RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

OXYGEN-DEPENDENT PHYSIOLOGICAL AND METABOLIC ADAPTATION OF *ASPERGILLUS FLAVUS* DURING BARLEY STRAW BIODEGRADATION

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ABSTRACT

Oxygen availability is a critical determinant of fungal biodegradation efficiency in lignocellulosic systems, particularly under large-scale or poorly aerated conditions. This study examined the physiological and metabolic responses of a locally isolated *Aspergillus flavus* strain during barley straw degradation under aerobic and oxygen-limited conditions over a 15-day incubation period. Biodegradation efficiency, cellulase activity, soluble carbohydrate and protein production were quantified, and structural alterations were evaluated using scanning and transmission electron microscopy. Secondary metabolites were qualitatively screened through chromatographic analysis. Statistical evaluation included Student's t-test and first-order kinetic modeling. Aerobic cultivation resulted in significantly higher dry matter loss (43.7%) compared with oxygen-limited conditions (19.3%) ($p<0.01$). Cellulase activity under aerobic conditions was approximately 2.3-fold greater than under oxygen limitation. Ultrastructural analysis revealed cell wall thickening, mitochondrial alterations, and lipid body accumulation under reduced oxygen availability, indicating adaptive responses to metabolic constraint. Qualitative screening did not reveal detectable aflatoxin within the analytical limits applied. These findings demonstrate that oxygen availability governs enzymatic efficiency and metabolic output in *A. flavus*, while partial biodegradation capacity is retained under oxygen limitation. The study provides insight into fungal adaptation relevant to lignocellulosic biomass processing under heterogeneous aeration conditions. This study provides new insights into oxygen-mediated metabolic modulation in Aspergillus species under controlled fermentation conditions.

***Keywords*:** *Aspergillus flavus*, Oxygen limitation, Fungal adaptation, Barley straw, Lignocellulosic waste, Biodegradation.

Article History (2026-053) || Received: 17-Feb-2026 || Revised: 07-Mar-2026 || Accepted: 18-Mar-2026 || Published Online: 15-Apr-2026

1. INTRODUCTION

The accumulation of agricultural residues such as barley straw presents both an environmental burden and a potential renewable resource (Kuittinen et al., 2022). Efficient conversion of lignocellulosic biomass into value-added products remains central to the development of sustainable bio-based systems (Haldar & Purkait, 2020; Wahyudi et al., 2024). However, the structural complexity of cellulose, hemicellulose, and lignin necessitates robust microbial degraders capable of sustained enzymatic activity (Thapa et al., 2020). Filamentous fungi, particularly species of the genus *Aspergillus*, are well recognized for their lignocellulolytic capacity and adaptability to diverse ecological niches (Ghorai, 2024). Most studies evaluating fungal degradation efficiency are conducted under fully aerobic conditions, where oxygen supports high respiratory throughput and optimal enzyme production (Kües, 2015; Aydin et al., 2017; Elyamine et al., 2021; Ahmad et al., 2025b). In practical systems such as solid-state fermentation, composting matrices, and densely packed bioreactors oxygen diffusion is often heterogeneous, leading to localized hypoxic environments. Oxygen limitation represents a significant physiological constraint for strictly aerobic fungi, hypoxia signaling (Bissaro et al., 2018; Chen et al., 2025). Oxygen sensing and hypoxia-responsive regulatory pathways in filamentous fungi have been increasingly recognized as central modulators of mitochondrial activity and metabolic adaptation (Cairns, 2023; Zhang et al., 2024). Reduced oxygen availability directly affects mitochondrial respiration, ATP generation, and the biosynthesis of extracellular hydrolytic enzymes (Hillmann et al., 2015). In addition, oxygen tension can influence the regulation of secondary metabolism, including the production of bioactive compounds. Despite these implications, integrated studies combining degradation kinetics, ultrastructural analysis, and metabolic profiling under controlled oxygen limitation remain limited.

Aspergillus flavus is metabolically versatile and widely distributed in arid and semi-arid environments. While it is known for its capacity to produce aflatoxins under specific conditions, its physiological adaptation to oxygen-

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restricted lignocellulosic systems has not been comprehensively characterized (Alzahrani, 2025). Understanding how this species responds structurally and metabolically to reduced oxygen availability is essential for evaluating its potential relevance in controlled biomass valorization systems (Ahmad et al., 2025a; Kovács et al., 2026).

The present study investigates the impact of oxygen limitation on barley straw biodegradation by a locally isolated *A. flavus* strain. By integrating biodegradation kinetics, enzymatic activity measurements, ultrastructural examination (SEM/TEM), and qualitative metabolite screening, this work aims to characterize adaptive responses under hypoxic conditions without extrapolating beyond the analytical limits of the applied methods. It was hypothesized that oxygen limitation would significantly modulate enzymatic efficiency and secondary metabolite production patterns.

2. MATERIALS AND METHODS

2.1. Fungal Isolation and Identification

Fungal isolates were obtained from agricultural soil and wastewater samples collected in the Jazan region of Saudi Arabia. Isolation was performed on Czapek's Dox agar and malt yeast extract agar supplemented with chloramphenicol to suppress bacterial growth. Pure cultures were obtained through repeated subculturing.

Identification was based on macroscopic and microscopic characteristics, including colony morphology, conidiophore structure, vesicle shape, and conidial arrangement, following established taxonomic keys for *Aspergillus* species (Samson et al., 2014). The isolate was confirmed as *Aspergillus flavus* and maintained on agar slants at 4°C for long-term storage. Identification was based on morphological characteristics; molecular confirmation was not performed.

