected by various factors, including the composition of the substrate, environmental conditions, and the presence of contaminants [109]. For example, the nutrient content of the substrate can affect the growth and metabolism of fungi, which in turn can impact the yield and quality of the final product [110]. Similarly, the pH level of the fermentation environment can affect the growth and activity of fungi, and the presence of antinutrients or mycotoxins in the substrate can inhibit fungal growth and metabolism [111]. However, there is no concrete data demonstrating a negative impact on the fungal fermentation of sorghum by *Aspergillus* strains. Table 10 describes some crucial factors that affect sorghum's suitability for fungal fermentation. Therefore, it is important to carefully consider the factors of substrate and physical and chemical parameters that can affect fungal fermentation.

4. Industrial significance of filamentous fungi

Filamentous fungi secrete certain enzymes from their hyphae into the surrounding medium, and this secretion can be utilized as a source of enzymes apart from the intact organism [110]. The emergence of biotechnology has played a pivotal role in making recombinant enzymes in a large-scale industrial process. Members of the association of manufacturers and formulators of enzyme products (AMFEP) have successfully commercialized over 260 enzymes. Approximately 60 % of these commercially available enzymes are derived from fungi and are produced using a fungal host organism [127]. *Aspergillus*, accounting for more than 25 % of all industrial enzymes, stands as the undisputed champion among microorganisms of industrial significance. The top five fungal contributors include *Trichoderma*, *Penicillium*, *Rhizopus,* and *Humicola,* collectively contributing to an additional 20 % of industrial enzymes [127].

Enzymes derived from fungi are highly suitable for industrial applications because they rely on external sources of organic energy and material for their maintenance, growth, and reproduction. While most species of fungi grow as multicellular filaments called hyphae, some species, like yeasts, exist as single cells. Heterotrophy is a characteristic of fungi wherein they acquire energy from simple sugars, polypeptides, or more complex carbohydrates. However, since fungi can only absorb small molecules through their cell walls, enzymatic digestion outside the mycelium is often necessary. Fungi can utilize various energy sources, starting with the simplest compounds, such as soluble sugars. Subsequently, they can digest starch, pectin, cellulose, lignin, and waxes in a sequential manner. These fungi secrete a diverse range of enzymes that are essential for effective digestion. The secretion of these enzymes simplifies the process of large-scale production and isolation. Moreover, extracellular enzymes produced by fungi have naturally adapted to function under harsh conditions, making them excellent candidates for industrial catalysts. Their ability to withstand extreme environments further enhances their desirability for industrial applications.

The fungi *A. niger, A. oryzae*, and *Trichoderma reesei* play significant roles in industrial fermentations. In Japan, non-pathogenic strains of *A. oryzae* have been utilized for thousands of years to convert rice into alcoholic sake [128]. *A. niger* gained industrial importance in 1919 due to its ability to produce citric acid, and it later became a valuable source of enzymes [129]. Although *A. niger* and *A. oryzae* can produce mycotoxins like ochratoxin A, but the production of toxins depends on the specific strain and growth conditions [130]. *Aspergillus* species such as *A. niger, A. sojae,* and *A. oryzae* have obtained a Generally recognized as safe (GRAS) status from the US Food and Drug Administration, which approves their use in the food industry [131]. Developing alternative approaches, media, and substrates is essential to meet the enormous demand for enzymes in the industry. *Aspergillus* strains will be one of the key players in the production of commercial enzymes as well as biofuels to mitigate the future challenge of fossil fuels. This species is known for its ability to release significant amounts of enzymes into its surrounding environment [132]. Over the years, many of these enzymes, produced through large-scale submerged fermentation, have been extensively applied in the food and beverage industry [133].

4.1. Aspergillus strains in enzyme production

One of the most widely used fungi for enzyme production is *Aspergillus*, which belongs to a group of filamentous fungi that can secrete a range of hydrolytic enzymes, such as proteases, cellulases, amylases, and lipases [134]. These enzymes can degrade various plant polysaccharides and lipids and have applications in food, feed, and industrial sectors. Several studies have reported the production and optimization of different enzymes by *Aspergillus* strains using sorghum as a substrate in solid-state fermentation (SSF) or submerged fermentation (SmF) processes [4,135].

4.1.1. Overview of Aspergillus species commonly used in enzyme production

Aspergillus strains are commonly employed for enzyme production because they have the capacity to release substantial amounts of enzymes into their surrounding environment and have widely used these enzymes (lipase, amylase, cellulases, aminopeptidase, pectinase, etc.) in the food and beverage industry for decades [133]. Various types of enzymes are produced by different *Aspergillus* strains, and these enzymes possess huge market value on a global scale (Summarized in Table 11). The most commonly used *Aspergillus* strains in enzyme production include *Aspergillus niger, Aspergillus oryzae, Aspergillus awamori,* and *Aspergillus flavus* [136]. Market analysis from 2022, pectinase led the global enzyme market with a valuation of 20,900 million USD, followed closely by xylanase at 19,100 million USD (Table 11). Glucose oxidase also held a significant position at 6543.7621 million USD. Notably, all these enzymes were produced using *Aspergillus* strain (Table 11), and these strains are known to produce several enzymes with numerous applications in various industries such as food, beverage, detergent, textile, paper, and pharmaceuticals [136,137].

Table 9
Comparison of enzyme production ability with different substrates and *Aspergillus* Sp.

Name of Enzyme	Fungal Sp.	Sorghum			Wheat			Corn			References
		Conditions	Hours	Max Yield	Conditions	Hours	Max Yield	Conditions	Hours	Max Yield	
β-glucosidase	*A. niger*	**FT:** SSF **pH:** NR **Temp:** 30 °C **Inc. size:** 05 MD (0.5 cm in diameter) **Vessel:** 250 EF **Sub:** BS Ref:*	120	54.90 U/g	**FT:** SSF **pH:** NR **Temp:** 30 °C **Inc. size:** 05 MD (0.5 cm in diameter) **Vessel:** 250 EF **Sub:** WB Ref: *	96	47.0 U/g	**FT:** SmF **pH:** 6.0 **Temp:** 28 °C **Inc. size:** 2.5 × 10^6 spores/flask **Vessel:** 250 EF **Sub:** CC **Volume:** 50 ml **RPM:** 170 Ref:#	200	48.7 IU/ml	* [4]; # [104]
β-xylosidase	*A. niger** *A. awamori*#	**FT:** SSF **pH:** NR **Temp:** 30 °C **Inc. size:** 05 MD (0.5 cm in diameter) **Vessel:** 250 EF **Sub:** BS Ref:*	144	64.88 U/g	**FT:** SSF **pH:** NR **Temp:** 30 °C **Inc. size:** 05MD (0.5 cm in diameter) **Vessel:** 250 EF **Sub:** WB Ref: *	144	63.2 U/g	**FT:** SmF **pH:**5.5 **Temp:** 28 °C **Vessel:** 500 EF **Sub:** CC **Volume:** 50 ml **RPM:** 220 rpm Ref: #	72	2.1 U/ml	* [4]; # [105]
β-xylanase	*A. niger** *A. awamori*#	**FT:** SSF **pH:** NR **Temp:** 30 °C **Inc. size:** 05 MD (0.5 cm in diameter) **Vessel:** 250 EF **Sub:** BS Ref:*	72	300.07 U/g	**FT:** SmF **Temp:** 30 °C **pH:** 5.5 **Inc. size:** NR **Vessel:**500 EF **Sub:** WS **Volume:** 50 mL Ref: #	72	55 U/ml	**FT:** SmF **Temp:** 28 °C **pH:**5.5 **Inc. size:** NR **Vessel:** 500 EF **Sub:** CC **Volume:** 50 mL **RPM:** 220 Ref: #	72	80 U/ml	* [4];# [105]
Xylanase	*T. lanuginosus*	**FT:** SSF **pH:** 6 **Temp:** 50 °C **Inc. size:** 10^7 spores/ml **Vessel:** 250 EF **Volume:** 20 ml **Sub:** 5 gm SS **Media St.:**15 Psi for 25 min	144	10,145 ± 141 U/g	**FT:** SSF **pH:** 6 **Temp:** 50 °C **Inc. size:** 10^7 spores/ml **Vessel:** 250 EF **Volume:** 20 ml **Sub:** 5 gm WS **Media St.:**15 Psi for 25 min	144	2191 ± 73 U/g	**FT:** SSF **pH:** 6 **Temp:** 50 °C **Inc. size:** 10^7 spores/ml **Vessel:** 250 EF **Volume:** 20 ml **Sub:** 5 gm CC **Media St.:**15 Psi for 25 min	144	2480 ± 87 U/g	[103]
α-Amylase	*Trichoderma* sp.* *R. oryzae*#	**FT:** SmF **pH:** 5.3 **Inc. size:** 1.0X10^7spores/mL **Vessel:** 500 EF **Sub:** S.St **Volume:** 100 mL **RPM:** 180 Ref:*	24	258 ± 2.8 U/L	**FT:** SSF **pH:** NR **Temp:** 35 °C **Inc. size:** 10^5/ml **Vessel:** 250 flask **Sub:** WB **Volume:**100 ml **Sub:** 5 gm WB Ref: #	24	635.0U/L	**FT:** SSF **pH:** NR **Temp:** 35 °C **Inc. size:** 105/ml **Vessel:** 250 flask **Sub:** CG **Volume:**100 ml **Sub:** 5 gm CG Ref: #	120	183.5 U/mL	* [41];# [106]
Glucoamylase	*Trichoderma* sp.* *Aspergillus* sp. # *A. niger*@	**FT:** SmF **pH:** 5.3 Inc. size: 1.0X10^7spores/mL **Vessel:** 500 EF **Volume:** 100 mL **Sub:** S.St **RPM:** 180 Ref:*	120	83 ± 1. 2 U/L	**FT:** SSF **pH:** 5 **Temp:** 55 °C **Inc. size:**10^6 spores/ml **Vessel:** 250 EF **Sub:** 5 gm WB Ref: #	96	(264 ± 0.64 U/gds)	**FT:** SSF **pH:** NR **Temp:** 28 °C **Inc. size:** 10^6 spores/ml **Vessel:** 250 EF **Volume:** 100 mL **Sub:** 5 % CF Ref: @	168	243.09 U/ml	* [41];# [107]; @ [108]

