



Substrate–Microorganism Interactions and Multi-Response Optimization of Enzyme Production in Cereal-Based Solid-State Fermentation

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ABSTRACT

Solid-state fermentation (SSF) offers a sustainable approach for producing industrial enzymes using cereal-based agricultural residues. This study aimed to evaluate substrate–microorganism interactions, compare enzyme-production performance, and identify favourable fermentation combinations through multi-response optimization. A quantitative secondary-data analysis was conducted using the publicly available Mendeley Data repository, Production of enzymes via solid state fermentation from cereals. The analysis included 487 enzyme-production records representing 209 experiments. Descriptive statistics, comparative analysis, Pearson correlation, and desirability-based optimization were applied to examine substrate characteristics, fungal microorganisms, fermentation duration, and enzyme activities. Wheat bran was the most frequently reported substrate (21.1%) and exhibited a median xylanase activity of 2,467.53 U/gds. Fungal co-cultivation involving *Trichoderma reesei* and *Aspergillus niger* demonstrated comparatively higher xylanase, CMCase, and FPase activities than the corresponding monocultures within selected experimental comparisons. Incubation duration showed a weak negative correlation with xylanase activity ($r = -0.223$). Multi-response optimization identified a rice straw–wheat bran mixture combined with fungal co-cultivation as the highest-ranked observed combination, achieving a composite desirability score of 1.000. These findings highlight the potential of substrate selection and microbial co-cultivation for improving enzyme production. However, heterogeneous experimental conditions and measurement differences necessitate controlled validation before industrial application.

Keywords: Solid-State Fermentation; Cereal-Based Substrates; Fungal Co-Cultivation; Enzyme Production; Multi-Response Optimization

1. Introduction

Solid-state fermentation (SSF) is one of the important biotechnological processes which facilitate microbial growth and production of metabolites in moist solid materials free or with limited free-flowing water. It is widely applied for the production of industrial enzymes, organic acids and other valuable biological products. The principle of SSF is advantageous in terms of water saving, reduced processing cost, effective use of agricultural residues etc. compared to the conventional submerged fermentation. The properties render it a promising option for sustainable bioprocessing and waste management (Chilakamarry et al., 2022). Agricultural by-products are also used in producing commercially valuable compounds, which is additionally contributing to resource efficiency and development of circular bioeconomy (Šelo et al., 2021). Furthermore, microbial biotechnology has helped to enhance the use of SSF to produce valuable industrial products from organic wastes (Yafetto, 2022).

As a result of their availability, affordability and nutritional value, cereal-based materials such as wheat bran, rice bran, rice straw, maize residues, as well as other agricultural by-products, are extensively studied as substrates in fermentation processes. Carbohydrates, proteins, minerals and structural polysaccharides are included in these materials for microbial growth and enzyme biosynthesis. Yet, various differences in Lignocellulosic composition, particle structure and nutrient availability can affect the fermentation efficiency (Iram et al., 2020). Wheat bran is a good substrate for microbial fermentation due to its physicochemical properties, which can be good for enzyme production, but may need to be altered to change the characteristics of the substrate, affecting enzyme activity (Ren et al., 2024). Moreover, the residues of cereals can be used to gain advantages in resource recovery by producing fungal enzymes and valorization of agricultural wastes (Teigiserova et al., 2021). However, the activities of cellulases and nutrients may significantly affect the cultivation conditions (Verma & Kumar, 2020).