2.2. Substrate Preparation and Cultivation Conditions

Barley straw was thoroughly washed, oven-dried, and milled into fine particles, which were used as a lignocellulosic substrate at 5% (w/v). For aerobic cultivation, Erlenmeyer flasks were incubated under ambient atmospheric conditions with continuous shaking. Oxygen-limited (hypoxic) conditions were established using sealed serum bottles fitted with butyl rubber stoppers and GasPak systems. Oxygen limitation reduced oxygen concentration to hypoxic levels without inducing complete anaerobiosis, effectively reducing oxygen availability. L-cysteine hydrochloride was added as a reducing agent under a CO_2 atmosphere to maintain a low redox potential.

2.3. Biodegradation Assay

Cultures were incubated at 28°C for 7, 10, and 15 days under either aerobic or hypoxic conditions. All experiments were performed in triplicate. Residual barley straw was recovered, washed, dried at 70°C to constant weight, and weighed. Biodegradation efficiency was calculated as the percentage of dry matter loss relative to the initial substrate weight.

2.4. Biochemical Analyses

Total soluble carbohydrates were quantified using the anthrone–sulfuric acid method (Hedge & Hofreiter, 1962), and total soluble proteins were measured using the Lowry assay (Lowry et al., 1951). Cellulolytic activity was evaluated by quantifying reducing sugars released using the dinitrosalicylic acid (DNS) method. Results were expressed as mg glucose L^{-1} culture filtrate (Miller, 1959).

2.5. Secondary Metabolite Screening

Culture filtrates were obtained by centrifugation and filtration through 0.22 µm membranes. Metabolite profiling was performed using a gas chromatography system coupled to a mass selective detector equipped with a DB-5 capillary column. The instrument was operated at the Central Laboratory Facility of Jazan University, Saudi Arabia. Compound identification was achieved by comparison with NIST and Wiley mass spectral libraries. In addition, chromatograms were examined to determine whether aflatoxin-related peaks were present within the GC–MS system's detection limits. The analysis was limited to qualitative screening.

2.6. Microscopy

Ultrastructural analyses were performed using Scanning and Transmission Electron Microscopy (SEM and TEM). Samples underwent standard fixation, dehydration, and staining procedures (Beauvais et al., 2018) and were examined using a JEOL JSM-IT200 (SEM) and JEOL JEM-1400 (TEM) microscope.

2.7. Statistical Analysis

All experiments were conducted in triplicate (n = 3), and results are presented as mean ± standard deviation (SD). Statistical comparisons between aerobic and oxygen-limited conditions were performed using Student's t-test and one-way analysis of variance (ANOVA) where appropriate. A p-value < 0.05 was considered statistically

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significant. First-order kinetic modeling and multivariate analysis were performed using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA), RSM is widely used for multivariate optimization of lignocellulosic bioprocesses (Behera et al., 2022).

3. RESULTS

3.1. Statistical Validation of Biodegradation Efficiency

Barley straw degradation was systematically monitored over 15 days. Under aerobic conditions, dry matter loss was 44 ± 1.5%, significantly higher than the 20 ± 0.8% observed under hypoxic conditions ($P < 0.01$). Biodegradation efficiency is summarized in Table 2.

- **Statistical Significance:** Student's t-test confirmed that oxygen availability is a primary determinant of degradation efficiency throughout the incubation period.
- **Kinetic Modeling:** The first-order rate constant (k) was markedly higher in aerobic systems (0.0364 day^{-1}) compared to hypoxic systems (0.0082 day^{-1}), quantitatively illustrating the oxygen-dependent reduction in metabolic rate.

3.2. Ultrastructural Adaptations and Cellular Integrity

SEM and TEM analyses provided direct visual evidence of the strain's structural resilience under oxygen stress.

- **Aerobic Morphology:** SEM revealed dense conidial chains and well-organized phialides, indicating optimal reproductive capacity (Fig. 1).
- **Hypoxic Adaptation:** TEM showed strategic thickening of the cell wall and an increased accumulation of lipid globules, highlighting morphological adjustments to sustain survival (Fig. 2).
- **Cellular Integrity:** Despite these stress-induced modifications, the overall cellular structure remained intact, explaining the strain's capacity to maintain a functional, albeit reduced, biodegradation rate of 20% under oxygen-limited conditions.

