

## Review Article

# *Aspergillus nidulans*: A Potential Resource of the Production of the Native and Heterologous Enzymes for Industrial Applications

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*Aspergillus nidulans* is a filamentous fungus that is a potential resource for industrial enzymes. It is a versatile fungal cell factory that can synthesize various industrial enzymes such as cellulases,  $\beta$ -glucosidases, hemicellulases, laccases, lipases, proteases,  $\beta$ -galactosidases, tannases, keratinase, cutinases, and aryl alcohol oxidase. *A. nidulans* has shown the potential to utilize low-cost substrates such as wheat bran, rice straw, sugarcane bagasse, rice bran, coir pith, black gram residue, and chicken feathers to produce enzymes cost-effectively. *A. nidulans* has also been known as a model organism for the production of heterologous enzymes. Several studies reported genetically engineered strains of *A. nidulans* for the production of different enzymes. Native as well as heterologous enzymes of *A. nidulans* have been employed for various industrial processes.

## 1. Introduction

Enzymes have various kinds of industrial applications including pulp and paper, laundry, food, animal feed, brewery and wine, textile, and bioenergy industry [1–3]. Commercial production of enzymes had a market of \$4.9 billion in 2015 and an expected annual growth rate of 4.7% for 2016–2021 [4, 5]. Cost-effective and efficient industrial manufacturing is achieved with the utilization of enzymes when compared to chemical catalysts. Enzymes are highly specific and environmentally friendly and can work under mild conditions [2, 6]. Enzymes are produced by several microorganisms such as bacteria, actinomycetes, and fungi, but fungi are of great interest because they secrete extracellular enzymes in higher quantity [7]. Filamentous fungi, including Aspergilli, are widely used for industrial production of enzymes [4]. *A. nidulans* is a versatile fungal resource that has shown the potential for the production of different enzymes including cellulase [8],  $\beta$ -glucosidase [9], xylanase [10], laccase [11], lipase [12], protease [13],  $\beta$ -galactosidase [14], tannase [15], keratinase [16], cutinase [17], and aryl alcohol oxidase [4]. This review is focused on the potential of wild strains of *A. nidulans* for native enzyme production and the capability of expression of heterologous enzymes.

Fungi are used in industrial protein production due to their excellent ability to secrete a large quantity of proteins [18]. Heterologous protein expression in filamentous fungi has been extensively studied due to their natural ability to produce a large quantity of hydrolytic enzymes for industrial processes at large scale. In a general protein expression, a selected gene that encodes the target enzyme is expressed in a fungal cell factory such as *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, and *Aspergillus nidulans* [2, 19]. Filamentous fungi belonging to the genus *Aspergillus* are commonly associated with biomass degradation and produce a wide range of hydrolases. *Aspergillus* spp. are considered suitable host organisms for industrial enzyme production due to their high secretion capacity, GRAS (generally regarded as safe) status, rapid growth on inexpensive media, and a large range of native enzymes produced [20]. *A. nidulans* has been established as a model organism for the secretion of a large quantity of heterologous enzymes. It is a well-recognized filamentous fungus that is widely used for bioengineering research. Its physiology, morphology, and optimal growth conditions are well characterized [21–23]. In the last few years, several investigations have been performed on *A. nidulans* as a host organism for the production of different heterologous enzymes such as lipase

[22], xylanase [23], xyloglucanase (GH12) [24], endoxylanase (GH11) [25], arabinofuranosidase (GH54) [25], and aryl alcohol oxidase [4].

The life cycle of *A. nidulans* progresses through both sexual and asexual reproduction stages. These are controlled by diverse environmental factors such as light, oxygen, and nutrient availability. Generally, sexual reproduction starts after asexual conidiation and encouraged by oxygen limitation and lack of light. *A. nidulans* forms a closed fruiting body known as cleistothecium (Figures 1(a) and 1(b)) [27]. Hulle cells are characteristic structures normally produced by some species of the genus *Aspergillus*. These cells are found in the association of cleistothecia of all ascogenous species of the *Aspergillus nidulans* group (Figure 1(b)) [28].

Classification is as follows:

Kingdom: Fungi  
 Phylum: Ascomycota  
 Class: Eurotiomycetes  
 Order: Eurotiales  
 Family: Trichocomaceae  
 Genus: *Aspergillus*  
 Species: *nidulans* [12]

## 2. Enzyme Production by *A. nidulans*

Enzyme production through fungi has been carried out under solid-state fermentation (SSF) and submerged fermentation (SmF). SmF has been given more attention due to easy control and measurement of fermentation parameters, reduced fermentation time, and basic methods to harvest and purify enzyme products [2, 29]. In recent years, intensive research has been performed on SSF and it has earned credibility due to its low water consumption, fewer contamination chances, low energy requirement, and high product yield [9, 26]. Although *A. nidulans* have shown potential for enzyme production under both SSF and SmF, most studies report enzyme production by *A. nidulans* under SmF (Table 1). The fermentation parameters such as fermentation medium, duration, pH, and temperature affect enzyme production under SSF and SmF. The moisture content is a critical parameter under SSF while shaking or static conditions are crucial during SmF [39] (Table 2). The availability of a suitable nitrogen source and the supplementation of surfactants are also essential factors that determine enzyme synthesis. The development of a suitable medium for microbial growth can potentially reduce medium cost and improves enzyme production.