FT: Fermentation type; SSF: Solid state fermentation; SmF: Submerged fermentation; Temp: Temperature; Inc. size: Inoculum size; MD: Mycelial disc; EF: Erlenmeyer flask; Sub: Substrate; Media St.: Media sterilization; BS: Biomass sorghum; SS: Sorghum straw; S. St: Sorghum starch; WB: Wheat bran; WS: Wheat straw; CC: Corn cob; CG: Corn grits; CF: Corn flour; NR: Not reported; Ref: Reference.

4.1.2. Comparative analysis of different Aspergillus strains in terms of enzyme productivity

In terms of enzyme productivity of different *Aspergillus* strains, several key strains stand out for their remarkable enzymatic capabilities. One of the most commonly used *Aspergillus* strains for enzyme production is *A. niger*. This strain is known for its capacity to produce a diverse range of enzymes, including amylases, proteases, pectinases, mannanase, xylanase etc. (Table 12). *A. niger* is also known for its high secretion ability, making it an ideal candidate for industrial enzyme production. Table 12 presents a comparative analysis for selecting the most suitable strain for specific enzyme production. For instance, in the case of α-amylase, it shows that *A. niger* (2659 ± 36.541 IU/mL) produces a higher quantity of enzyme compared to *A. awamori* (247.2 U/mL) and *A. oryzae* (1984.08 IU/mL) [165–167]. Therefore, for industrial α-amylase production, *A. niger* would be the preferred choice, with a focus on optimizing parameters to enhance yield further.

Table 10
Factors affecting fungal fermentation during enzyme production.

Factors should be considered for Sorghum during fermentation			
Factors	Description	Effect on Fermentation	References
Sorghum variety	➢ Different varieties exhibited different mineral contents, particularly in manganese and zinc.	➢ Change the pH value	[112]
Moisture Content	➢ Optimal moisture content (60%–80 %) is crucial for fungal growth and fermentation.	➢ High Moisture causes low fungal growth due to limited oxygen transfer at high levels of SSF. ➢ High moisture can lead to mold growth. ➢ Low moisture can inhibit fermentation.	[113–115]
Nutrient Content	➢ Nutrient content, such as nitrogen and phosphorus, are important for fermentation. ➢ Sorghum contains starch 70–72 % , protein 8.9–11.02 % , fiber 1.40–2.70 %, and lipid 2.30–2.80 %[1].	➢ Higher nitrogen and phosphorus content can support better fungal growth and fermentation.	[88,112][1]
Presence of Antinutrients	➢ Antinutrients hinder fungal growth. ➢ Pre-treatment may be necessary to reduce their levels. ➢ Range of tannin content among all sorghum varieties 0.02 %–3.59 %[1].	➢ Sorghum contains tannins and phytase, which can delay fungal growth	[116,117][1]
Presence of Mycotoxin	➢ Proper pre-screening of sorghum batches is essential. ➢ Sorghum contains aflatoxin (13 %), fumonisins (17 %), and sterigmatocystin (15 %)[1]	➢ Sorghum contaminated with mycotoxins can negatively impact fungal fermentation and pose health risks.	[111,118][1]
Factors should be considered for *Aspergillus* during fermentation			
Factors	**Description**	**Effect on Fermentation**	**References**
Temperature	➢ Critical factor for fermentation ➢ Fungal-dependent parameters that affect enzyme production. ➢ Temperature range of 25 °C–35 °C for SSF and it depends on growth kinetics of the microorganism.	➢ Affect enzyme production ➢ Temperature varies with substrate type, enzyme type, and fungal strain (Shows in Table 9)	[119,120]
pH	➢ Fungal fermentation is sensitive to pH levels. ➢ pH is easily maintained during SmF but difficult to control during SSF. ➢ pH can also affect the thermodynamics and kinetics of microbial respiration. ➢ Optimum pH range of 3.8–6.0 for filamentous fungi[1].	➢ Sorghum with an optimal pH range can promote desirable fungi growth and inhibit unwanted microorganisms' growth. ➢ Metabolic activities of microorganisms can change the pH of the medium during SSF.	[121–123][1]
Incubation Time	➢ Important factor for different types of enzyme production (explained in Table 9). ➢ For example, β-glucosidase (3–8 days)[1], Xylanase (4–6 days)[2], α-amylase (1–5 days)[3], Glucoamylase (4–7 days)[4].	➢ Shortening the incubation period for optimal enzyme production is advisable to minimize the risk of contamination and lower production costs.	[4,119][1] [105],[2] [106];[3] [108];[4]
Aeration	➢ Oxygen availability ➢ Vital factor for submerged fermentation (SmF). Aeration rate 0.5-2vvm for *Aspergillus* strain[1]	➢ Ensure oxygen availability. ➢ Helps to maintain fungus metabolic activity and produce enzymes efficiently.	[110,124][1]
Substrate Particle Size	➢ Smaller particles of the substrate provide a larger microbial surface. However, particles that are too small might lead to substrate accumulation that interferes with the aeration of microorganisms, resulting in inadequate development. ➢ Larger particles increase the aeration efficiency but provide a small area for microbial action. ➢ Particle size of 2–3 mm for sorghum straw is optimal for xylanase production[1], while sorghum bran larger than 1.0 mm is best for glucoamylase production[2].	➢ Affects the substrate's capacity to interact with the growth of microorganisms, as well as its ability to facilitate thermal transfer and mass transfer in solid-state fermentation (SSF)	[103,123,125][1] [126],[2]
Agitation	➢ Helps in distributing nutrients evenly. ➢ Agitation rates may vary between 100 and 500 rpm for *A.niger*[1]	➢ Preventing substrate accumulation and enhancing mass transfer of gases ultimately promotes *Aspergillus* growth and enzyme synthesis.	[110,124][1]

Another highly productive *Aspergillus* strain is *A. oryzae*. This strain is also known for its ability to produce high levels of proteases, amylases, glucoamylases, and xylanases [168]. In addition to these strains, other *Aspergillus* species have also been shown to be productive in enzyme production, including *A. nidulans, A. fumigatus,* and *A. flavus* (Table 12). However, the productivity of these strains varies depending on the specific enzyme being produced and the fermentation conditions.

4.1.3. Factors influencing the efficiency of enzyme production by Aspergillus strains

The parameters of the fermentation process, such as fermentation medium, duration, pH, and temperature, affect enzyme production under SSF and SmF (Summarized in Table 9). The moisture content of the substrate (Described in Table 10) is a critical parameter under SSF, while shaking or static conditions are crucial during SmF [182]. For sorghum, submerged fermentation (SmF) has been reported to occur at pH values of 5.3 and 6.0 [41], while a pH of 6.0 has been observed in solid-state fermentation (SSF) [103]. Although there is a lot of data available regarding pH levels in SmF, but there is a noticeable lack of data concerning pH levels in solid-state fermentation (SSF). Several studies have investigated particle size distribution (PSD) during wheat fermentation, but there is limited data available for sorghum grain. Sonia et al. [103] found that sorghum straw sizes ranging from 2 to 3 mm yielded the highest xylanase activity (26,121 U/g); however, reduced xylanase activity was observed with sorghum straw sizes of 0.5–1.0 mm (18,980 U/g) and 5.0–7.0 mm (16,171 U/g) under solid state fermentation (SSF). Makanjuola et al. [126] reported that a particle size of sorghum bran >1.0 mm is optimal for glucoamylase production in submerged fermentation. Typical moisture content for SSF has been reported to be in the range of 60–80 % [115], which is optimum for fungal growth and enzyme production. Thanapimmetha et al. [135] mentioned an optimum of 77.5 % moisture during SSF of sorghum but did not mention the particle size range. Generally, moisture and PSD are investigated separately, and there is no data related to the optimum PSD of sorghum.

Table 11
Comparison of enzyme production and their market value by using *Aspergillus* strains.