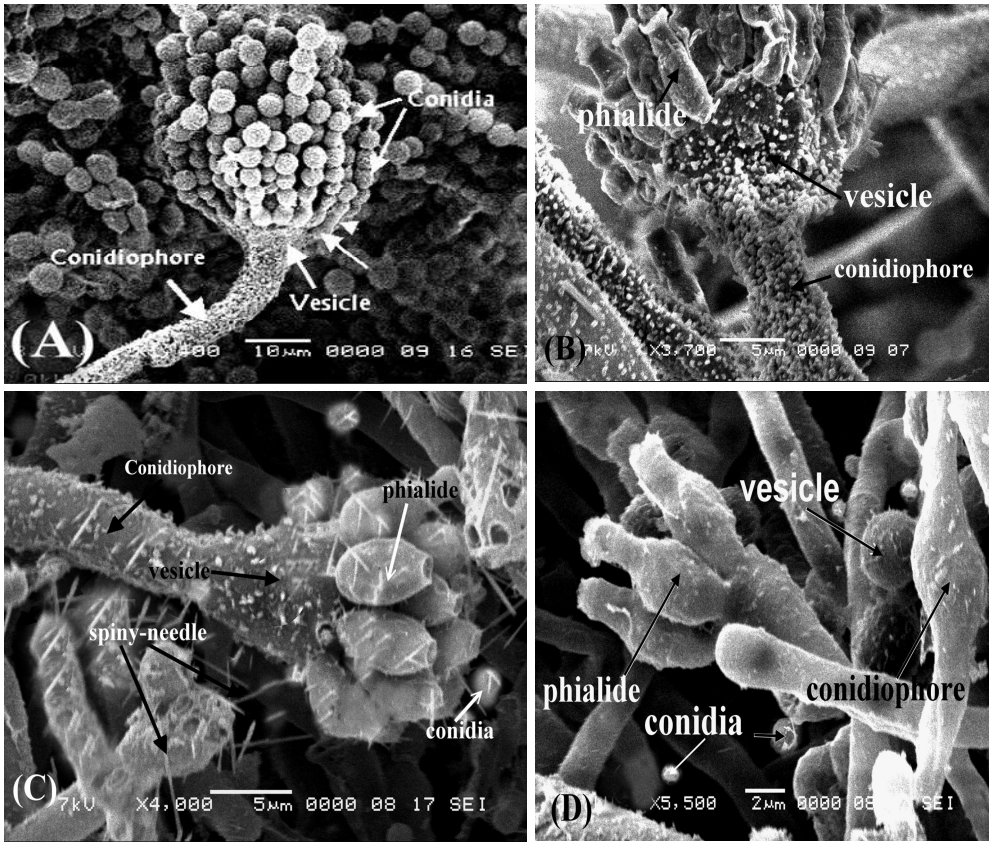

Fig. 1: Morphological characteristics of *Aspergillus flavus* grown under aerobic conditions as observed by scanning electron microscopy (SEM).

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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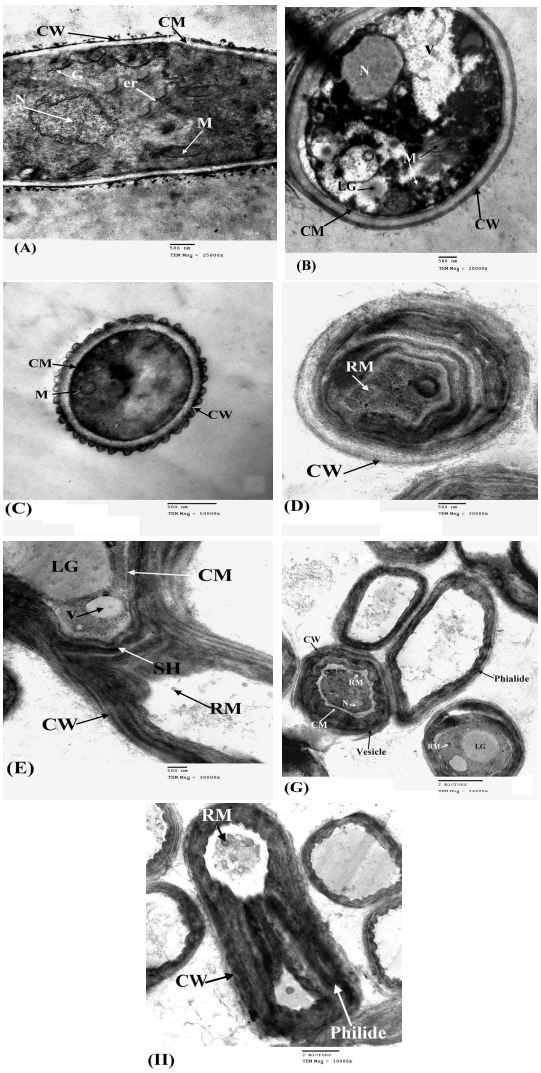

Fig. 2: Ultrastructural features of *Aspergillus flavus* grown under oxygen-limited conditions as observed by transmission electron microscopy (TEM).

3.3. Secondary Metabolite Profile and Aflatoxin Screening

GC–MS analysis revealed qualitative differences in metabolite composition between aerobic and oxygen-limited cultures. Aerobic samples showed greater metabolite diversity compared to hypoxic cultures, indicating oxygen-dependent regulation of secondary metabolism.

Screening of chromatograms did not reveal peaks corresponding to aflatoxin within the detection limits of the applied GC–MS method under either cultivation condition. The analysis was restricted to qualitative presence/absence determination. No further interpretation regarding regulatory gene expression was made based on this data.

3.4. Enzyme Activity and Protein Flux

The biochemical response of the strain was quantified through spectrophotometric assays. Aerobic cultures exhibited significantly higher cellulolytic activity compared to oxygen-limited cultures ($P<0.05$). The amount of reducing sugars released reached 228 mg glucose L^{-1} under aerobic conditions after 10 days of incubation, representing a 2.3-fold increase relative to oxygen-limited cultures.

3.4.1. Protein and Carbohydrate Dynamics: Polynomial regression analysis revealed a strong correlation between incubation time and the release of soluble proteins and carbohydrates. Aerobic systems consistently achieved higher global desirability (0.93) in the RSM models.

Qualitative and quantitative changes in secondary metabolite production were detected using GC–MS (Table 1). Under aerobic conditions, 10 metabolites were identified, with nonadecanol (21.16%) and heneicosanoic acid (12.85%) as the most abundant. Under oxygen-limited conditions, metabolite diversity decreased, with only seven detectable compounds. The most abundant metabolites under hypoxia were 1-chlorododecane (24.60%) and acetyl cedrene (21.04%). This reduction in metabolite diversity underscores the regulatory role of oxygen in secondary metabolism in *A. flavus* (Keller, 2019).