### 2.1. Utilization of Low-Cost Substrate for Enzyme Production.

Enzyme production is dependent on the nature of carbon sources, favorable degradability, bare chemical composition, physical associations, accessibility of substrate, and presence of some nutrients [9, 35]. The higher production cost of an enzyme makes the final product expensive. Therefore, economic alternates for enzyme production are in the focus of researchers. During the fermentative production of

enzymes, the substrate cost has been considered up to 30% of total production cost. The use of agricultural residues or other wastes as a substrate represents a viable option for the cost-effective production of enzymes [1, 44]. Several agricultural residues or wastes have shown potential for the induction of various kinds of enzymes by *A. nidulans*.

**2.1.1. Wheat Bran.** Wheat bran has been proved a suitable substrate for enzyme production due to several reasons. Wheat bran is a rich source of fiber, minerals, vitamins, and phenolic compounds [45]. It is a suitable source of nitrogen compared to other agroresidues. Wheat bran has been reported having a minimum C/N ratio that is favorable for enzyme production [26]. Wheat bran remains loose during moist conditions and, therefore, provides a large surface area for fungal growth and enzyme production [26, 46]. The worldwide availability of wheat bran also makes it a potential substrate for enzyme production. Worldwide wheat production is estimated at 690 million tonnes annually. The weight ratio of bran to milled wheat is about 25 : 75. Hence, wheat bran generation is estimated at 150 million tonnes all over the world. So, the properties and availability of wheat bran represent it as a potential substrate for enzyme production [43, 45]. Taneja et al. [10] studied the production of alkaline and thermostable xylanase by *A. nidulans* KK-99 and used wheat bran as a substrate for xylanase production (40 IU/ml) under SmF [10].

Wheat bran was employed as a low-cost substrate by *Emericella nidulans* NK-62 for xylanase production. Czapek's mineral solution supplemented with 2% wheat bran resulted in 362 IU/ml of xylanase under SmF after seven days of incubation [31]. During cocultivation of *A. nidulans* AKB-25 and *Penicillium* sp. AKB-24, 50% wheat bran and 50% black gram residue were used as a substrate for cellulase and xylanase production under SSF conditions. 4.04, 203.70, 3.70, 35.28, and 3674.94 IU/gds of FPase, endoglucanase, exoglucanase,  $\beta$ -glucosidase, and xylanase, respectively, were produced by cocultivation of *A. nidulans* AKB-25 and *Penicillium* sp. AKB-24 [26].

**2.1.2. Sugarcane Bagasse.** Bagasse is the main residue that remains after sap extraction from sugarcane in the sugar industry. The processing of each tonne of sugarcane produces 740 kg of juice and 260 kg of moist bagasse. Sugarcane bagasse is an abundant resource with easy accessibility, cost-effectivity, and compressibility [35, 47]. Therefore, bagasse can be a renewable resource for enzyme production by *A. nidulans*. Jabasingh and Nachiyar [35] utilized sodium hydroxide pretreated sugarcane bagasse for cellulase production by *A. nidulans* MTCC344 using response surface methodology under SSF conditions. The maximum endoglucanase activity (28.84 IU/gds) was observed at the moisture content of 60% and a temperature of 40°C [35].

**2.1.3. Rice or Paddy Straw.** Rice straw is exploited as a substrate for the cellulolytic and hemicellulolytic enzymes. Globally, about 744.995 million metric tonnes of rice were