The fundamental importance of microorganisms in SSF is that they help to determine the utilization of the substrates and the synthesis of enzymes. The species of fungi especially *Aspergillus*, *Trichoderma* and *Penicillium* are commonly studied for the production of various industrial enzymes such as cellulases, xylanases, proteases etc. These microorganisms can produce extracellular enzymes which convert complex plant polymers to simpler compounds. Selected strains of fungi have been shown to produce cellulase and xylanase in a solid-state culture (Espinoza-Abundis et al., 2023). So, the production of lignocellulolytic enzymes from agro-industrial residues is gaining greater and greater attention as an environmentally friendly biotechnology (Leite et al., 2021). But production of enzymes by these microorganisms is reliant on the compatibility of the substrates, the enzymes produced, and the presence of appropriate fermentation conditions (Nargotra et al., 2022). Variations in enzyme yields and production efficiency (Pérez-Contreras et al., 2024) are also due to the differences among the fungal strains and their mechanisms of degradation of lignocellulose.

Enzymes can be produced depending on the microbial performance which can vary with the composition of substrate–microorganism interactions and fermentation conditions. Microbial metabolism and enzyme activities may be affected by factors like temperature, pH, moisture, incubation time, and nutrient supplementations. However, statistical modeling also offers opportunities to identify important fermentation variables, and to assess their relationship to the production outcomes (Jin et al., 2024a). The control of the fermentation environment to boost the productivity and consistency of the process of microorganisms has also been emphasized by the progress of fermentation engineering (Jin et al., 2024b). Moreover, process strategies need to be developed for efficient and economical production of industrial enzymes using low-cost agricultural raw materials (Sharma et al., 2022).

Although these developments have been made, the comparison of enzyme production from various cereal substrates and microorganisms is still difficult due to differences in experimental conditions, the methods of measurement and the responses of the enzymes to fermentation. The example of multiple microbial enzymes proves that more than one enzymatic response needs to be evaluated in the research (Singh et al., 2024). Performance of the cellulolytic enzyme through bioprocess optimization also suggests the usefulness of systematic approaches to discover favourable fermentation conditions (Bhattacharya et al., 2024). The results reported here, however, need to be compared with the results from the various combinations of substrate–microorganism and various enzyme activities, and further research is required. Hence, the present study was designed to compare the enzyme production by various microorganisms over different cereal-based substrates under SSF, explore the interaction of the microorganism and substrate and study the relationship between the combination of the two and fermentation performance and to determine the favourable combination by exploratory multi-response optimization. The study aims to combine comparative enzyme activity analysis with multi-response evaluation to gain insight into sustainable production of enzymes using cereal-based material.

2. Methodology

2.1 Research Design and Data Source

This study utilized the quantitative design in the secondary data research approach to study the interaction between the substrate and microorganism and the production of enzymes under cereal based solid state fermentation (SSF). Secondary data were retrieved from Mendeley Data repository: Production of enzymes via solid state fermentation from cereals, Version 2 (Bourguine et al., 2021), which is publicly accessible. It included 487 enzyme production records of 209 reported experiments using cereal substrates, fungal microorganisms and various fermentation variables. These previous experimental measurements were used to explore the various aspects of substrate properties, microbial enzyme-production profiles, fermentation parameters, and various enzyme-response relationships.

2.2 Data Extraction and Variable Identification

To determine the experimental variables which are relevant to the research goals, secondary data was used. Selected data were those related to substrates based on cereals, fungal microorganisms, production of enzymes and solid-state fermentation conditions. The following variables were taken into account for the analysis: experiment identification number, type of substrate, microorganism, type of enzyme, enzyme activity, unit of measurement, incubation time, treatment of the substrate, presence of nutritional supplement, optimization technique, fermentation parameters, publication year, and reference from the original paper. The activity of the enzymes was considered as the primary response variables, and substrate properties, microbial species and fermentation conditions, as explanatory variables. Measures taken to distinguish related enzyme measures and to keep measures consistent across observations are retained, as these can be used as experimental identifiers and as a source reference. The data obtained was systematically arranged and analysed descriptively, compared and contrasted between substrate–microorganism combinations and assessed for several enzyme production responses.