Table 1: Secondary metabolites produced by *Aspergillus flavus* cultivated on barley straw under aerobic and oxygen-limited (hypoxic) conditions

Aerobic Conditions	%	Oxygen-Limited Conditions	%
Nonadecanol	21.16	3,4-Tridecadien-1-ol	4.25
Benzobutyl,trimethyl-penten-3-ol	17.80	1-Chlorododecane	24.60
Isopropylthio-5-trifluoro-acetyl	7.30	9,12-Octadecadienoic acid	9.90
L-1,2-Dioleoyl Glyceryl phosphocholine	17.62	Fumaric acid, (tert-butyl)dimethyl ester	8.20
2-Chlorobenzofuran	4.22	Benzo furo(3,2-b)benzofuro(2,3-e)pyran-6-one	10.21
L-Proline	0.75	3,4-Tridecadien-1-ol (isomer)	12.18
2-Isopropylthio-1,3-oxathiolyium-4-oxalat	2.25	Acetyl cedrene	21.04
1-Hexadecanone,1-cyclopentyl	5.83	-	-
Cyclopenten-1-one,3-chloro	12.85	-	-
Heneicosanoic acid	4.34	-	-

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Table 2: Biodegradation efficiency (%: Dry matter consumed) of barley straw by *Aspergillus flavus* over time

Conditions \ Period	7 days	10 days	15 days
	%	%	%
Aerobic	24.30 ± 0.70	38.40 ± 0.69	43.70 ± 1.57
Oxygen-Limited	14.17 ± 0.65	19.30 ± 0.70	20.10 ± 0.36

Table 3: Production of total soluble carbohydrates (T.C) and proteins (T.P) during barley straw biodegradation (µg/100 mL)

Period	Conditions	(T.C)	(T.P)
7 days	Aerobic	305.7 ± 4.3	205.0 ± 2.0
	Oxygen-Limited	294.5 ± 4.0	97.7 ± 0.1
10days	Aerobic	311.4 ± 2.6	213.4 ± 1.1
	Oxygen-Limited	292.4 ± 1.6	105.2 ± 2.7
15 days	Aerobic	360.0 ± 1.0	232.8 ± 0.2
	Oxygen-Limited	305.3 ± 2.2	118.0 ± 0.6

Analysis of culture supernatants revealed significant differences in macromolecular accumulation with oxygen availability (Table 3). Concentrations of total soluble carbohydrates and proteins were consistently higher in aerobic cultures throughout the incubation period. Cellulase activity, a key indicator of hydrolytic capacity, showed strong oxygen dependence, with activity under aerobic conditions approximately 2.3 times higher than under oxygen limitation (Table 4). This reduction aligns with the energy-intensive nature of hydrolytic enzyme biosynthesis, which relies on aerobic respiration.

3.5. Kinetic Modeling and Process Evaluation

The degradation of barley straw dry matter followed first-order kinetics. Multivariate optimization and response surface analysis were performed using SPSS software (Fig. 3).

Degradation was considerably faster under aerobic conditions, with a rate constant (k) of 0.0364 day^{-1} and a half-life ($t_{1/2}$) of 19.1 days. In contrast, oxygen limitation severely hampered degradation, resulting in a lower rate constant (0.0082 day^{-1}) and a longer half-life (84.3 days). These kinetic parameters confirm that oxygen availability is critical for efficient substrate utilization.

Table 4: Cellulase activity of *Aspergillus flavus* measured as concentration of reducing sugars released (mg glucose L^{-1} culture filtrate; mean ± SD, n = 3)

Period	Conditions	mg glucose L^{-1} culture filtrate
7 days	Aerobic	200.6 ± 0.3
	Oxygen-Limited	86.42 ± 0.2
10days	Aerobic	216.2 ± 0.6
	Oxygen-Limited	89.1 ± 0.6
15days	Aerobic	228.3 ± 1.0
	Oxygen-Limited	72.5 ± 0.2

Polynomial regression analysis adequately described temporal trends in soluble protein and carbohydrate production (Fig. 4 and 5). Multivariate analysis using response surface methodology identified aerobic cultivation as the most favorable condition for maximizing process performance, yielding a global desirability value of 0.93, whereas oxygen-limited cultivation showed limited optimization potential (Fig. 6).

The disparity between the two oxygen regimes is visually summarized in the Fig. The bar chart illustrates a direct comparison of the optimum values achieved for a key output (e.g., enzyme activity or product yield) under aerobic versus oxygen limited, with aerobic performance being significantly greater.

Optimization Results (RSM + Desirability)

Response Surface Methodology (RSM) was applied to evaluate the combined effects of incubation period and cultivation conditions on biodegradation efficiency, enzyme activity, and metabolite production. Aerobic cultivation yielded the highest global desirability value (0.93), indicating optimal process performance, whereas oxygen-limited conditions exhibited significantly lower optimization potential.

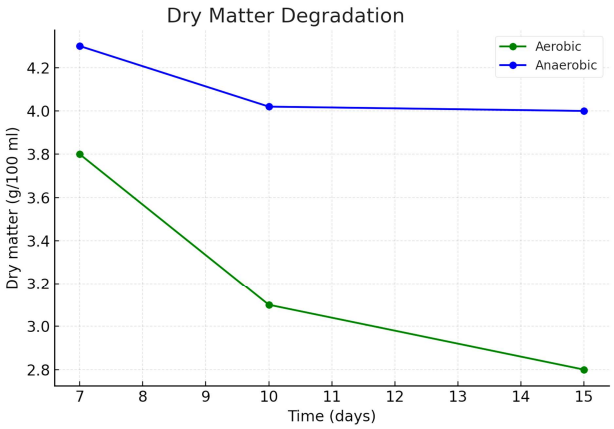

Fig. 3: Dry matter loss kinetics of barley straw incubated with *Aspergillus flavus*: comparison between aerobic and oxygen-limited conditions after 7, 10, and 15 days; Values are presented as mean ± SD (n = 3).