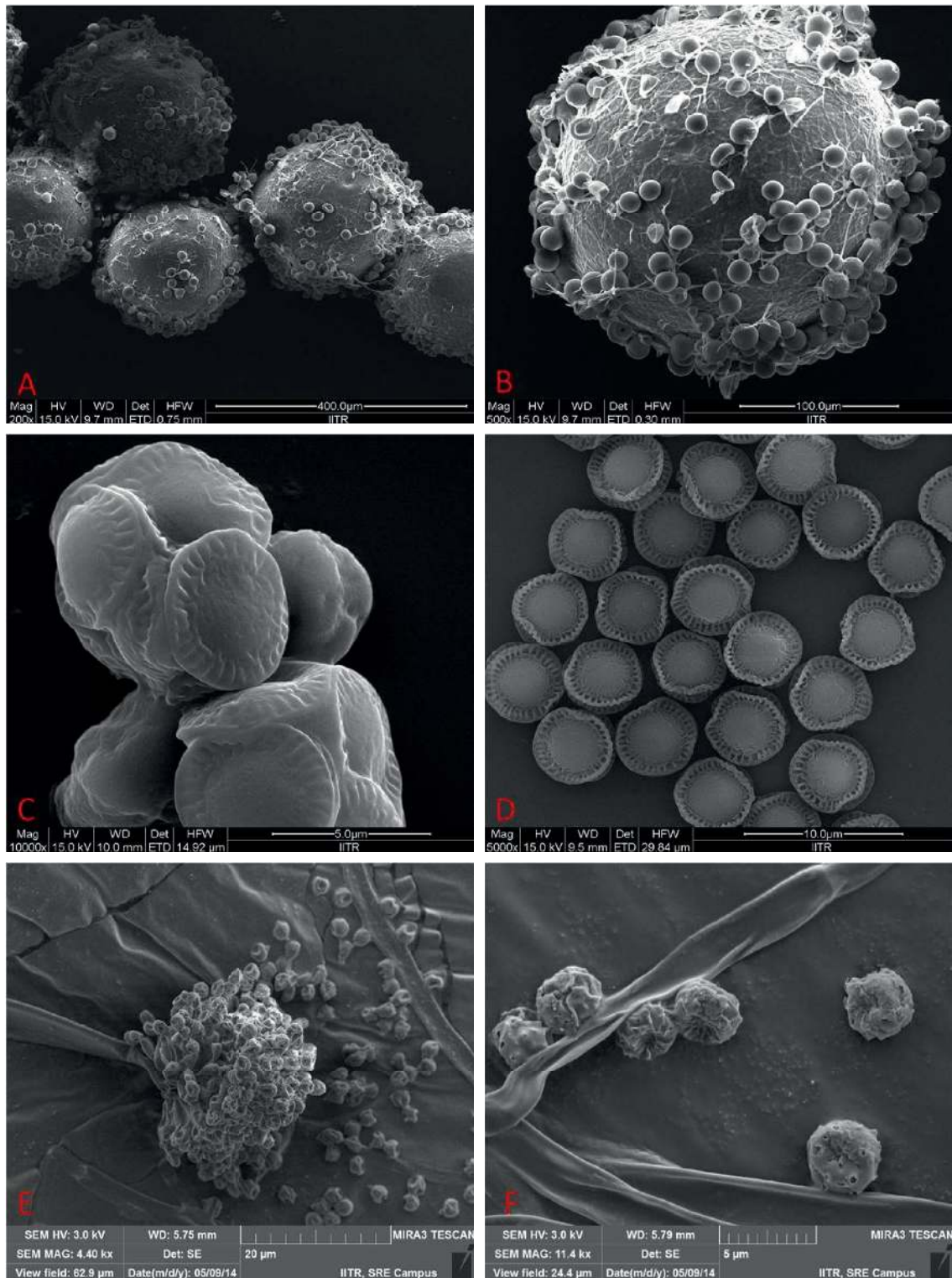


FIGURE 1: Sexual and asexual structures of *A. nidulans* AKB-25: (a) Cleistothecia, (b) close-up of peridium of cleistothecium with hulle cells, (c) developing ascus, (d) ascospores, (e) conidiophores, and (f) conidia [26].

produced during the year 2013-2014. Rice crop produces rice straw with a ratio of 1 : 1.5 for rice grain to rice straw. So, the huge generation of rice straw can be a cost-effective substrate for enzyme production [48, 49]. Bagga and Sandhu [33] utilized different carbon sources for cellulase

production by *A. nidulans* and observed that delignified bagasse, paddy straw, and wheat straw resulted in higher production of cellulase compared to carboxymethyl cellulose, xylan, pectin, lactose, and glycerol. Among agro-residues, tested paddy straw produced maximum cellulase

TABLE 1: Production of industrial enzymes from *Aspergillus nidulans*.