Enzyme Name	*Aspergillus* strain	Field	Enzyme Use	Global Market (USD) & key Manufacturer (KM)	References
α-Amylases	*A. niger* *A. oryzae*	• Brewing • Beverage, • Textile, • Pulp • Etc.	➢ Dough softening, ➢ Increased bread volume, ➢ Sugar production during fermentation ➢ Removal of starch from textile fibers ➢ Antimicrobial activity ➢ Break down starch	1840.8 Million (2023)* **KM:** - Novozymes - Dupont Danisco - DSM - Amano Enzyme	[138–141]*
Aminopeptidase	*A. niger* *A. oryzae* *A. sojae*	• Food • Medicine	➢ Brewing and soy sauce ➢ Remove the bitter taste of protein hydrolysate ➢ hydrolysis of protein ➢ remove neurotoxic chemical agents	15 Million (2023)* **KM**: - Abbexa - Prospec TechnoGene - Merck - Medline	[142–144]*
β-Glucanase	*A. niger* *A. aculeatus*	• Grain feed industry • Beer industry	➢ Improve feed digestion and absorption ➢ Reducing the viscosity and releasing reducing sugar during fermentation	387.7 Million (2022)* **KM:** - Cargill Inc. - Alltech Life Sciences	[143–145]*
Cellulases	*A. japonicus* *A. niger* *A. awamori*	• Pulp and paper • Textile • Biofuel & biorefinery • Food/feed processing	➢ Deinking and reduces the heavy metals in the newspaper pulp ➢ Textile waste hydrolysis for recovery of glucose and polyester	1685.8 Million (2022)* **KM:** - Genencor - Danisco - Novozymes - Sigma Aldrich etc	[144, 146–148]*
Catalase	*A. niger* *A. flavus* *A. fumigatus*	• Food processing, textile, • Pharmaceuticals • Therapeutics	➢ Removal of hydrogen peroxide from cotton bioprocessing ➢ Aflatoxins biosynthesis ➢ Fungal development, ➢ Production of enzymatic antioxidant	387.4 Million (2022)* **KM:** - Sigma-Aldrich Co. LLC. - MP Biomedicals LLC. - Megazyme Inc.	[144, 148–150]*
Glucose oxidase	*A. niger*	• Breadmaking • Dairy • Wine/beer • Processing • Textile	➢ Removal of hydrogen peroxide from cotton bioprocessing ➢ Food preservation ➢ Preventing the oxidative deterioration during the processing of beer	6543.7621 million (2022)* **KM:** - Sigma-Aldrich Co. BBI - Solutions, Roche - Diagnostics	[144,151, 152]*
Glucoamylase	*A. awamori* *A. oryzae* *A. niger*	• Food & Beverage • Confectionary	➢ Flour to improve its quality slow down the staling of dough ➢ Make high glucose and/or fructose syrups for making candy ➢ Catalyse the hydrolysis of starch into fermentable sugars	834.2 million (2023)* **KM:** - Novozymes - Amano Enzyme - Genencor - AB Enzyme	[144,153, 154]*
Glutaminase	*A. oryzae* *A. Sojae*	• Food • Medical	➢ Used as anti tumour drugs ➢ Used as bio sensor ➢ Soya sauce fermentation	149.7 million (2022)* **KM:** - Ajinomoto, - Daesung, - Kyowa Hakko Kirin	[143,144, 155]*
Lactase	*A. niger* *A. oryzae*	• Milk and dairy products • Pharmaceuticals	➢ Dietary Supplements ➢ Helps to digest milk	234.74 Million (2023)* **KM:** - Novozymes, - Merck - KGaA, - Kerry Inc.	[144,156]*
Lipase	*A. niger* *A. oryzae*	• Paper • Food, • Detergent, • Textile industries • Cosmetics & perfume • Pharmaceuticals	➢ Washing strain from fabrics ➢ Used as stabilizer in food creation. ➢ Production of biodiesel ➢ Fat removal ➢ Remove hard strains and breakdown oil	222.0 million in 2022* **KM:** - Novozymes A/S, - DSM N.V., - Hansen Holding A/S	[144,157, 158]*
Pectinases	*A. niger*	• Food & beverage • Textile • Wine • Food/feed	➢ Efficiency in pectin degradation from grape juice ➢ Bioscouring of cotton fibers ➢ Fruit juice purification	20,900 Million(2022)* **KM:** - AB Enzymes, - Genecor, - Jinyuan Biochemical Co Ltd. - Sunson Novozymes	[144,159, 160]*
Protease	*A. oryzae* *A. flavipes*	• Leather, • Feed • Textile industry • Detergent • Pharmaceutical	➢ Biofilm removal ➢ Brewing process ➢ Nutritional improvers ➢ High digestibility supplement ➢ Proteolytic tenderization	2100 Million(2022)* **KM:** - Novozymes - Advanced Enzymes - Technologies Limited, - Royal DSM N.V	[144, 161–163]*
Xylanases	*A. niger*	• Food and beverage • Pulp and paper • Textile	➢ Improve yield and clarity of juice ➢ Bioleaching, desizing, bioscouring ➢ Biofinishing of cellulosic fabrics	19,100 million (2023)*; **KM:** - DuPont Danisco - Novozymes - DSM - Altech	[144,148, 164]*

KM: Key Manufacturer.

Table 12
Comparison of the best enzyme-producing strains of *Aspergillus*.

Strain	Cellulase (CMCase)	Protease	Pectinase	β-Glucosidase	Mannanase	Lipase	Glucoamylase	Xylanase	α-Amylase	References
A. niger	**A:** 1.49 ±0.015[1] **Unit:** IU/mL **Sub:** Starch	**A:**184.08 ±6.8[2] **Unit:** U/g **Sub:** WB	**A:**3.67 ±0.024[1] **Unit:** IU/mL **Sub:** Starch	**A:**0.370 ±.011[1] **Unit:** IU/mL **Sub:** Starch	**A:**0.65 ±0.018[1] **Unit:** IU/mL **Sub:** Starch	**A:**9.14[3] **Unit:** IU/g **Sub:** WB	**A:** 90.17[4] **Unit:** U/g **Sub:** PP	**A:**1.43 ±0.09[1] **Unit:** IU/mL **Sub:** **Starch**	**A:**2659 ±36.54[1] **Unit:** IU/mL **Sub:** **Starch**	[166,169][2] [170][;3][171][;4]
A. awamori	**A:** 4.636[3] **Unit:** U/ml **Sub:** MS	**A:**70.32[1] **Unit:** U/g **Sub:** WB	**A:** 38.0[4] **Unit:** U/g **Sub:** **GP**	–	–	–	**A:**336.08[1] **Unit:** U/g **Sub:** WB	**A:** 581.42[1] **Unit:** U/g **Sub:** WB	**A:** 247.2[2] **Unit:** U/mL/h **Sub:** CS	[167][1][172][;2][173][;3][174][;4]
A. oryzae	**A:** 38.80[3] **Unit:** U/ml **Sub:** CC	**A:** 1327.76[2] **Unit:** U/g **Sub:** WB	**A:** 120[3] **Unit:** U/ml **Sub:** **SR**	–	**A:** 104.2[4] **Unit:** U/mg **Sub:** BG	**A:** 35.66[3] **Unit:** U/ml **Sub:** S	**A:**121.62[2] **Unit:** U/g **Sub:** WB	**A:**138.71[2] **Unit:** U/g **Sub:** WB	**A:** 1984.08[1] **Unit:** IU/mL **Sub:** PS + WB	[165][1] [167][;2]; [175][3] [176][;4]
A. fumigatus	**A:** 4.07[1] **Unit:** U/g **Sub:** CH	–	–	–	–	–	–	–	**A:** 60–130[2] **Unit:** U/mg **Sub:** **M**	[177][1] [178][;2]
A. nidulans	**A:** 39.56 **Unit:** U/ml **Sub:** CMC	**A:** 2262.4 **Unit:** U/ml **Sub:** G and C	–	**A:** 35.11 **Unit:** IU/gds **Sub:** BGR	–	**A:** 102 **Unit:** IU/ml/min **Sub:** **RB** Rice bran	–	**A:** 40 **Unit:** IU/ml **Sub:** WB	–	[119]
A. tubingensis	**A:** 750 **Unit:** U/gds[1] **Sub:** CM	–	–	–	**A:** 1023.0[4] **Unit:** U/gds **Sub:** CM	**A:** 7.6 ± 0.6[2] **Unit:** U/g **Sub:** WB + OP		**A:** 615.5 ± 19.21[3] **Unit:** U/g **Sub:** SR		[177][1] [179][;2] [180][;3] [181][;4]

[**Note:** A: Enzyme Activity; Sub: Substrate; WB: Wheat bran; PP: Pea peel; MS: Molokhia stalks; GP: Grape pomace; CS: Cassava starch; CC: Corn cobs; SR: Soybean residue; BG: Bean gum; S: Sorghum; PS: Potato starch; CH: Coconut husk; M: Maltose; G: Glucose; C: Casein; BGR: Black gram residue; RB: Rice bran; CM: Copra meal; OP: Olive pomace; SR: Sorghum residue; CMC: Carboxy methyl cellulase].

5. Fungal fermentation process for enzyme production

Fungal fermentation is a widely used process for enzyme production. Filamentous fungi, such as *Aspergillus* sp. and *Trichoderma* species, offer several advantages, including low material costs, high productivity, and ease of enzyme recovery from the culture medium [132]. In fungal fermentation for enzyme production, the choice of carbon source plays a crucial role in determining the yield and quality of the enzymes. For example, wheat bran, sorghum straw, and lignocellulosic substrates have been investigated for their impact on enzyme productivity. Besides this, some pivotal factors such as pH, temperature, incubation time, substrate particle size, nutrient content, and moisture content of the substrate (summarized in Table 10), fermentation type (SSF, SmF), and appropriate carbon source can significantly enhance enzyme activity and overall production efficiency.