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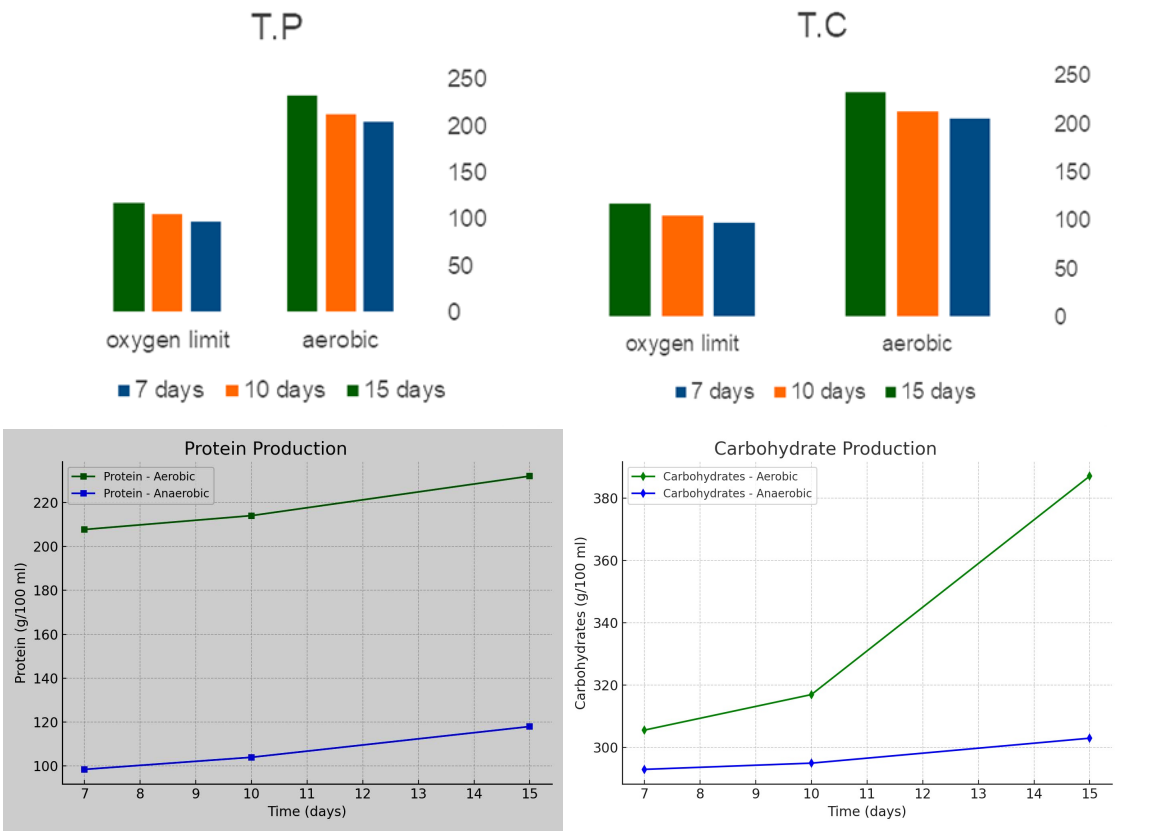

Fig. 4: Changes in protein and total soluble carbohydrates concentration during cultivation of *Aspergillus flavus* under aerobic and oxygen-limited conditions; Values are presented as mean ± SD (n = 3).

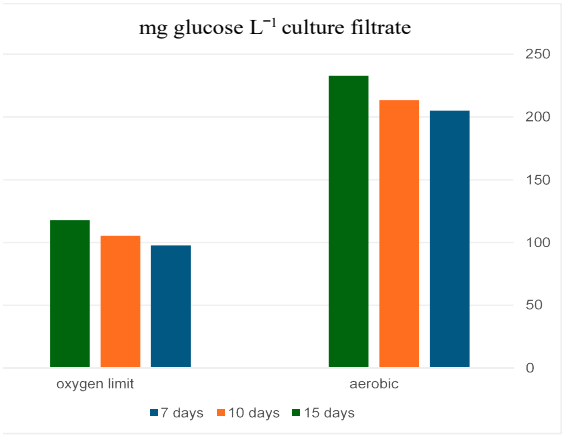

Fig. 5: Changes in mg glucose L^{-1} culture filtrate concentration during cultivation of *Aspergillus flavus* under aerobic and oxygen-limited conditions: Values are presented as mean ± SD (n = 3).

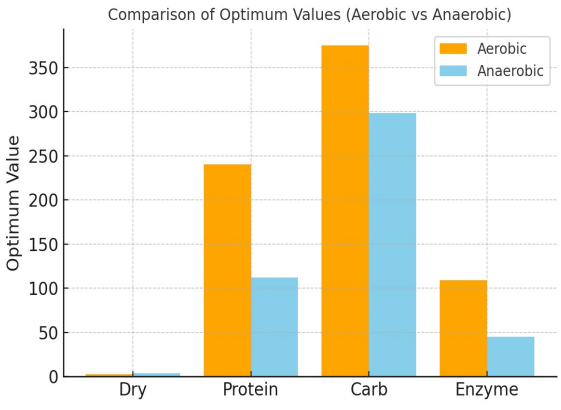

Fig. 6: Representative contour plots from the RSM analysis, showing the relationship between process variables and responses like protein yield and enzyme activity.

4. DISCUSSION

The present study demonstrates that oxygen availability is a master regulator of physiological performance, enzymatic hydrolysis, and metabolic profiling in *Aspergillus flavus* during barley straw biodegradation. By integrating kinetic, ultrastructural, and biochemical analyses, we provide evidence that while aerobic conditions

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maximize lignocellulose conversion, the fungus retains a measurable, though reduced, metabolic capacity under oxygen-limited conditions. These findings contribute to a mechanistic understanding of fungal adaptation to heterogeneous aeration, a condition frequently encountered in solid-state fermentation systems and natural composting environments.