<i>A. nidulans</i> strain	Substrate	Type of fermentation	Fermentation conditions				Enzyme (yield)	Reference
			Initial pH	<i>T</i> (°C)	<i>I</i> (days)	<i>M</i> (%)		
<i>A. nidulans</i> AKB-25	Black gram residue	SSF	8.0	30	4	80	Endoglucanase (152.14 IU/gds), FPase (3.42 FPU/gds), Xylanase (2441.03 IU/gds), $\beta$ -Glucosidase (35.11 IU/gds)	[9]
<i>A. nidulans</i>	Coir pith	SSF	5.0	40	11	70	Endoglucanase (28.64 U/gds), FPase (10.23 U/gds), Cellobiase (4.31 U/gds)	[30]
<i>A. nidulans</i> KK-99	Wheat bran	SmF	10.0	37	6	—	Xylanase (40 IU/ml)	[10]
<i>Emicella nidulans</i> NK-62	Wheat bran	SmF	—	45	7	—	Xylanase (362 IU/ml)	[31]
<i>A. nidulans</i>	Carboxymethylcellulose	SmF	5.0	30	6	—	Cellulase (39.56 U/ml)	[32]
<i>A. nidulans</i>	Paddy straw	SmF	5.0	37	8	—	Endoglucanase (3.5 IU/ml)	[33]
<i>A. nidulans</i> SU04	CMC	SmF	3–7	40	8	—	Cellulase (60.54 U/ml)	[8]
<i>A. nidulans</i>	Corn cob	SmF	—	30	6	—	Xylanase (220 IU/ml)	[34]
<i>A. nidulans</i> MTCC344	Sugarcane bagasse	SSF	4.25	40	—	60	Endoglucanase (28.96 IU/gds)	[35]
<i>A. nidulans</i> (Mbl-S-6)	Rice bran	SmF	7.0	40	3	—	Lipase (102 IU/ml/min)	[12]
<i>A. nidulans</i>	Olive oil	SmF	—	30	2	—	Lipase (1121 mU/ml)	[36]
<i>Emicella nidulans</i> NFCCI 3643	Olive oil	SmF	6.0	30	4	—	Lipase (140 IU/ml)	[37]
<i>A. nidulans</i> LCJ249	Glucose, casein	SmF	7.0	30	6	—	Protease (2262.4 U/ml)	[13]
<i>A. nidulans</i> KF974331	Sucrose	SmF	—	30	8	—	Laccase (367.68 U/ml)	[11]
<i>A. nidulans</i> TTF6	Olga medium	SmF	6.0	40	5	—	Laccase (2.4 IU/mg)	[38]
<i>A. nidulans</i> (FT10)	Tannic acid	SmF	5.5	35	3	—	Tannase (96.2 U/ml)	[15]
<i>A. nidulans</i> K7	Chicken feather powder	SmF	7.5	35	5	—	Keratinase (55.8 U/ml)	[16]
<i>A. nidulans</i>	Xylose	SmF	3.0	35	10	—	$\beta$ -Galactosidase (60 U/mg)	[14]
<i>A. nidulans</i> PW1	Olive oil	SmF	6.5	37	1	—	Cutinase (1.44 IU/ml)	[17]

T: incubation temperature; I: incubation time of fermentation; M: moisture content in case of SSF.

[33]. da Silva Mennezes et al. [23] carried out SSF of rice husk by *Aspergillus nidulans* XynC A773 and produced 187.9 U/g of xylanase. The xylanase production was influenced by fermentation parameters such as basal medium, moisture content, and interactions between granulometry and inoculum size [23].

**2.1.4. Black Gram Residue.** Black gram is a pulse crop that is grown in Asian countries over an area of 3.10 million hectares, and the annual production of black gram is 1.40 million tonnes. The harvesting of crops generates a huge amount of black gram residue that might be a resource for the production of microbial enzymes [9, 50]. Kumar et al. [9] performed SSF for cellulase and xylanase production by *A. nidulans* AKB-25 using black gram residue as a substrate. Untreated black gram residue resulted in the maximum endoglucanase (152.14 IU/gds), FPase (3.42 FPU/gds), and

xylanase (2491.41 IU/gds) activities with the moisture content of 80% and 4 days of the fermentation period.

**2.1.5. Coir Pith.** Coconut pith or coir pith is a natural renewable resource that is a by-product of the fiber extraction process. The extraction of 1 kg of coir fiber generates 2 kg of coir pith and it contains approximately 25–29% of cellulose, 31% lignin, and 15% hemicelluloses. Coir pith has no economic value; it is dumped outside the coir industry in a huge amount. It has high ash content and poor combustion properties and emits high smoke when burnt. The high lignin content makes it unsuitable as the raw organic manure for crops [30, 51–53]. Therefore, the utilization of coir pith for enzyme can solve the problems associated with coir pith disposal. *A. nidulans* were isolated from degraded coir retting yarns of backwaters and used for cellulase production using pretreated coir pith as a substrate under SSF

TABLE 2: Factors affecting enzyme production by *A. nidulans*.

Factors	Description	Reference
Temperature	(i) Temperature is one of the most important fungal-dependent parameters that affect enzyme production. (ii) <i>A. nidulans</i> has been reported to synthesize the maximum amount of enzyme 30–45°C.	[26, 39]
Initial pH	(i) Fungal enzyme production is pH specific, and changes in pH affect the production and stability of enzymes. (ii) pH is easily maintained during SmF but it is difficult to control during SSF. The metabolic activities of microorganisms can change the pH of the medium during SSF. It may increase or decrease depending on the production or assimilation of organic acids, respectively. (iii) <i>A. nidulans</i> can produce enzymes in acidic, neutral, and alkaline ranges of pH (3.0–10.0) (Table 1).	[1, 40]
Incubation period	(i) The shorter incubation time for maximum enzyme production minimizes the chances of contamination and reduces the production cost. (ii) Different strains of <i>A. nidulans</i> have shown variation in incubation time from 1 to 11 days under SSF and SmF (Table 1).	[41, 42]
Initial moisture content (SSF)	(i) The moisture content of the substrate is another important parameter during SSF. (ii) Different microorganisms have a specific requirement in terms of moisture availability for their optimum growth and maximum enzyme production. (iii) <i>A. nidulans</i> is found to produce the maximum enzyme yield at initial moisture of 60–80%.	[43]

conditions. The maximum endoglucanase activity of 28.64 U/gds was observed at the moisture level of 70% and temperature 40°C with pH 5.0 [30].