5.1. Solid state fermentation (SSF) and submerged fermentation (SmF)

Solid-state fermentation (SSF) and Submerged fermentation (SmF) are efficient methods for enzyme production using *Aspergillus* Species. However, according to the previous research study and information from Table 9, Solid-state fermentation (SSF) is a preferred method for fungal enzyme production as it offers advantages such as lower production costs, efficient use of raw materials, and higher productivity. In the context of β-xylosidase production, solid-state fermentation (SSF) exhibits a superior yield when sorghum (64.88 U/g) and wheat (63.2 U/g) are used as substrates; meanwhile, corn only shows β-xylosidase activity at 2.1 U/ml under submerged fermentation (SmF) [4,105]. Conversely, when sorghum was used as a substrate for α-amylase production in SmF, the yield was significantly low (258 ± 2.8 U/L), in comparison with wheat yields (635.0 U/L), and corn yields (1835.0 U/L) respectively under SSF [41,106]. This indicates that SSF is the preferred method for fermentation to efficiently meet the demand for industrial enzymes while keeping production costs low.

A. niger, *A. awamori*, and *A. oryzae* have been reported as excellent producers of enzymes like xylanase, β-xylosidase, and β-glucosidase through SSF using lignocellulosic substrates (Tables 9 and 12). SSF involves growing fungi on solid substrates like grains or plant materials, mimicking their natural habitat and promoting enzyme secretion [110]. In contrast, submerged fermentation (SmF) is the most common method used in the fermentation industry, with research focused on improving productivity, yields, and process economics [183,184]. SmF offers advantages such as better control of environmental parameters, lower labor costs, reduced space needs, and easier scale-up compared to solid-state fermentation (SSF). However, SmF also faces challenges, especially when using filamentous fungi, including long fermentation times, excessive foam production, and costly media [185]. de Barros Soares et al. [185] reported that submerged fermentation (SmF) for transglutaminase production faces challenges, including lengthy fermentation times, excessive foam hindering oxygen transfer, and the use of costly culture media. Professor A.P.J. Trinci famous quote, as referenced by Pandey et al. [186]: "God did not create filamentous fungi

to grow in a fermenter." He suggested that submerged fermentation (SmF) is an unnatural environment for filamentous fungi, as they naturally thrive in solid-state conditions [186]. Solid-state fermentation (SSF) is the optimal choice for filamentous fungi for enzyme production.

Table 13
Comparison of SSF vs SmF.

Type of advantages	SSF	SmF	References
Biological	Produced in a high volume of product	Product yield is low compared to SSF	[188,189]
	Stability of products is high	Low stability	
	Tolerance to elevated substrate concentration	Not feasible for high-substrate concentration	
	Provide solid support for microorganisms	Liquid medium	
	Possible fermentation of water-insoluble substrate	Not possible	
	Spore inoculation	Easy inoculation	
	Batch process	Continuous process	
	Low water demand minimize the risk of contamination	Requires high volume of water increased chance of contamination	
	Best for fungal spore production and long-term preservation	Not suitable for fungal spore production and long-term preservation	
Processing	Difficult to control pH and temperature	Easy	[189–192]
	Challenging to mix	Easy	
	Medium consists of a moist substrate with a high concentration of the product.	Liquid medium with low product concentration	
	Facilitates an easy extraction process	Complex extraction process	
	Inoculum size is large, more than 10 %	Inoculum size low, less than 10 %	
	No foam formation during fermentation	Foam formation during fermentation	
	Simple Bioreactor for fermentation	High tech design	
	Low raw material cost	High cost	
	The culture system consists of three phases (gas, liquid, and solid)	The culture system mainly consists of liquid	
	Challenging to scaleup	Easy scaleup	
Environmental	Sustainable technology for bioremediation and biodegradation of hazardous compound	Capacity of bioremediation and biodegradation is low	[189]
	Best for the recycling process	Not suitable for the recycling process	
	High biomass conversion	Low conversion rate	
	Solving waste problem as biological detoxification	Not practical for biological detoxification	
	Produce less liquid waste	Produce high amount of liquid waste	
	Minimize pollutants and harmful waste during the product process	Not minimized	
Economic	Substrate usually natural materials	Costly commercial materials	[188,189]
	A modified bioreactor (rotor tray) is simple, cheap, and user-friendly	Expensive bioreactor, complex	
	Easy downstream processing	Challenging in downstream processing	
	Low recovery cost	High recovery cost	
	High yield	Low yield	

Table 13 highlights critical biological, processing, economic, and environmental comparisons between SSF and SmF. SSF boasts higher volumetric productivity, reduced water and energy usage, and is cost-effective, making it ideal for utilizing agro-industrial by-products. However, SSF faces challenges in parameter control and slower growth, while SmF may encounter variations in conditions affecting nutrient availability [187]. The choice between SSF and SmF depends on the specific requirements of the production process, the fungal strain being used, and the desired end products.

5.2. Steps involved in fungal fermentation of sorghum for enzyme production

The process begins with the characterization of sorghum biomass, encompassing both straw and grain components, including total reducing sugars (TRS), free amino nitrogen (FAN), ash, phosphate, total nitrogen, etc. Size fractionation and milling techniques are then employed to finely prepare the biomass for extraction, ensuring optimal efficiency at each stage of the process. Identification and selection of suitable fungal strains and parameters are the crucial and critical steps of the fermentation process. By considering all the physical and chemical factors outlined in Table 10 and Fig. 5, it is possible to achieve the desired production efficiently. The extracellular enzyme can be easily separated and purified during downstream processing using filtration, centrifugation, precipitation, and lyophilization. Furthermore, filamentous fungi have the capability to produce extracellular enzymes, making them readily accessible in the medium [110,133]. This eliminates the need for cell disruption and significantly reduces both time and cost compared to intracellular enzymes. Details of the steps are described in Fig. 4.

5.3. Optimization strategies for enhancing enzyme yields using Aspergillus strains

Optimizing the enzyme production process is essential for enhancing efficiency, reducing costs, and ensuring high-quality enzyme products. By restructuring workflows and minimizing the use of resources, companies can improve their yield and profitability. Efficient processes also enable faster scale-up and commercialization, giving businesses a competitive edge in the market. Moreover, optimization contributes to environmental sustainability by reducing waste generation and energy consumption. Overall, optimizing enzyme production processes fosters innovation, drives product development, and supports the long-term success of enzyme manufacturers in a dynamic and competitive industry landscape.

There is no exact procedure for maximum enzyme production using fermentation, including fungal fermentation, so optimization plays a crucial role in fermentation to enhance product yield and quality. Optimization strategy can be classified into two major ways- 1. Process optimization, and 2. Host cell modification (Strain development by genetic engineering or random mutagenesis approaches). Details Classification are illustrated and described in Fig. 5.

5.3.1. Process optimization

This optimization also enhances cost efficiency by minimizing resource wastage and maximizing raw material utilization, which is particularly vital in industrial settings. Additionally, it ensures consistent product quality, meets standards, and enhances reliability. Firstly, it maximizes productivity by fine-tuning parameters like nutrient concentrations, pH levels (3.8–6.0 for filamentous fungi), incubation time (2 days–7 days), temperature (25°C–35 °C for SSF), etc., resulting in higher yields of desired enzymes [4,41,103–105]. Time efficiency is improved through shortened fermentation cycles, which promptly meet market demands. The traditional method of enzyme production using external carbon and nitrogen sources such as glucose (350 USD for 25 kg), yeast extract (1600 USD for 25 kg), and urea (740 USD for 1 ton)