2.3 Data Screening and Preparation

Data screening was done to evaluate completeness, consistency and appropriateness for quantitative analysis. Experimental records were checked for enzyme activity missing, inconsistent enzyme-substrate names, differences in the identification of microbial strains and enzyme measurement units. The enzymes activity is reported in the original database in 6 categories of units: U/gds, U/g, U/mL, U/g protein, U/cm² and other enzyme activity expressions related to the substrate. Substrate mixtures and fungal co-cultures were differentiated from individual substrates and single organism fermentation experiments. Observations where there were not complete incubation measurements were retained for categorical analysis but not used for calculations of numerical incubation duration. The experimental identifiers were used to link records and not consider multiple enzyme measurement from the same experimental replicate as independent experimental replicates.

2.4 Classification of Cereal-Based Substrates and Fungal Microorganisms

The cereal-based substrates were classified based on the contents of the database and these substrate types were wheat bran, wheat straw, rice bran, rice straw, corn-based substrates and other cereal residues. Mixed substrates with cereal materials and other by-products from agriculture were classified separately because of the need to maintain the reported compositions of these materials. The fungal microorganisms were classified into genera, species and strains. The fungus genera recorded were from *Aspergillus*, *Trichoderma* and *Pleurotus*. Experiments with a single strain of fungi differed from those with microbial co-cultures. Frequencies and percentages were used to evaluate the distribution of the substrates and microorganisms. Substrate–microorganism combinations were then evaluated for their presence in the experimental records and for the availability of enzyme production measurements for certain combinations.

2.5 Assessment of Fermentation Conditions and Enzyme Production

The incubation time, methods of pretreatment, nutritional supplementation, optimization methods and process parameters recorded in the database were used to evaluate the fermentation characteristics. Incubation time was measured in hours and was the main fermenting variable available in a numerical form by the principal. The other fermentation parameters such as temperature, pH, moisture content, inoculum size, preparation of substrate were studied with the descriptive data available in the experimental data sheets. The other variables were only included in the numerical analysis if their value and measurement unit could be determined. The activities of xylanase, carboxymethyl cellulase (CMCase), filter paper activity (FPase),

β -glucosidase, β -xylosidase, protease and other enzymes were reported and used to evaluate the performance of enzyme production. The activities of enzymes were determined independently by enzyme type and suitable measurement units. This enabled descriptive comparisons of experimental procedures, as presented in the source publications, and took into consideration the variation in the experimental procedures.

2.6 Evaluation of Substrate–Microorganism Interactions

The interactions between substrate and microorganism were studied by making comparisons on production of enzymes by different combinations of cereal substrate and fungal organism reported. Observations were classified into substrate type, microorganism type, enzyme type, unit type and source experiment. Comparisons were drawn between differences in enzyme activities of the various fungal strains grown on the same substrates and the ability of each individual microorganism to grow on the various cereal materials. Single organism and co-culture experiments were differentiated to determine their different enzyme production patterns. Wherever possible, comparisons within publications were given precedence to reduce the effects of analytical procedure difference that would create a heterogeneity. Activity patterns were analysed descriptively and compared graphically. The data set was not consistently presented in independent experimental replicates, so that the differences between substrate–microorganism combinations were not seen as interaction effects, but as observation patterns.

2.7 Multi-Response Evaluation and Optimization

Multi-response optimization was tackled by making comparative experiments that gave more than one enzyme activity. Experimental identifiers were analyzed to look for several enzyme responses for a reported fermentation system. Special attention was paid to xylanase, CMCase and FPase as they were included in multiple experiments and offered complementary information in relation to the production of enzymes to break down the lignocellulose. Combined quantitative analysis was only done for observations taken with compatible measurement units. If several answers were provided, the enzyme activities were normalized to deal with the differences in the numerical size of the answers. The relative performance of the fermentation combinations observed was evaluated by a dimensionless desirability and the higher the enzyme activities were considered favourable. Normalized responses were used to calculate the composite desirability, which was a geometric mean with equal weights. The scores obtained were only in relation to the previously reported experimental conditions and were not to represent an optimum or an experimentally validated optimum. Combined response profiles were interpreted taking into account the differences in incubation times of individual enzyme measurements.