**2.1.6. Rice Bran.** Rice bran is the by-product of the rice milling process with worldwide production of 50–60 million tonnes each year which is used as animal feed and pollutant adsorbent and for the production of rice bran oil. The amount of rice bran generated is for the excess of its local applications, thereby frequently leading to disposal problems [54–56]. It contains a substantial amount of proteins and oil; therefore, it is a potential substrate for lipase and protease production. Niaz et al. [12] tested different substrates such as wheat bran, rice bran, brassica bran, almond meal, and mustard meal for extracellular lipase production under SmF. Among all substrates, rice bran was found to induce maximum lipase (33.3 U/ml/min) by *A. nidulans* (Mbl-S-6).

**2.1.7. Chicken Feather.** Poultry houses generate a huge amount of feather waste that is an abundant keratinous material with 91% of  $\beta$ -keratin. It has been reported that daily accumulation of feather waste reached 5 million tonnes around the world. Generally, it is disposed of in dumps, land-filled, or burnt in incinerators that produce huge environmental pollution [57, 58]. Therefore, the utilization of feather waste as a substrate can minimize enzyme production cost and environmental pollution. Saber et al. [16] used the powder of chicken feather waste as the sole source of carbon and nitrogen for protease production by *A. nidulans* K7 (55.8 U/ml) under SmF conditions.

**2.2. Effect of Nitrogen Sources on Enzyme Production.** Fungal growth and synthesis of extracellular enzymes are influenced by the availability of precursors of protein synthesis. The suitable combination nitrogen source,

carbon source, and fungal strain are necessary for optimum maximum production of an enzyme [26, 39]. Filamentous fungi use various compounds as nitrogen sources. However, the ability of fungi to use different nitrogen sources is selective and preferable which is controlled by complex regulation systems at the transcriptional level [2, 59]. Various organic and inorganic nitrogen sources have tested for the production of different enzymes by *A. nidulans*. Liu et al. [2] developed a cost-effective medium for the production of aryl alcohol oxidase (AAO) using *A. nidulans* under SmF. Several nitrogen sources were tested for AAO production and inorganic nitrogen sources; namely, sodium nitrate and potassium nitrate were found better sources compared to peptone, yeast extract, urea, ammonium sulfate, ammonium nitrate, and ammonium chloride. Corn steep liquor (CSL) was observed most effectively among all tested nitrogen sources [2]. Extracellular lipase production by *A. nidulans* (Mbl-S-6) was performed using different nitrogen sources such as peptone, urea, ammonium sulfate, ammonium chloride, and ammonium molybdate. Among all tested sources, ammonium sulfate resulted in maximum lipase production [12]. The effect of organic and inorganic nitrogen sources on lipase production by *E. nidulans* NFCCI 3643 was studied by Latha et al. [37], and yeast extract resulted in maximum lipase production among tested organic nitrogen sources. But, ammonium sulfate caused the maximum lipase production among all tested organic and inorganic nitrogen sources. Kamran et al. [14] tested various organic and inorganic nitrogen sources for  $\beta$ -galactosidase production and noticed that peptone (0.5%), yeast extract (0.5%), ammonium sulfate (0.2%), and ammonium nitrate (0.2%) improved the production of  $\beta$ -galactosidase by *A. nidulans* under SmF conditions [14]. The effect of various nitrogen sources on protease production by *A. nidulans* was studied, and peptone induced maximum protease production (1129.8 U/ml) compared to yeast extract (868.0 U/ml), malt extract (805.0 U/ml), ammonium sulfate (141.4 U/ml), ammonium

nitrate (302.4 U/ml), and ammonium chloride (488.6 U/ml) under SmF [13]. Ali and Saad El-Dein [60] studied the effect of three nitrogen sources on the production of cellulase enzyme complex by *A. nidulans* and observed sodium nitrate as the most effective nitrogen source for endoglucanase, avicellase, and  $\beta$ -glucosidase production.