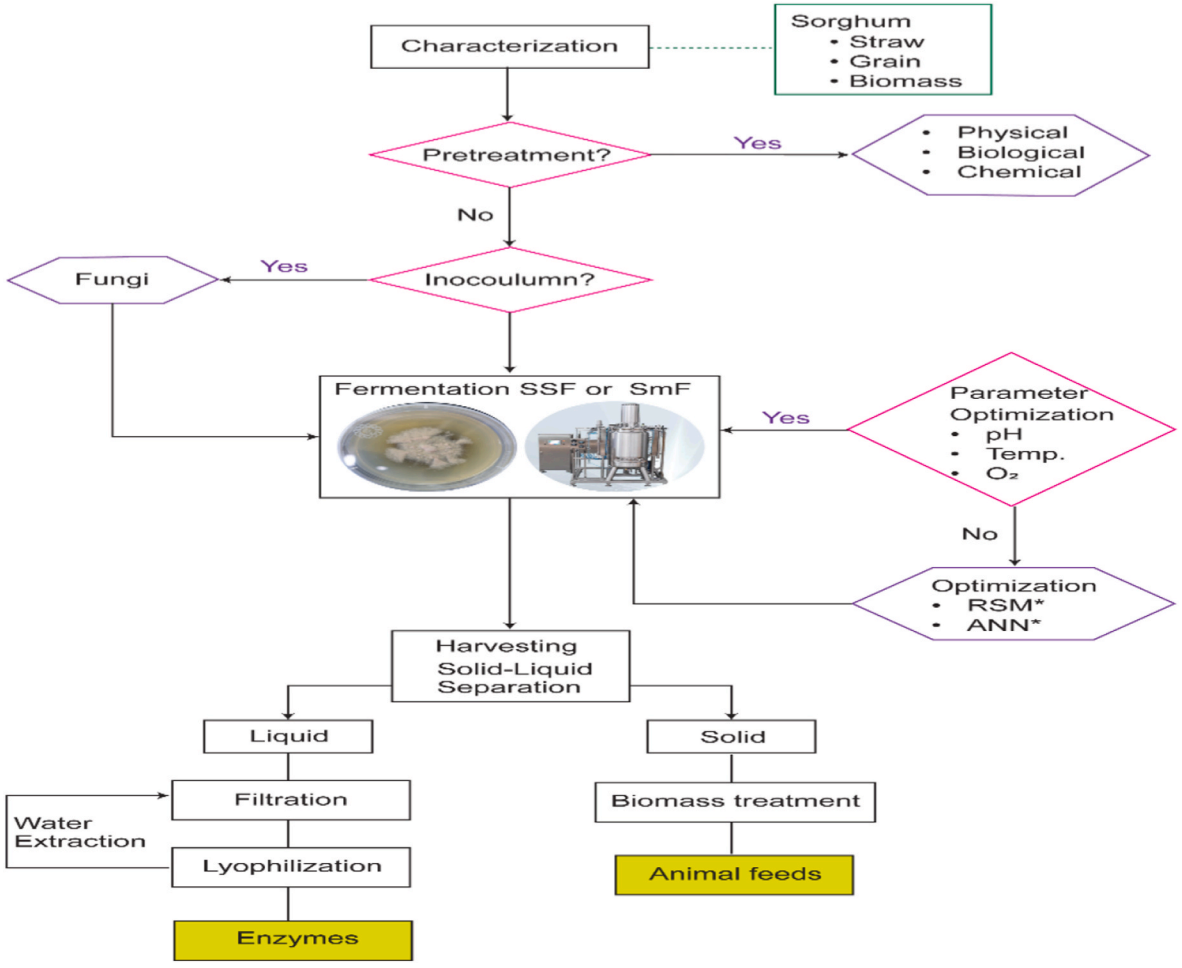

Fig. 4. Overall flowchart for enzyme production process using sorghum.
(Note: ANN: Artificial Neural Network; RSM: Response Surface Methodology; Temp: temperature).

proves to be costly, especially when industrially conducted through bacteria or fungi. As a result, the traditional method of enzyme production using bacteria or fungi is a costly process. To address this, utilizing grains and biomass of crops like sorghum, wheat, and corn for enzyme production is suggested as a carbon and nitrogen source, although processing cellulosic feedstock presents a major challenge. To overcome this challenge, this study proposes developing an integrated biomedium capable of producing enzyme cocktails (α-amylase, glucoamylase, and xylanase) using *Aspergillus awamori*. Additionally, *Aspergillus oryzae* will be employed to produce protease in a separate fermenter, which will convert protein into free amino nitrogen (FAN) as a nitrogen source, according to the study of Wang et al. [193], where a pre-fungal fermentation will be performed before the final fermentation as depicted in Fig. 6.

The integrated method illustrated in Fig. 6 outlines potential approaches for enzyme and biofuel production. This process can be approached in two different scenarios. Scenario 1: involves producing two different fungal strains in the same bioreactor (solid-state fermentation). For protease and free amino nitrogen (FAN) production, *Aspergillus oryzae* and *Aspergillus nidulans* are the best options, as shown in Table 12. Conversely, *Aspergillus awamori* and *Aspergillus niger* are optimal for producing amylase and glucoamylase. These strains can generate a sufficient amount of total reducing sugars (TRS) and FAN through the saccharification of starch and lignocellulosic materials from sorghum (shown in Fig. 7). In this scenario, commercial enzymes like α-amylase, glucoamylase, and protease, as well as nitrogen sources like yeast extract and urea, are not required. After fungal fermentation, solid-liquid separation via filtration allows the liquid portion to be used for biofuel or specific enzyme production, as it already contains high amounts of sugar, protein, and FAN for the second fermentation process. The solid biomass, rich in protein, digestible starch, and short-chain fatty acids, can be used as animal feed, which is in high demand for ruminants. Additionally, using fungal strains for enzyme production simplifies product recovery because the enzymes are secreted, eliminating the need for high-tech equipment like homogenizers for cell disruption or ion-exchange chromatography (IEX) and hydrophobic interaction chromatography (HIC) for protein purification, making the process cost-effective.

Scenario 2: involves conducting two separate solid-state fermentations for TRS, FAN, and enzyme production, which are then integrated into a single bioreactor (Fig. 5). This process requires two fermenters, making maintenance relatively more challenging, but its yields will be higher compared to Scenario 1. The disadvantage of Scenario 1 is that the optimal conditions for the two different fungal strains and enzymes are not the same. Additionally, protease can degrade other enzymes, such as amylase, glucoamylase, and cellulase, leading to lower product yields. To address this issue, a protease inhibitor could be added to the medium, but this would increase production costs. Therefore, Scenario 2 is proposed as an integrated method, illustrated in Fig. 6.

This biomedium will produce sufficient amounts of sugar and FAN for enzyme as well as bioethanol production, eliminating the need for additional carbon and nitrogen sources and leading to substantial cost savings. Additionally, it offers an efficient approach to converting lignocellulosic material into sugar for second-generation biofuel

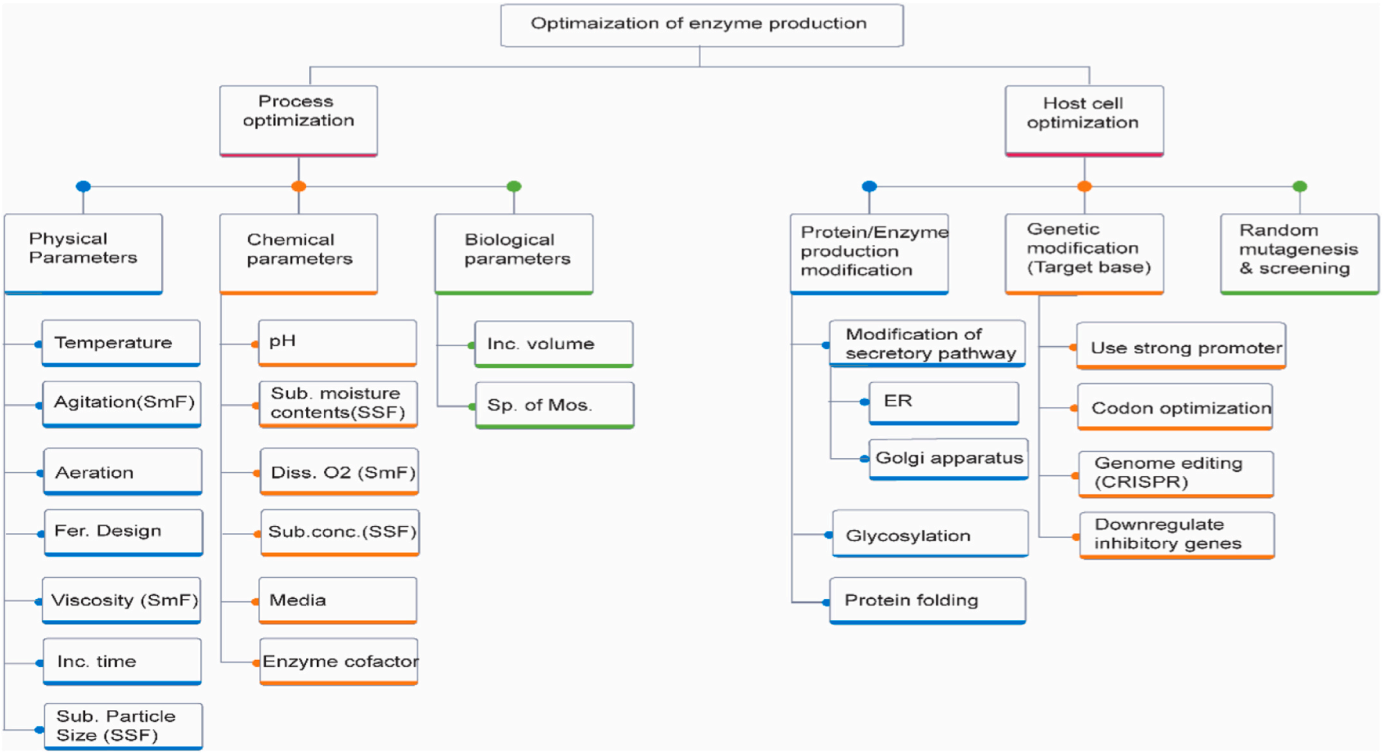

Fig. 5. Factors and their classification for optimization.
(**Note**: **Fer:** Fermentation; **Inc:** Incubation; **Sub:** Substrate; **Diss**: Dissolve; **Conc:** Concentration; **Mos:** Microorganisms, **Sp**: Species; **ER:** Endoplasmic Reticulum; **SSF**: Solid State Fermentation; **SmF:** Submerged Fermentation).