2.8 Statistical Analysis and Data Visualization

To summarize the categories of substrates, microbial population, enzyme activity and fermentation characteristics, descriptive statistical methods were used. Frequencies and percentages were used to represent the categorical variables and the mean $\pm$ standard deviation (SD) or median with interquartile range (IQR) was used for numerical variables, as appropriate. Enzyme production measurements were classified as to enzyme type and activity unit for the purpose of maintaining comparability. Where possible, numerical observations were used for exploratory assessment of the relationships between incubation duration and enzyme activity by means of Pearson correlation analysis. Cautious interpretation of correlations between records from different source studies was made due to variations in substrate, microorganism identity and experimental conditions. Comparisons were not formally tested if there was insufficient evidence of independent replication.

2.9 Ethical Considerations

The current study was exclusively based on secondary experimental data which are freely available in a scientific repository. No humans were involved, no animals were used, no personal information was disclosed and such experiments as were conducted were new ones. Therefore, ethical approval from the institutions was not necessary for the secondary analysis. The original source of the data was cited appropriately and the scientific meaning of the experimental measurements that were reported were not changed. To keep the results transparent, reproducible, and of research integrity, differences in units of measurement, experiment procedure, and reporting were taken into account.

3. Results

The present study used secondary experimental data from the Mendeley Data repository, Production of

enzymes via solid state fermentation from cereals. The substrates analysed were cereal-based substrates, fungal microorganisms, enzyme-production characteristics, fermentation time, and multi-response performance.

3.1 Distribution of Cereal-Based Substrates and Fermentation Records

A total of 487 records of enzymes that were produced in experiments were recorded in the database, corresponding to 209 experiments. A total of 45 labels of substrate, 111 labels of microorganism and 34 labels of enzyme were identified. Wheat bran was the most common substrate with a total of 103 records (21.1%), and wheat bran mixed with sugarcane bagasse (11.5%) and wheat bran mixed with cottonseed meal (8.4%). The other materials mentioned, rice straw and corn stover, each accounted for 7.4% of observations. The remaining observations were made on other individual cereal substrates and mixtures. The data on the distribution of the major substrates are provided in Table 1.

Table 1. Distribution of Cereal-Based Substrates in Solid-State Fermentation Records

Substrate	Frequency (n)	Percentage (%)
Wheat bran	103	21.1
Wheat bran + sugarcane bagasse	56	11.5
Wheat bran + cottonseed meal	41	8.4
Rice straw	36	7.4
Corn stover	32	6.6
Oat + sugarcane bagasse	32	6.6
Wheat straw	28	5.7
Other substrates and mixtures	159	32.6
Total	487	100.0

The most common categories of substrates reported are shown in Figure 1. Wheat bran was the main material followed by wheat bran and sugarcane bagasse, and wheat bran and cottonseed meal. The frequencies are the result of observations of enzyme production, not of independent experiments.