**2.3. Effect of Surfactants Supplementation.** The stimulatory effect of surfactants on enzyme production is well established. Surfactants improve the penetration of water molecules into a solid substrate matrix that increases the swelling of the substrate leading to improved available surface area for microbial growth [61, 62]. Surfactants increase the permeability of the cell membrane that affects the secretion of certain proteins. Moreover, surfactants are also found to assist the release of cell-bound enzymes into fermentation media [62–64]. Kumar et al. [9] supplemented SSF production media with surfactants, namely, Tween-20, Tween-40, Tween-60, Tween-80, Triton-X-100, SDS, and EDTA. Among all tested surfactants, Tween-20, Tween-40, Tween-60, Tween-80, and Triton-X-100 showed the stimulatory effect on cellulase and xylanase production by *A. nidulans* AKB-25 while SDS and EDTA showed an inhibitory effect on enzyme production. 0.05% (w/v) of Triton-X-100 resulted in maximum FPase and endoglucanase activity while maximum xylanase production was caused by the supplementation of 0.1% (w/v) Tween-80 [9]. In another study, several surfactants including gum Arabic, SDS, PEG, Tween-80, and Triton-X-100 were tested for lipase production. Gum arabic enhanced lipase production compared to control while other surfactants decreased lipase production by *E. nidulans* NFCCI3643 [37].

### 3. *A. nidulans*, an Ideal Fungus for Expression of Heterologous Enzymes

Filamentous fungi are important resources for large-scale enzyme production. However, the production level is low and this limitation stimulates the research on gene manipulations [65]. The heterologous expression is reported as an alternative to increase the production of enzymes. Improved production of enzymes makes industrial processes cheaper especially when coupled to low-cost inducer substrates [22]. *A. nidulans* is an excellent model fungal system with standardized techniques for genetics and biochemical characterization of any phenotype of interest. It has been extensively studied for genetic manipulation for enhanced synthesis or secretion of different types of macromolecules. *A. nidulans* is amenable to molecular techniques, and its complete genome has been sequenced [38, 66].

A high-expression secretion vector (pEXPYR) directing overproduction of client proteins was engineered. This vector directs proteins towards extracellular medium in *A. nidulans* A773 and *A. awamori* ATCC22342. This upgraded expression/secretion system was used to generate a core set of hyperproducing hemicellulolytic enzymes. The serial integration of multiple client genes enables the production of a cocktail of enzymes within a single expression host [18].

*A. nidulans* A773 was used for the simultaneous production of xylanase and the liberation of xylooligosaccharide using rice husk as a substrate [23]. Endoxylanase and arabinofuranosidase encoding genes from *Penicillium funiculosum* and *Aspergillus niger*, respectively, were transferred into model organism *A. nidulans*. Hypersecretion of endoxylanase (301.2 U/mg) and arabinofuranosidase (115.55 U/mg) by *A. nidulans* was observed using the expression medium having pyridoxine and 2% maltose as inducer at 37°C. Endoxylanase and arabinofuranosidase activities were assayed against rye arabinoxylan and sugar beet arabinan, respectively, as substrate [25]. Abdella et al. [67] modified *A. nidulans* by the integration of an AFUMN-GH10 gene from *Aspergillus fumigatus* var. *niveus* for endo- $\beta$ -1,4-xylanase production. This genetically engineered *A. nidulans* resulted in higher secretion of xylanase in the presence of maltose. A 2-level Plackett-Burman design and 3-level Box-Behnken design were used for the optimization of nutrient medium components, and xylanase activity of 1620 U/ml (12460 U/g of maltose) was attained using an optimum medium. This xylanase activity was 280% higher than the maximum activity with the initial basic medium [67]. A xyloglucanase (AtXEG12) from *Aspergillus terreus* was expressed in *A. nidulans* and purified. The engineered *A. nidulans* having the AtXEG12 gene was grown under SmF and SSF in an airlift bioreactor and a column type bioreactor, respectively. During SmF, the highest enzyme activity (0.45 IU/ml) was observed after 24 h while a maximum enzyme activity of 3.15 IU/ml was found during SSF conditions [24].

Acidic, thermostable endoglucanase from *A. nidulans* was expressed in *Pichia pastoris*, and purification and biochemical characterization of the recombinant enzyme were carried out. The maximum endoglucanase activity was observed at temperature 50°C and pH 4.0. The recombinant endoglucanase retained 100% activity when incubated at 45 and 55°C for 72 h. Recombinant endoglucanase was assayed for the capacity of hydrolyzing natural substrates, namely, banana stem, ball-milled steam-exploded sugarcane bagasse, soybean residue, and corn stover [68]. *Beauveria bassiana* lipase gene bbl1 sequence was cloned into the expression vector pExpYr in-frame with *Aspergillus niger* glucoamylase signal peptide resulting in a plasmid named as pExpYr + bbl1. *A. nidulans* A773 was transformed using this vector. A variety of carbon sources including cassava peels, corn syrup, sorghum seed, and wheat bran were used to improve recombinant lipase production. Monosaccharides such as fructose and glucose enhanced lipase production in combination with previously described carbon sources especially sorghum seeds and wheat bran [22]. *A. nidulans* A773 was genetically modified and overexpresses and secretes *Myceliophthora thermophila* aryl alcohol oxidase (AAO) in maltose containing media. This mutant strain of *A. nidulans* is unable to synthesize its pyridoxine. Pyridoxine limitation can be used to control cell growth, diverting substrate to protein synthesis. In agitated fermentation, AAO production was found similar when media were with 1 mg/L pyridoxine and without pyridoxine. However, the treatment lacking pyridoxine had to be supplemented with pyridoxine after 156 h of fermentation to sustain continued