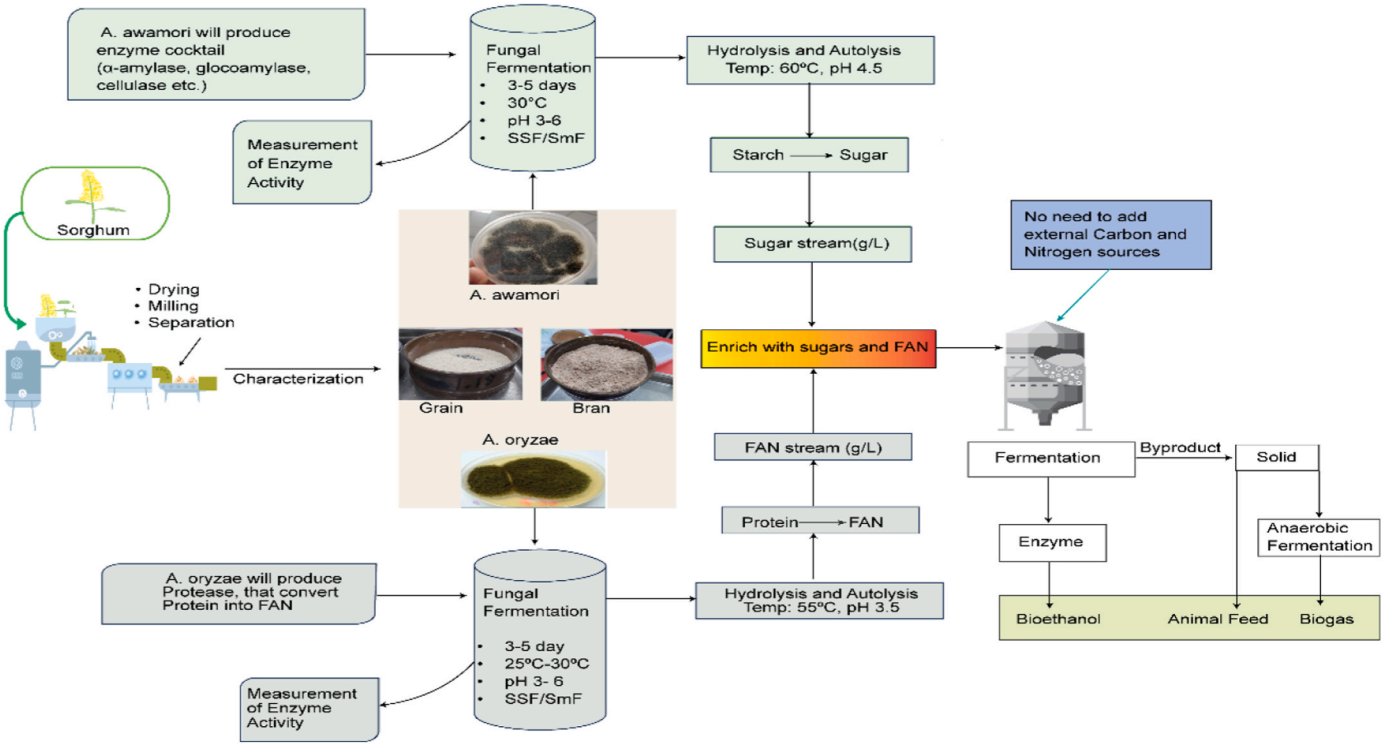

Fig. 6. Integrated method for enzyme production.

production without depending on external enzymes and costly pre-treatment methods for saccharification. (Details in Fig. 7).

As a result, the cost of enzyme and second-generation biofuel production will be reduced. As per the proposed method for 1L media volume to produce sufficient sugar as a carbon source, FAN, and enzyme cocktail fermentation, the cost will be only $0.00035. Cost estimation has been calculated by the following equation:

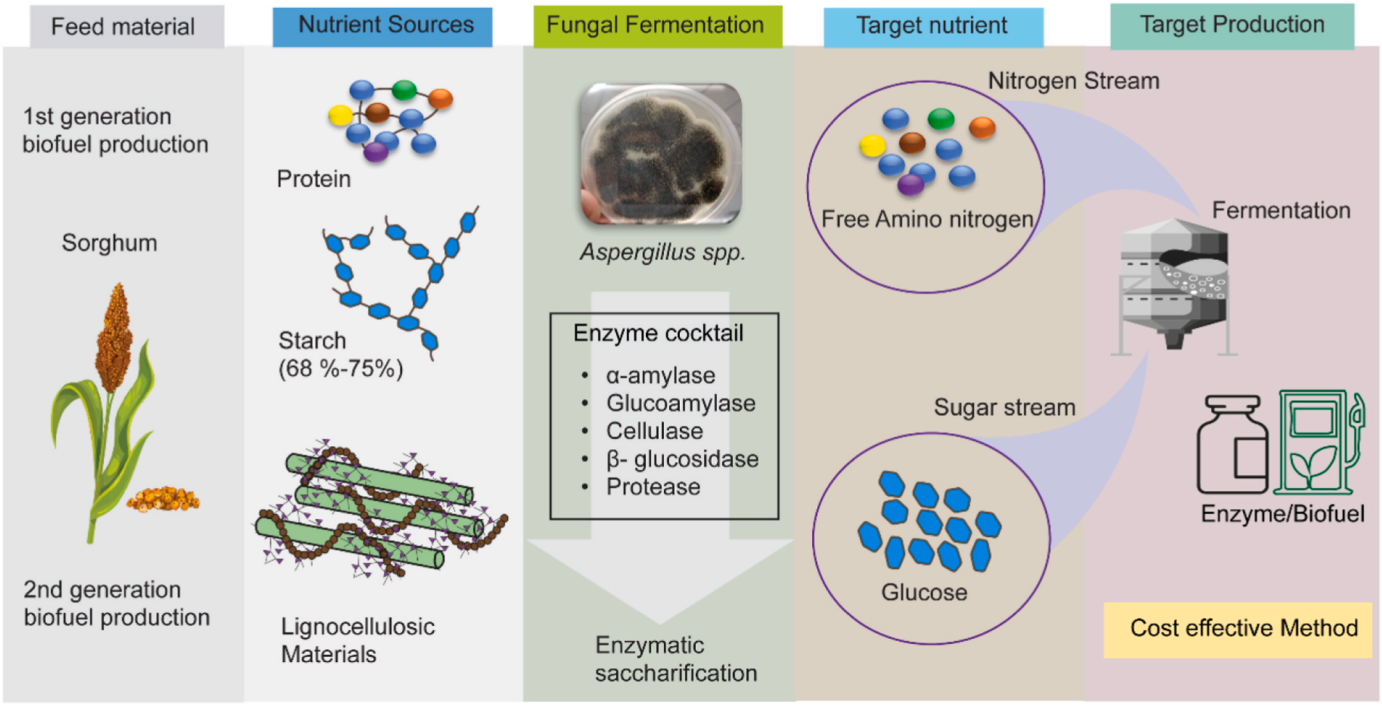

Fig. 7. Cost-effective 1st and 2nd generation biofuel production with the integrated method using Sorghum grain and biomass.

Cost : 1000g X 4.184 J / g X ΔT. equation 1

So, energy requirement, 1000 x 4.148 x 10 = 41840J.
Electricity cost: 41840J/3600000.
= 0.0116kWh, [If, 1kWh cost 30 cents].
=0.348 cent or $0.00348.Where,
Culture volume: 1000 mL or 1000g.
ΔT: Temperature increase 20–30 °C (10 °C), energy requirement for 10 °C increase 4.184J/g.

This process offers two key benefits: 1. Hydrolysis of cellulosic material and conversion to sugar and free amino nitrogen, and 2. Elimination of the need for external carbon, nitrogen, and enzyme sources.

In Table 10, numerous factors related to the fermentation process have been discussed, indicating that a significant amount of data will be generated for the optimization process. Consequently, it becomes challenging for an individual to analyze this vast amount of data efficiently and identify the optimal parameters. So, artificial neural network (ANN) or response surface methodology (RSM) would be an effective method for process optimization and finding the best parameters to produce the maximum yield of the enzyme. In a study conducted by Thanapimmetha et al. [135], Response surface methodology (RSM) employing Box-Behnken Design (BBD) was utilized to optimize moisture content (77.5 %), inoculum size (10.5 %), and incubation time (56 h) during the LSF of sorghum. However, recent research suggests that pH, temperature, substrate particle size, aeration, and agitation are equally crucial parameters for both solid-state and submerged fermentation processes [125]. In addition, Manan and Webb [125] mentioned some important physical characterization techniques that are crucial for optimizing solid-state fermentation (SSF), such as porosity measurement for air circulation, bulk density, and particle density assessments for understanding structural changes, specific surface area measurements to enhance enzyme-substrate contact, and tortuosity analysis to evaluate gas diffusion within the substrate bed. Utilizing these methods, researchers and industrial practitioners can select suitable substrates, adjust moisture content, and enhance microbial growth and product formation efficiently.

Venkateswarulu et al. [194] reported that, after variables optimization using RSM lactase production increased significantly to 91.32 U/ml, representing a remarkable 3.48-fold improvement over the traditional process. Another study conducted by Iram et al. (2022) revealed that the activity of cellulase increased from 0.6 IU/ml to 0.82 IU/ml, and xylanase activity also increased from 3.99 IU/ml to 52.76 IU/ml after undergoing optimization. They have done an optimization process, utilizing Response surface methodology (RSM) with *A. niger*, which involved employing a 6.5 % inoculum size, 310 rpm agitation rate, and 1.4 vvm aeration rate.