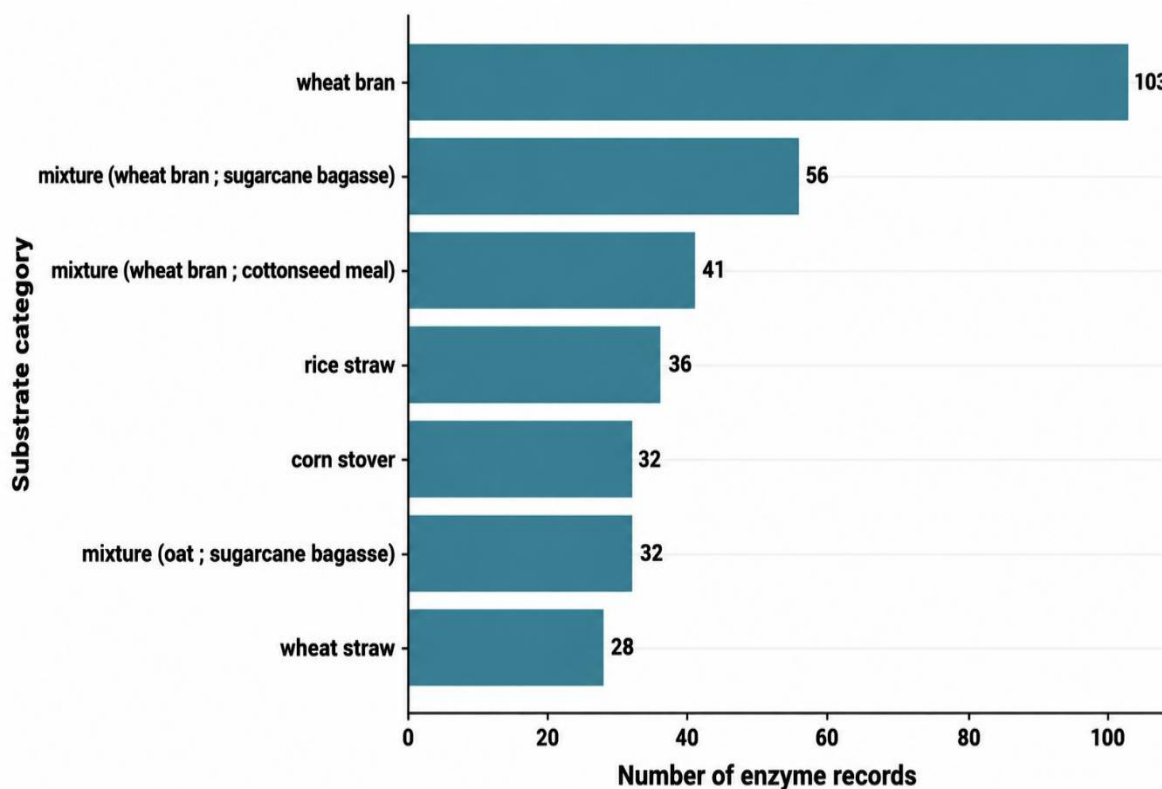


Figure 1. Distribution of Major Cereal-Based Substrates

3.2 Comparative Enzyme Production Across Cereal-Based Substrates

Production of enzymes was different with different cereal-based substrates. Activities were analysed separately for comparability, in units per gram of dry substrate (U/gds). Wheat bran exhibited a median xylanase activity of 2,467.53 U/gds (11 records) while rice straw gave a median of 1,298.70 U/gds (11 records). The median values of CMCase activity were 97.36 U/gds and 44.11 U/gds for wheat bran and rice straw respectively. The median activity of the CMCase and FPase of corn stover was 266.78 and 73.15 U/gds, respectively, with the latter value being obtained from only two observations.

Table 2. Comparative Enzyme Activities Across Selected Cereal-Based Substrates

Substrate	Enzyme	Records (n)	Median activity (U/gds)
Wheat bran	Xylanase	11	2,467.53
Wheat bran	CMCase	12	97.36
Wheat bran	FPase	10	22.81
Rice straw	Xylanase	5	1,298.70
Rice straw	CMCase	4	44.11
Rice straw	FPase	4	12.54
Corn stover	Xylanase	1	17.00

Corn stover	CMCase	2	266.78
Corn stover	FPase	2	73.15
Wheat straw	Xylanase	1	1,023.00
Wheat straw	CMCase	2	68.55
Wheat straw	FPase	4	27.30

The descriptive comparisons are summarized in Table 2, while Figure 2(a–c) illustrates the variation in the three enzyme responses across four substrates.