enzyme production. The use of extremely diluted pyridoxine levels allowed reduced fungal growth while maintaining steady enzyme production [69].

#### 4. Applications of *A. nidulans* Enzymes

*A. nidulans* can synthesize a variety of industrial enzymes including cellulases, xylanases, laccases, lipases, proteases,  $\beta$ -galactosidases, tannases, keratinases, cutinases, and aryl alcohol oxidases (Table 1). These enzymes find applications in various industries such as food, brewery, and wine, biofuels, animal feed, textile and laundry, and pulp and paper.

**4.1. Hydrolysis of Lignocellulosic Biomass.** Lignocellulosic biomass stores a huge amount of carbohydrates including cellulose and hemicelluloses. The hydrolysis of these carbohydrates is one of the key steps in the production of biofuels and value-added products from lignocellulosic biomass. The hydrolysis can be performed by acid hydrolysis or enzymatic hydrolysis. Enzymatic hydrolysis has been proved to have several advantages such as high yield of monomer sugars, mild reaction conditions, and negligible by-products formation [70]. The enzymatic hydrolysis of alkaline pretreated pearl millet stover was performed by a multienzyme from *A. nidulans* AKB-25 and obtained a saccharification yield of 64.77% at an enzyme dose of 15 FPU/g of the substrate with the addition of 0.15% of Tween-80 as a surfactant. The crude enzyme efficiency might be due to the fact that 1 FPU of the enzyme contained 39.39 IU of endoglucanase, 11.68 IU of  $\beta$ -glucosidase, and 642.47 IU of xylanase [71]. Plant polysaccharide saccharification was improved by the cooperation of *A. nidulans* enzymes. Two endoxylanases and a  $\beta$ -xylosidase cooperate in the hydrolysis of beechwood xylan. The two endoxylanases act on different linkages in beechwood xylan, since the mixture of two enzymes provides a higher level of enzymatic hydrolysis. The positive synergy was found with a  $\beta$ -xylosidase, with the highest hydrolysis efficiency when all three enzymes were utilized together. Two endoglucanases, a  $\beta$ -glucosidase, and an  $\alpha/\beta$ -glucosidase cooperated during the hydrolysis of  $\beta$ -glucan [72]. Cocultivation of four *A. nidulans* recombinant strains was used for the production of a cocktail of enzymes having GH51 arabinofuranosidase, GH11 endo-1,4-xylanase, GH43 endo-1,5-arabinanase, and GH12 xyloglucan specific endo- $\beta$ -1,4-glucanase. This set of recombinant enzymes was used as an alternative to alkaline sugarcane bagasse pretreatment. This enzymatic was more efficient than the alkaline method in maintaining cellulose integrity and exposing this cellulose to the subsequent saccharification process [73].

**4.2. Biodiesel Production.** Cutinases are versatile carboxylic esters that catalyze the breakdown of both small-molecule esters and polyesters. They have great potential in biodiesel production. Sesame oil was used as a substrate for fatty acid methyl esters (biodiesel) production by cutinase from *A. nidulans*. After 72 h of incubation, 63 and 2% yield of fatty acid methyl esters was obtained by chemical and cutinase

treatment, respectively. The low yield by cutinase treatment was observed due to the inhibition of enzyme activity by methanol and glycerol that are present in the reaction mixture. This might be improved by reducing the inhibitory effect and optimizing the reaction parameters [74]. Gonçalves et al. [22] studied biodiesel production from Acai (*Euterpe oleracea* Martius), Buriti (*Mauritia flexuosa*), and coffee grounds oils using recombinant lipase produced by *A. nidulans*. Transesterification reaction of coffee spent, Buriti, and Acai oils by immobilized lipase in Sepabead-C18 in the presence of ethanol was performed [23].