5.3.2. *Host cell optimization*

Since the development of the initial commercially recombinant enzyme, research in fungal biotechnology has focused on enhancing the yield of enzyme(s) within a specific host organism. Choosing the best fungal host to produce a recombinant enzyme is challenging. Sometimes, a prediction-based model for host selection is not suitable, so screening for multiple hosts is the best option whenever possible. Ideally, the cellular machinery (secretion, glycosylation, protein folding, etc.) of the host should match what's needed for enzyme production. Another additional reason to choose fungal hosts most broadly for enzyme production is that several species, such as *A. niger*, *A. oryzae*, and *Trichoderma reesei* been used safely for enzyme production for a long time [195].

Enzyme production can be enhanced through host cell modification using three strategies. The first involves classical mutagenesis and screening, where genetic modification is not predetermined. This method entails inducing random mutations within the genome and subsequently assessing the host's ability to produce the desired enzyme or protein. Classical mutagenesis and screening methods have long been employed to enhance the production of small molecules [196] as well as enzymes. Cherry et al. [197] developed a strain of *A. niger*, which can produce more glucoamylase production than the normal *A. niger* strain using classic mutagenesis techniques.

The second approach is targeting base genetic modification, where modification is predetermined, and this method ensures the expression of the target gene. Genetic modification can be done in various ways, including using stronger promoters (glaA, gpdA, chb1) [198], optimizing codon sequences for better translation, deleting genes that

encode endogenous proteases (pyrG), using gene fusions to enhance secretion, and overexpressing accessory cell machinery like chaperones and protein fusions [199,200]. Sui et al. [201] reported that *A. niger* DS03043, an industrial strain containing seven copies of the glucoamylase-coding gene *glaA*, produces significantly higher yields of glucoamylase compared to the normal strain. Altering the amino acid sequence of the enzyme for increased secretion and/or yield may also be a valid approach [202,203]. Increasing carbohydrate-degrading activity by activating the ACE3 transcription factor and the regulatory pathways CreA/Cre1 and PkaC/PkaC2 (signal transduction pathways), along with the activation of the *TEMER07589* gene to enhance cellulase degradation are important mechanisms for enhancing enzyme production using *Aspergillus* strains [198,204,205]. Details are described in Table 14.

In the field of industrial biotechnology, the use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has revolutionized the process of strain development. A review by Deng et al. [208] explores CRISPR systems and tools for genome editing in filamentous fungi. By using CRISPR-Cas9-mediated gene editing, precise alterations such as specific point mutations and gene deletions can now be achieved with efficiencies nearing 100 % [203]. Liu et al. [209] reported that using CRISPR/Cas9, they successfully mutated the imported *amdS* gene in the genome, targeting genes involved in the cellulase production pathway, including *cre-1, res-1, gh1-1,* and *alp-1*. This genome-editing approach generated multiple strains with enhanced hyper-cellulase production, resulting in a 5-fold increase in extracellular protein secretion and a 13-fold increase in lignocellulase activity compared to the parental strain in *M. thermophila*.

Optimization of protein secretion is the third step, and very little is understood about the secretory mechanism. Protein secretion pathways consist of the transfer of a nascent protein to the endoplasmic reticulum (ER), its appropriate folding, posttranslational modification, and maturation in the Golgi apparatus. If possible, shifting enzyme production from intracellular to extracellular pathways by modifying the secretory mechanism will offer numerous advantages in industrial bioprocesses, including simplifying purification processes, reducing purification steps, time, and, ultimately, production cost. It is a great advantage to use fungal host cells for commercial enzyme production because most of the enzymes are secreted in the medium during fungal fermentation. Fungi such as *A. niger* and *A. oryzae* can adjust the amount of protein in the secretory pathway, which may be advantageous for their survival in natural environments. Adding another protein with the desired protein (fusion protein) can be a viable approach to change the secretory pathway. Ward et al. [210] successfully increased the yields of chymosin by fusing endogenous glucoamylase with calf chymosin in *A. awamori*.

During optimization processes, vast amounts of experimental and predicted data are generated, posing challenges for manual calculation and precision. Recent advancements in omics technology, including transcriptomics, proteomics, and metabolomics, along with artificial intelligence, offer the potential to predict or create precise models for optimization to achieve optimal results. Artificial neural network (ANN) is a modern optimization process that gives more accurate predictions than RSM. Research by Venkateswarulu et al. [194] indicates that accurate predictions were achieved by both the Response surface methodology (RSM) and the Artificial neural network (ANN) models. However, when it came to predicting lactase production, the ANN model outperformed RSM with a higher coefficient of determination (R^2 = 0.99456) compared to RSM (R^2 = 0.9496).

Artificial neural network (ANN) tools can be effectively used for the optimization of fermentation processes by modeling complex relationships between input variables (such as nutrient concentrations, pH, temperature, etc.) and the output variables (such as product yield, biomass concentration, etc.). Fig. 8 demonstrates the optimization process.

Table 14
Mechanisms and target pathways of host cell modification to enhance the production capability of *Aspergillus*.

Mechanism	Target gene/pathway	Significance	Host cell	References
Increase carbohydrate degrading activity	**Transcription Factors (TF):** ➢ Activation of ACE3 TF and binds to cellulase-coding genes. ➢ Trigger cellulase production **Regulatory Pathways:** ➢ CreA/Cre1 ➢ CreB and CreC **Signal Transduction Pathways**: Gene involved PKA pathway (pkaC1 and pkaC2, acyA or acyB)	➢ Breakdown of complex carbohydrates into simpler sugars ➢ Convert biomass into products such as biofuels and biochemicals. ➢ Enhance Carbohydrate Active Enzymes (CAZymes) by filamentous fungal cells.	• *T. reesei* • *A. niger* • *A. oryzae* • *A. sojae*	[198]
Increase Cellulase degradation	➢ Activation of TEMER07589 gene (belongs to GH61 family)	➢ Increases the activity of cellobiohydrolase (CBH) and beta-glucosidase (BG) ➢ Leading to enhance cellulose degrading activity	• *A. fumigatus*	[205]
Enhance glucose conversion	➢ Inactivation of agdA gene	➢ Inhibit cell wall component degradation of fungus	• *A. niger*	[206]
Protect enzyme from endogenous protease	➢ Disruption of endogenous protease pyrG	➢ Minimizing proteolysis ➢ Enzyme production can be significantly improved.	• *A. oryzae* • *A. niger*	[204]
Increase enzyme production (Strong promoter)	**Commonly used strong promoter** ➢ Glucoamylase gene (glaA) in *A. oryzae* ➢ glyceraldehyde-3-phosphate dehydrogenase gene (gpdA) in *A. nidulans* ➢ α-amylase gene (amyB) in *A. oryzae* ➢ Cellobiose hydrolysis enzyme gene (cbh1) in *T. reesei*	➢ RNA polymerase more readily and efficiently, leading to higher rates of gene transcription ➢ Yield of the desired enzyme increase	• *A. oryzae* • *A. nidulans* • *T. reesei*	[198]
Integrate high copy number of gene	➢ Amplification 216 kb region of glaA genes produce more glucoamylase compared to single copy gene	➢ Enhance glucoamylase production	• *A. niger*	[197]
heterologous protein production	➢ Long non-coding RNA (Hax1) helps to increase protein production	➢ overexpressing Hax1 leads to a significant increase in cellulase activity	• *T. reesei*	[207]
Modification of secretory pathway	➢ Using protein localization tags ➢ Protein fusion	➢ Extracellular enzymes produce ➢ Easy to purification ➢ Cost effective	• All filamentous fungi	[203]