4.1. Oxygen Availability Governs Lignocellulose Degradation Kinetics

The substantial reduction in dry matter loss (from 43.7% to 19.3%) and cellulase activity (approximately 2.3-fold decrease) under oxygen limitation underscores the critical role of aerobic respiration in supporting the energy-intensive biosynthesis of hydrolytic enzymes. This finding aligns with the well-established principle that filamentous fungi, as obligate aerobes, depend on oxidative phosphorylation for efficient ATP generation required for extracellular enzyme production (Hesse et al., 2002; Paulussen et al., 2017; Mattila et al., 2020). The first-order kinetic modeling further quantifies this dependency, with the rate constant (k) decreasing from 0.0364 day^{-1} under aerobiosis to 0.0082 day^{-1} under hypoxia. This kinetic penalty is consistent with observations in other fungal species where oxygen restriction slows the initial attack on lignocellulosic polymers (Siedt et al., 2023). However, the persistence of approximately 20% substrate degradation under hypoxic conditions indicates that *A. flavus* does not enter a state of complete metabolic arrest. This residual activity suggests the operation of fermentative or alternative metabolic pathways that partially sustain cellular functions and basic hydrolytic capacity. Recent advances in understanding fungal hypoxia responses have highlighted the importance of redox homeostasis, particularly the NAD+/NADH balance, in modulating metabolic flux under oxygen-limited conditions (Shimizu, 2018). The accumulation of lipid bodies observed in our TEM analysis (Fig. 3) likely reflects a redirection of carbon flux toward storage compounds when respiratory capacity is constrained, a survival strategy documented in other fungi facing energy limitation (Hillmann et al., 2015).

4.2. Ultrastructural Remodeling as an Adaptive Strategy

The structural modifications observed under oxygen limitation provide direct cytological evidence of fungal adaptation to energy stress. The thickening of the cell wall, clearly visible in TEM micrographs (Fig. 3), is a well-characterized stress response in *Aspergillus* species, often associated with enhanced resilience against environmental challenges. This remodeling may serve to reinforce the cellular barrier against osmotic or oxidative stress that can accompany hypoxic conditions. Importantly, the preservation of overall cellular integrity under hypoxia explains the strain's capacity to maintain partial functionality, distinguishing this adaptive response from irreversible degenerative changes. Mitochondrial alterations observed under oxygen-limited conditions are particularly noteworthy. Given that mitochondria are the primary consumers of molecular oxygen, their structural adjustment is an expected consequence of reduced respiratory activity (Yu et al., 2024). The increased abundance of lipid bodies suggests a shift in metabolic strategy: under aerobic conditions, lipids would be mobilized for energy generation via β-oxidation, whereas under hypoxia, their accumulation may represent either a bottleneck in fatty acid catabolism or a deliberate storage response for when conditions improve. This phenomenon has been previously reported in *A. fumigatus* during hypoxic stress, reinforcing the concept of a conserved fungal response to oxygen deprivation (Grahl et al., 2012).

4.3. Oxygen-Dependent Regulation of Secondary Metabolism

The qualitative differences in secondary metabolite profiles between aerobic and oxygen-limited cultures (Table 1) confirm that oxygen availability modulates the metabolic output of *A. flavus* beyond simple biomass conversion. The greater metabolite diversity under aerobic conditions likely reflects the full engagement of oxygen-dependent biosynthetic pathways, including those requiring molecular oxygen as a substrate for key enzymatic reactions (Keller, 2019). In contrast, the reduced diversity and distinct compound profile under hypoxia indicate a reprogramming of secondary metabolism, potentially favoring pathways compatible with low-oxygen environments. The absence of detectable aflatoxin in our chromatographic analysis, within the defined analytical limits of the GC–MS method, warrants careful interpretation. This observation may reflect the specific culture conditions, the particular strain used, or the possibility that aflatoxin biosynthesis requires specific triggers beyond simple oxygen availability. Recent molecular investigations have revealed complex regulatory networks governing aflatoxin production in *A. flavus*, with oxidative stress and specific transcription factors playing critical roles. Peng et al. (2024) demonstrated that the transcription factor AtfA, which responds to oxidative stress signals, is intimately involved in coordinating aflatoxin metabolism and virulence. Similarly, Lohmar et al. (2025) identified FhpA as a key regulator linking stress response to secondary metabolite production. These findings suggest that aflatoxin biosynthesis is not simply a function of oxygen presence or absence, but rather a finely tuned response to specific environmental and physiological cues (Cho et al., 2025). Our study's qualitative screening approach does not preclude the possibility of aflatoxin production under alternative conditions or detection with more sensitive quantitative methods.

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4.4. Bioprocess Implications and Translational Potential

From a biotechnological perspective, our findings carry dual implications. First, they reaffirm that maintaining aerobic conditions is paramount when maximum degradation rates and enzyme yields are the primary objectives, particularly in bioreactor systems where oxygen transfer can be controlled and optimized. The higher global desirability value (0.93) for aerobic cultivation in our RSM models quantitatively supports this conclusion. Second, the demonstrated tolerance of this *A. flavus* isolate to oxygen-limited conditions suggests potential applicability in systems where uniform aeration is challenging or economically prohibitive. Large-scale agricultural waste management, including composting piles and static biomass accumulations, inevitably contains hypoxic microzones . In such contexts, a fungal strain capable of maintaining partial activity under oxygen restriction could contribute to overall biodegradation more effectively than strictly aerobic organisms that would cease function entirely. The adaptation of *A. flavus* to arid environments, as documented in Saudi Arabia (Alzahrani, 2025), may have pre-adapted this isolate to tolerate fluctuating oxygen tensions, a hypothesis worthy of further investigation. Moreover, the observed shift in metabolite profiles under hypoxia raises the possibility of exploiting oxygen limitation as a tool for directing fungal metabolism toward specific compounds of interest. Studies on other fungal genera have successfully used hypoxic stress to enhance the production of valuable secondary metabolites, suggesting that controlled oxygen restriction could be integrated into bioprocess strategies for *A. flavus* as well. This study demonstrates that *A. flavus* exhibits a graded response to oxygen availability, with aerobic conditions supporting maximal lignocellulolytic activity and metabolic diversity, while oxygen limitation triggers adaptive ultrastructural remodeling and metabolic reprogramming that sustain partial functionality. The integration of these findings with recent molecular insights into fungal stress responses provides a more complete picture of how *A. flavus* navigates heterogeneous oxygen environments (Chien et al., 2025). For biotechnological applications, this work supports the development of aeration strategies tailored to process goals, whether maximizing degradation rates under controlled aerobic conditions or leveraging hypoxic tolerance in less-engineered systems.