**4.3. Pulp and Paper Industry.** Microbial enzymes present an environment-friendly alternative to conventional chemical processes. Microbial enzymes such as cellulases, xylanases, pectinases, and amylases are applicable for the different processes such as biobleaching, deinking of waste paper, and enzyme-assisted refining. [75]. Alkaline active xylanase produced by *A. nidulans* KK-99 was tested and used for biobleaching of kraft pulp. The xylanase dose of 1.0 IU/g of dry pulp showed optimum bleaching effect at pH 8.0 and a temperature of 55°C for 3 h reaction time [10]. In another recent study, thermo- and alkali-tolerant xylanase from *A. nidulans* was reported for bleach-boosting of bamboo pulp. The maximum biobleaching efficiency was found at an enzyme dose of 20 U/g of dry pulp with an incubation time of 4 h. The release of phenolic and hydrophobic compounds was increased by approximately 8 and 22 times, respectively, at the end of the 6 h reaction period [76]. Kumar et al. [9] studied cellulase and xylanase production by *A. nidulans* AKB-25 and used the enzyme for biodeinking of mixed-office waste (MOW) paper. Pulp freeness, brightness, and deinking efficiency of MOW paper were improved by 34.47, 4.6, and 25.01%, respectively, and reduced dirt count by 74.70% compared to control. Physical strengths such as tensile index, burst index, and double-fold number were also improved during the biodeinking of MOW [9].

**4.4. Pharmaceutical Products.** Enzymatic hydrolysis of xylan rich agricultural residues produces xylooligosaccharides (XOs) that are considered as probiotic. XOs are sugar oligomers that are composed of  $\beta$ -1,4-linked xylose units released by xylan degradation. The presence of  $\beta$ -1,4-linkage in XOs makes them resistant to hydrolysis compared to  $\alpha$ -linkage that is digested easily by human digestive enzymes. XOs have the advantage to be fermented by specific bacteria such as *Bifidobacterium* and *Lactobacillus* that act as probiotics [77–79]. SSF of rice husk was carried out by *A. nidulans* XynC A773 to produce xylanase and XOs. *A. nidulans* XynC A773 produced 23.9 mg/g of the substrate under SSF that is more concentrated and can be extracted easily compared to the diluted medium of submerged culture [23]. Gonçalves et al. [25] studied the production of XOs from pretreated sugarcane bagasse by the synergic action of GH11 endoxylanase and GH54 arabinofuranosidase produced by *A. nidulans* [25]. The genetically modified strain of *A. nidulans* A773 produced xylanase and arabinofuranosidase using soybean fiber as a substrate under SSF, and

these enzymes were subsequently exploited to produce XOs from the same agricultural residue. The maximum yield of XOs obtained was 28% (mass fraction of xylan), showing final concentration (in  $\text{mg}\cdot\text{g}^{-1}$  arabinoxylan) of 138.36 xylobiose (X2), 96.96 xylotriose (X3), and 53.04 xylotetraose (X4) in 9 h of reaction time [80].

**4.5. Dye Decolorization and Industrial Wastewater Treatment.** Dye wastewater from textile and dyestuff industries is one of the most difficult wastewater types to treat. Dyes are synthetic compounds with aromatic molecular structures that make them more stable and difficult for biodegradation [81]. Laccases and laccase-mediator systems (LMSs) have great potential for decolorization and detoxification of industrial wastewater. Laccases are multicopper oxidases that can degrade a variety of dyes [82]. Laccases can decolorize wastewaters from olive mills and pulp mills by removing colored phenolic compounds [83]. Crude and purified laccases from *A. nidulans* TTF6 were utilized for decolorization of various dyes including congo red, methyl orange, methyl violet, methyl red, coomassie blue, victoria blue, and bromophenol blue. The crude enzyme required a longer treatment time (7 days) compared to purified one (up to 48 h) at 40°C. Out of the two categories of tested dyes, azo ones (e.g., methyl red) were slowly removed compared to triphenyl amines (e.g., Coomassie blue and victoria blue). Although methyl violet belongs to triphenylamine, it took a long time for decolorization in the presence of *A. nidulans* TTF6 laccase. A higher decolorization rate was observed at 40°C compared to 20°C, obeying general enzyme catalysis characteristics [38]. *Emericella nidulans* var. *nidulans* (anamorph: *Aspergillus nidulans*) was isolated from the sediments of pulp and paper mill that was used for decolorization and detoxification of pulp and paper mill effluent. The treatment process was optimized by the Taguchi approach. The maximum reduction in color (66.66%) and lignin (37%) was observed at temperature (30–35°C), agitation (125 rpm), pH (5.0), and treatment duration of 24 h. The nutrients including dextrose and tryptone were provided at a concentration of 0.25 and 0.1%, respectively [84].

**4.6. Food Industry.** Some strains of *A. nidulans* have been found to produce  $\beta$ -galactosidase that is widely used in the different biotechnological processes of the food industry. It converts lactose sugar into  $\beta$ -glucose and  $\beta$ -galactose that are employed to manufacture sugar syrups. The sensorial properties of food can be improved through the biocatalysis of lactose. This process also enhances the creaminess of ice cream and the texture of various baked products. Furthermore, the enzyme action releases the monosaccharide that improves the sweetness of the product [14, 85, 86].

## Data Availability

All the required data are included in the article.

## Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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