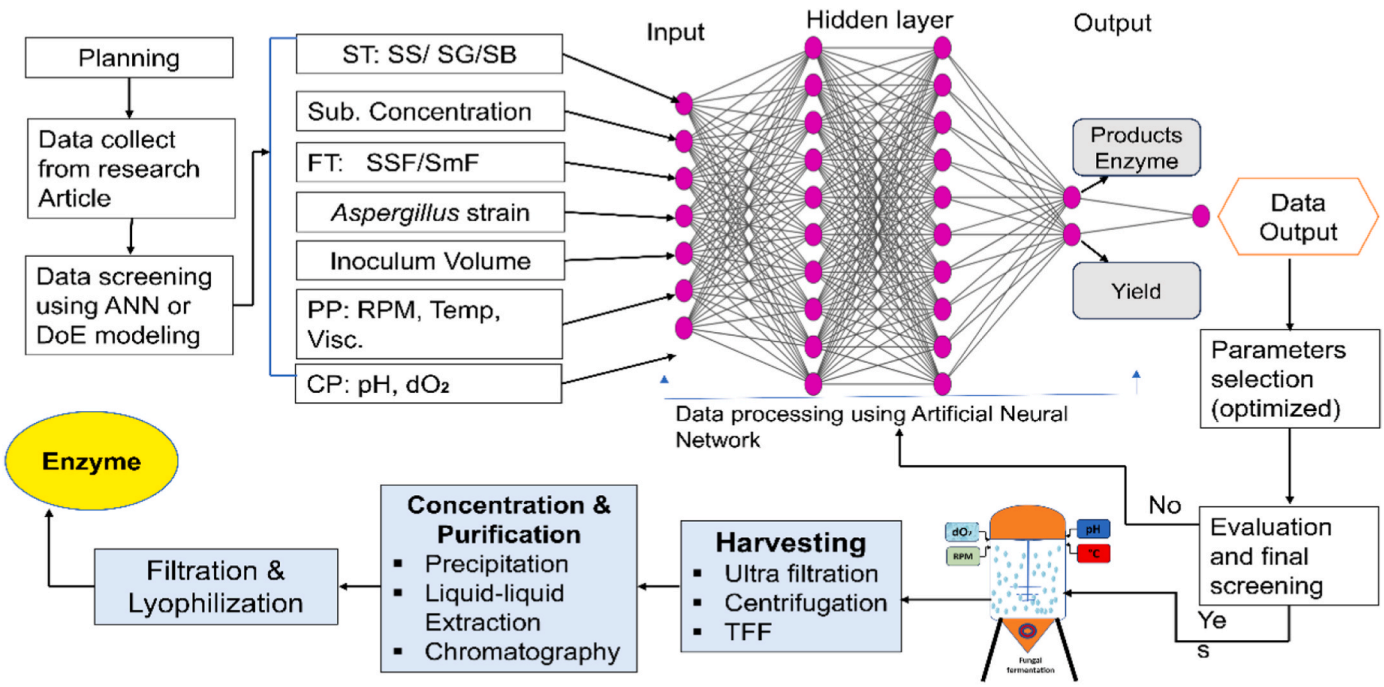

Fig. 8. Optimization and production process flowchart.
(Note: ST: Substrate type; SS: Sorghum straw; SG: Sorghum grain; SB: Sorghum bran; FT: Fermentation type; SSF: Solid state fermentation; SmF: Submerged fermentation; PP: Physical parameter; CP: Chemical parameter; Sub: Substrate; Visc: Viscosity; Temp: Temperature).

To illustrate, if our goal is to enhance the production of a particular enzyme, we must consider all the factors related to fermentation. We can then use techniques like Artificial Neural Network (ANN), or Response Surface Methodology (RSM) to screen these parameters. Finally, it enables smooth scale-up from lab to commercial production, which is essential for industrial applications.

6. Challenges and solutions in the fungal fermentation process

The fungal fermentation process for industrial enzyme production presents several challenges that need to be addressed for optimal yield and efficiency. Challenges include controlling the morphology of filamentous fungi, managing high viscosity in the fermentation broth that limits oxygen transfer, and ensuring proper aeration for productivity. The morphology of fungi, ranging from dispersed mycelium to dense pellets, can impact aeration and productivity. High viscosity in the fermentation broth hinders oxygen transfer, leading to reduced enzyme yield.

6.1. *Technological challenges for enzyme production*

There are many technological challenges associated with enzyme production from lignocellulosic material. For example, lignocellulosic biomass is depolymerized into monomers through complex enzymatic pathways, where the biomass-to-enzyme ratio, time, and agitation speed are crucial factors for efficient hydrolysis. However, residual lignin acts as an inhibitor, and the synergistic action of cellulolytic enzymes enhances sugar generation despite the presence of inhibitors [211]. High substrate loading and mass transfer of enzymes and soluble products slow down due to diffusional limitations, which increase with elevated substrate concentration. Higher substrate and enzyme concentrations also raise viscosity, hindering enzyme diffusion and resulting in lower glucose yield. A higher agitation speed and optimizing the enzyme-to-substrate ratio can improve sugar production from pre-treated biomass [212]. de Godoy et al. [213] demonstrates that, hydrolysis was conducted in both batch and fed-batch modes using various substrates at a concentration of 15 FPU/g enzyme. In the fed-batch operation, a 15 % (w/v) substrate was supplemented with 5 % substrate after 6 h, repeated three times. This method achieved a 66.16 % glucose yield from 24 % biomass loading, with 127 g/L glucose produced at 20 % solid loading in fed-batch mode, compared to 115.54 g/L in batch mode. So, fed batch culture is more effective than batch fermentation for the hydrolysis process.

Microbial contamination is common in fermentation processes, as substrates are often not sterile [214]. Proven strategies to minimize the risk of contamination include steam pre-treatment of the substrate, aseptic conditions and clean rooms. In addition, solid-state fermentation with filamentous fungi operates in the absence of free water and at low pH (3–5), which prevents bacterial growth. Batch fermentation is also preferred to prevent unwanted contamination. Table 15 summarizes some technological challenges of SSF and also discusses solutions as adopted by Manan and Webb [2].

Table 15
Technological challenges associated with SSF and potential solutions.

Challenges of SSF	Idea to solve the challenges
Engineering challenges arise from temperature buildup, pH control, oxygen transfer, and the gradients of mass, heat, substrate, and moisture.	Monitor the accumulation of temperature, oxygen, and carbon dioxide gradients in real-time to manage and regulate the system effectively.
Inconsistent distribution of cell mass, nutrients, temperature, pH levels, and moisture content.	Real time monitoring with all parameters and set critical quality attributes range for every parameters.
Achieving consistent aeration across the substrate is challenging.	Controlled by forced aeration which simultaneously regulates the temperature.
Heat generated by microbial metabolism and growth raises the solid substrate temperature, leading to moisture loss or the formation of a watery substrate.	Creating temperature gradients that can effectively dissipate metabolic heat
Growth and kinetics studies still difficult due to the limited and scattered nature of available information.	Mathematical models for accurate data prediction and optimization.

6.2. Logistical challenges

Logistical challenges also an important factor along with process (bioreactor, saccharification tank, centrifuge machine, filtration unit, seed fermenter, freeze dryer, solvent recycler, Ion exchange column) storage(temperatures sensors, freezer, moisture controller) and utilities facilities (water, electricity) and operation (material cost, engineer, labour, operator cost) [215]. Table 16 presents the logistical challenges, their consequences, and potential solutions for developing a sustainable production facility.

Overall, by addressing these challenges through a combination of technological advancements and scientific innovation, fungal fermentation processes can be optimized for increased enzyme production, reduced costs, and enhanced sustainability.

7. Future perspective

The future perspectives of enzyme production processes using *Aspergillus* strains and sorghum as substrates hold significant promise for sustainable and efficient bioprocessing applications. As the demand for industrial enzymes continues to grow across various sectors, including food, feed, biofuels, and pharmaceuticals, the utilization of *Aspergillus* species known for their robust enzyme-producing capabilities presents a valuable opportunity [218]. Using the proposed integrated method for biofuel, production costs will be reduced by 5 % and 8 % for 1st and 2nd generation of biofuel production respectively [219] as there is no need to add commercial enzymes for hydrolysis. By leveraging the enzymatic potential of *Aspergillus* strains in conjunction with sorghum biomass as a renewable and cost-effective substrate rich in cellulose and hemicellulose, future enzyme production processes can be optimized for enhanced productivity and resource efficiency. Fungi, particularly *Aspergillus* species, stand out as significant contributors to enzyme production, supplying a substantial portion of commercial enzymes. This fungal species exhibits remarkable stability and can thrive in harsh environments, making them ideal candidates for large-scale industrial enzyme production. Conversely, sorghum, a sustainable and abundant agricultural crop, as a substrate presents an eco-friendly alternative to conventional feedstocks.

8. Conclusions

This paper has reviewed the potentiality of sorghum and *Aspergillus* strains for enzyme production, several research gaps (effect of particle size distribution, moisture content for solid-state fermentation), optimizing enzyme production processes, and developing an integrated process to produce 1st and 2nd generation biofuel at low cost. Additionally, comparing submerged (SmF) and solid-state fermentation (SSF) methods, assessing yield variations for enzyme production. The advancements in genetic engineering and strain optimization techniques offer opportunities to enhance enzyme yields and tailor enzyme properties to meet specific industrial requirements. Integrating omics technologies and computational modeling enables deeper insights into metabolic pathways and fermentation dynamics, facilitating the design of more efficient enzyme production processes. The exploration of novel fermentation strategies, such as consolidated bioprocessing and integrated methods, holds promise for streamlining enzyme and biofuel production processes and reducing overall costs. Overall, the synergy between *Aspergillus* strains and sorghum substrates, coupled with technological innovations, underscores a promising future for enzyme production processes, driving sustainable and efficient biomanufacturing practices.

Table 16
Logistical challenges, consequences with solutions.

Logistical challenges	Consequences	Solution	References
1. **Supply chain** management ➢ Consistency of raw materials ➢ Seasonal variations	➢ Affect the production flow	➢ Establish a strong partnership with suppliers ➢ Engagement with waste management companies	[215,216]
2. **Transportation Issue** ➢ Transportation cost ➢ Contamination risk	➢ Increase production cost ➢ Product quality may decrease	➢ Route optimization ➢ Schedule collection	[216,217]
3. **Regulatory compliance** ➢ Waste management ➢ Product Safety standards	➢ Required additional infrastructure ➢ Final products can add complexity to the logistics	➢ Modular processing unit (modular bioreactor system that can be scaled up and down ➢ Integrated waste processing (market demands and availability)	[215,217]
4. **Enhance quality** control	➢ lack of quality control may lead to deviations in product yield and quality	➢ Training staff ➢ Regular quality assessments ➢ Monitoring and automation ➢ Conduct pilot projects	[216]

CRediT authorship contribution statement

Pratul Dipta Somadder: Conceptualization, Planning, Writing-manuscript, tables and figures. **Antoine Trzcinski:** PhD: Planning, Conceptualization, Writing – review & editing, Project administration. **Guangnan Chen:** PhD: Planning, Writing – review & editing, Project administration. **Yvonne Chow:** PhD: Planning, Conceptualization, Writing – review & editing. **Musaalbakri Abdul Manan:** PhD: Planning, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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