5. CONCLUSION

This study demonstrates that oxygen availability plays a critical role in regulating enzymatic efficiency and secondary metabolite production in *Aspergillus flavus*. Controlled hypoxic conditions significantly influenced lignocellulosic degradation dynamics, highlighting oxygen tension as a key modulator of fungal metabolic adaptation. The integration of response surface methodology with metabolite profiling provided a comprehensive understanding of the interaction between environmental constraints and biochemical responses. These findings contribute to optimizing fermentation strategies and offer practical insights for improving bioconversion processes under regulated oxygen conditions.

Declarations

Funding: This research work was funded by Jazan University, Saudi Arabia, under the research project (No. JU-20250222-DGSSR-RP-2025).

Acknowledgment: The authors gratefully acknowledge the financial support provided by Jazan University and extend their sincere thanks to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia.

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability: The data supporting this study are included within the article. Further inquiries can be directed to the corresponding author.

Ethics Statement: This research did not involve human participants, animal subjects, or any data requiring ethical approval from an institutional review board.

Author's Contributions: Mona S. Ashoor: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration.
Wafa Mohammed Asiri: Methodology, Validation, Data curation; Amal Alshammari: Methodology, formal analysis and investigation
Dhouha Taib Jellali formal analysis, Software, Visualization, Writing – review editing.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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REFERENCES

Ahmad, T., Wang, S., & Liu, Y. (2025a). Aspergillus flavus and Aflatoxins (3rd Edition). Toxins, 17(7), 326. https://doi.org/10.3390/toxins17070326

Ahmad, U., Ajaz, H., Perveez, A., & Azam, S.E. (2025b). Assessment of aflatoxin M1 (AFM1) in milk from Chakwal city using ELISA technique. Agrobiological Records, 19, 92-99. https://doi.org/10.47278/journal.abr/2025.011

Alzahrani, K. K. (2025). Unveiling the distribution and research patterns of Aspergillus spp. in Saudi Arabia: A systematic and bibliometric analysis. Frontiers in Microbiology, 16, 1638271. https://doi.org/10.3389/fmicb.2025.1638271

Aydin, S., Karaçay, H. A., Shahi, A., Gökçe, S., Ince, B., & Ince, O. (2017). Aerobic and anaerobic fungal metabolism and omics insights for increasing polycyclic aromatic hydrocarbons biodegradation. Fungal Biology Reviews, 31(2), 61–72. https://doi.org/10.1016/j.fbr.2016.12.001

Behera, S. S., Ray, R. C., & Das, S. (2022). Application of response surface methodology for optimization of lignocellulosic biomass degradation processes. Bioresource Technology Reports, 18, 101034. https://doi.org/10.1016/j.biteb.2022.101034

Beauvais, W., Gart, E. V., Bean, M., Blanco, A., Wilsey, J., McWhinney, K., & Ivanek, R. (2018). The prevalence of Escherichia coli O157: H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: A randomized controlled trial. PLoS One, 13(2), e0192149. https://doi.org/10.1371/journal.pone.0192149

Bissaro, B., Várnai, A., Røhr, Å. K., & Eijsink, V. G. H. (2018). Oxidoreductases and reactive oxygen species in conversion of lignocellulosic biomass. Microbiology and Molecular Biology Reviews, 82(4), e00029-18. https://doi.org/10.1128/MMBR.00029-18

Cairns, T. C. (2023). Hypoxia signaling and metabolic adaptation in filamentous fungi. Fungal Biology Reviews, 45, 1–12. https://doi.org/10.1016/j.fbr.2023.01.002

Chen, B. L., Zhang, T. T., Arooj, A., Xu, C., Yang, J. C. E., Sillanpää, M., & Zheng, Y. M. (2025). Enhancing anaerobic methane production from corn straw: Promoting lignocellulosic biomass biodegradation via aeration in liquid fraction of digestate for green and efficient pretreatment. Journal of Environmental Chemical Engineering, 13(5), 116234. https://doi.org/10.1016/j.jece.2025.119386

Chien, T. Y., Lo, H. C., Liu, M. L., Hsu, T. H., Lee, S. C., Hsu, W. K., & Chao, S. C. (2025). IoT-driven fermentation system for enhanced cordycepin production in Cordyceps militaris under hypoxic conditions. International Journal of Medicinal Mushrooms, 27(4), 1–14. https://doi.org/10.1615/IntJMedMushrooms.2024057399

Cho, T. E., Zhang, G., & Ling, J. (2025). Enhancement of bioactive metabolites from solid-state fermentation of Cordyceps fungus using various substrates on ameliorating oxidative stress to liver health. Journal of Industrial Microbiology and Biotechnology, 52, kuaf012. https://doi.org/10.1093/jimb/kuaf012

Elyamine, A. M., Kan, J., Meng, S., Tao, P., Wang, H., & Hu, Z. (2021). Aerobic and anaerobic bacterial and fungal degradation of pyrene: Mechanism pathway including biochemical reaction and catabolic genes. International Journal of Molecular Sciences, 22(15), 8202. https://doi.org/10.3390/ijms22158202

Ghorai, S. (2024). Filamentous fungi: The production powerhouses of cellulolytic and hemicellulolytic enzymes. In Biofuels (pp. 66–82). CRC Press. https://doi.org/10.1201/9781003350606-6

Grahl, N., Shepardson, K. M., Chung, D., & Cramer, R. A. (2012). Hypoxia and fungal pathogenesis: To air or not to air? Eukaryotic Cell, 11(5), 560–570. https://doi.org/10.1128/EC.00031-12

Haldar, D., & Purkait, M. K. (2020). Lignocellulosic conversion into value-added products: A review. Process Biochemistry, 89, 110–133. https://doi.org/10.1016/j.procbio.2019.10.001

Hedge, J. E., Hofreiter, B. T., & Whistler, R. L. (1962). Carbohydrate chemistry. Academic Press, New York, 17, 371-80.