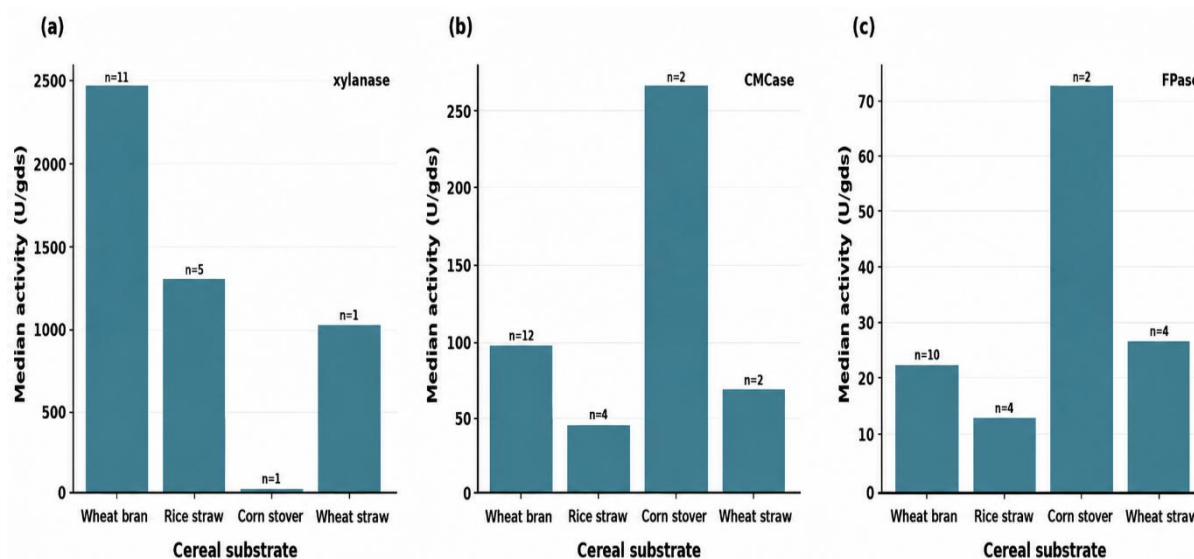


Figure 2. Median enzyme activities across selected cereal-based substrates. (a) Xylanase, (b) CMCase, and (c) FPase. Activities are expressed in U/gds, and observation numbers are shown above individual bars.

3.3 Distribution and Activity Profiles of Major Enzymes

There were 34 enzyme labels in the database, most of which were xylanase (92 records), CMCase (83), β -glucosidase (74) and FPase (61). The U/gds activity for xylanase within the U/gds measurement group was $999.01 \pm 1,702.68$ U/gds with a median of 286.56 U/gds. The mean activities of CMCase, β -glucosidase and FPase were 90.66 ± 161.18 U/gds, 36.87 ± 48.43 U/gds and 34.26 ± 74.84 U/gds respectively. Several enzyme categories had significant differences between the mean and median, suggesting that the distributions for these enzymes were skewed to the right and there was extensive variability between reported experiments. The characteristics of the enzyme activity are listed in Table 3 and the frequency of reporting is shown in Figure 3.

Table 3. Descriptive Activity Profiles of Major Enzymes in Solid-State Fermentation

Enzyme	U/gds records (n)	Mean $\pm$ SD (U/gds)	Median (U/gds)
Xylanase	60	$999.01 \pm 1,702.68$	286.56
CMCase	60	90.66 ± 161.18	31.83
β -Glucosidase	51	36.87 ± 48.43	22.47