Hesse, S. J., Ruijter, G. J. G., Dijkema, C., & Visser, J. (2002). Intracellular pH homeostasis in the filamentous fungus Aspergillus niger. European Journal of Biochemistry, 269(14), 3485–3494. https://doi.org/10.1046/j.1432-1033.2002.03042.x

Hillmann, F., Shekhova, E., & Kniemeyer, O. (2015). Insights into the cellular responses to hypoxia in filamentous fungi. Current Genetics, 61(3), 441–455. https://doi.org/10.1007/s00294-015-0487-9

Keller, N. P. (2019). Fungal secondary metabolism: Regulation, function and drug discovery. Nature Reviews Microbiology, 17(3), 167–180. https://doi.org/10.1038/s41579-018-0121-1

Kovács, S., Brendzsák, B., Pfliegler, W. P., Nagy, L., Hamow, K. Á., Pócsi, I., & Pusztahelyi, T. (2026). Mutations and Metabolic Factors Affecting Interaction of Toxigenic and Atoxigenic Aspergillus flavus. Microorganisms, 14(2), 473. https://doi.org/10.3390/microorganisms14020473

Kües, U. (2015). Fungal enzymes for environmental management. Current Opinion in Biotechnology, 33, 268–278. https://doi.org/10.1016/j.copbio.2015.03.006

Kuittinen, S., Hietaharju, J., Bhattarai, I., Hassan, M. K., Kupiainen, L., Kangas, J., & Pappinen, A. (2022). Technoeconomic analysis and environmental sustainability estimation of bioalcohol production from barley straw. Biocatalysis and Agricultural Biotechnology, 43, 102427. https://doi.org/10.1016/j.bcab.2022.102427

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Lohmar, J. M., Gross, S. R., Carter-Wientjes, C. H., Mack, B. M., Wei, Q., Lebar, M. D., & Cary, J. W. (2025). The putative forkhead transcription factor FhpA is necessary for development, aflatoxin production, and stress response in Aspergillus flavus. PLoS ONE, 20(3), e0315766. https://doi.org/10.1371/journal.pone.0315766

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry, 193(1), 265–275.

Mattila, H. K., Mäkinen, M., & Lundell, T. (2020). Hypoxia regulates enzymatic wood decomposition and intracellular carbohydrate metabolism in filamentous white rot fungus. Biotechnology for Biofuels, 13(1), 26. https://doi.org/10.1186/s13068-020-01677-0

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3), 426–428. https://doi.org/10.1021/ac60147a030

Paulussen, C., Hallsworth, J. E., Álvarez-Pérez, S., Nierman, W. C., Hamill, P. G., & Blain, D. (2017). Ecology of aspergillosis: Insights into the pathogenic potential of Aspergillus species. Journal of Fungi, 3(2), 22. https://doi.org/10.1111/1751-7915.12367

Peng, S., Hu, L., Ge, W., Deng, J., Yao, L., Li, H., & Mo, H. (2024). ChIP-Seq analysis of AtfA interactions in Aspergillus flavus reveals its involvement in aflatoxin metabolism and virulence under oxidative stress. International Journal of Molecular Sciences, 25(22), 12213. https://doi.org/10.3390/ijms252212213

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H. W., & Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. Studies in Mycology, 78(1), 141–173. https://doi.org/10.1016/j.simyco.2014.07.004

Shimizu, M. (2018). NAD+/NADH homeostasis affects metabolic adaptation to hypoxia and secondary metabolite production in filamentous fungi. Bioscience, Biotechnology, and Biochemistry, 82(2), 216–224. https://doi.org/10.1080/09168451.2017.1422972

Siedt, M., Teggers, E. M., Linnemann, V., Schäffer, A., & van Dongen, J. T. (2023). Microbial degradation of plant residues rapidly causes long-lasting hypoxia in soil upon irrigation and affects leaching of nitrogen and metals. Soil Systems, 7(2), 62. https://doi.org/10.3390/soilsystems7020062

Thapa, S., Mishra, J., Arora, N., Mishra, P., Li, H., O'Hair, J., & Zhou, S. (2020). Microbial cellulolytic enzymes: Diversity and biotechnology with reference to lignocellulosic biomass degradation. Reviews in Environmental Science and Bio/Technology, 19(3), 621–648. https://doi.org/10.1007/s11157-020-09536-y

Wahyudi, A., Hendraningsih, L., Mahmud, A., Mulatmi, S. N. W., & Prima, A. (2024). Solid-state fermentation characteristic of rice straw using herbivore's cecum microbes. Online Journal of Animal and Feed Research, 14(1), 47–52. https://doi.org/10.51227/ojafr.2024.6

Yu, H., Xiao, H., Deng, H., Frew, A., Hossain, M. A., Tan, W., & Xi, B. (2024). Upgrade from aerated static pile to agitated bed systems promotes lignocellulose degradation in large-scale composting through enhanced microbial functional diversity. Journal of Environmental Sciences, 145, 55–66. https://doi.org/10.1016/j.jes.2023.09.008

Zhang, Y., Liu, X., Chen, H., & Wang, J. (2024). Oxygen-dependent regulation of fungal secondary metabolism and adaptive responses. Journal of Fungi, 10(2), 98. https://doi.org/10.3390/jof10020098

Citation: Ashoor MS, Asiri WM, Alshammari AN and Jellali DT, 2026. Oxygen-dependent physiological and metabolic adaptation of *Aspergillus flavus* during barley straw biodegradation. Agrobiological Records 24: 27-36. https://doi.org/10.47278/journal.abr/2026.022