FPase	35	34.26 ± 74.84	17.58
β -Xylosidase	30	3.39 ± 4.66	1.63

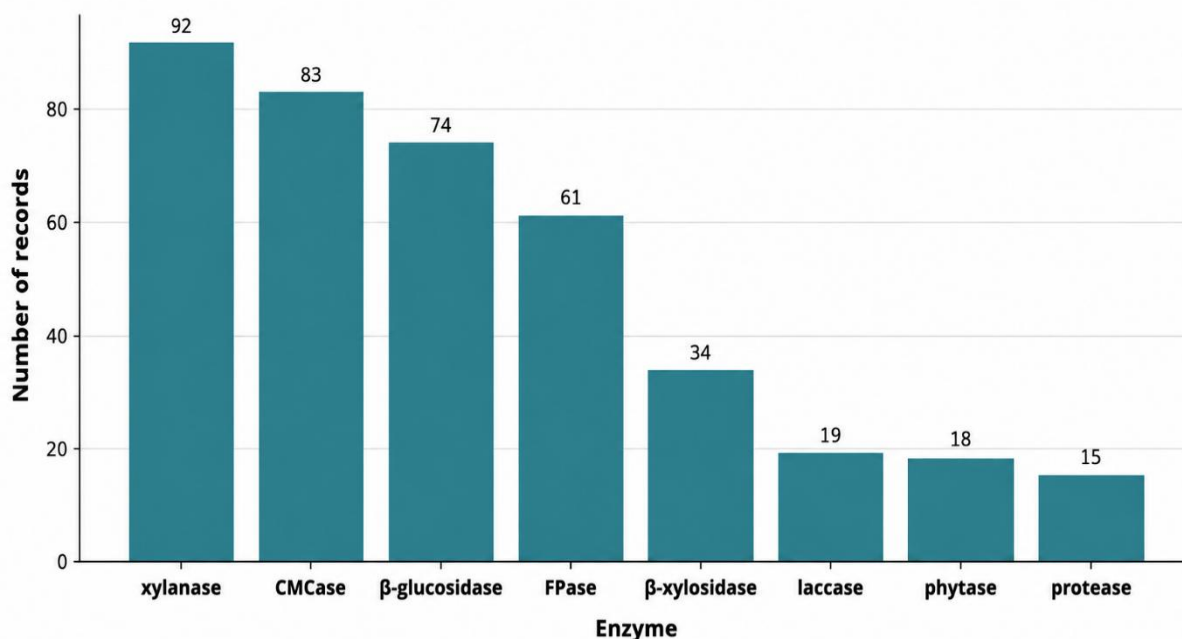


Figure 3. Frequency Distribution of Major Enzymes

3.4 Substrate–Microorganism Interactions and Enzyme Production

A subset of experiments included the use of *Trichoderma reesei* RUT C-30, *Aspergillus niger* BC-1 and their co-culture as substrate–microorganism combinations. The co-culture activity of xylanase, CMCase and FPase was 2710.62, 117.71 and 24.17 U/gds respectively for wheat bran. These activities were greater than the activities reported at the same time during either monoculture. The pattern was similar in case of rice straw, the co-culture showed the activity of xylanase as 2,144.52 U/gds, while the other two fungi *T. reesei* showed 1,298.70 U/gds, and *A. niger* showed 936.06 U/gds. The enzyme production measured (optical density) were summarized in Table 4 while Figure 4(a–c) shows the activity pattern among the fungal treatments.

Table 4. Enzyme Activities Across Selected Substrate–Microorganism Combinations

Experiment	Substrate	Microorganism	Xylanase	CMCase	FPase
201	Wheat bran	<i>T. reesei</i>	2,467.53	68.57	22.89
202	Wheat bran	<i>A. niger</i>	2,604.06	48.22	13.57
203	Wheat bran	Co-culture	2,710.62	117.71	24.17
204	Rice straw	<i>T. reesei</i>	1,298.70	58.16	16.05
205	Rice straw	<i>A. niger</i>	936.06	30.06	9.02
206	Rice straw	Co-culture	2,144.52	74.00	17.58
207	Rice straw + wheat bran	Co-culture	2,757.20	99.53	24.98

208	Rice straw + wheat bran	Co-culture	2,859.60	119.51	30.99
209	Rice straw + wheat bran	Co-culture	3,106.34	131.34	35.80

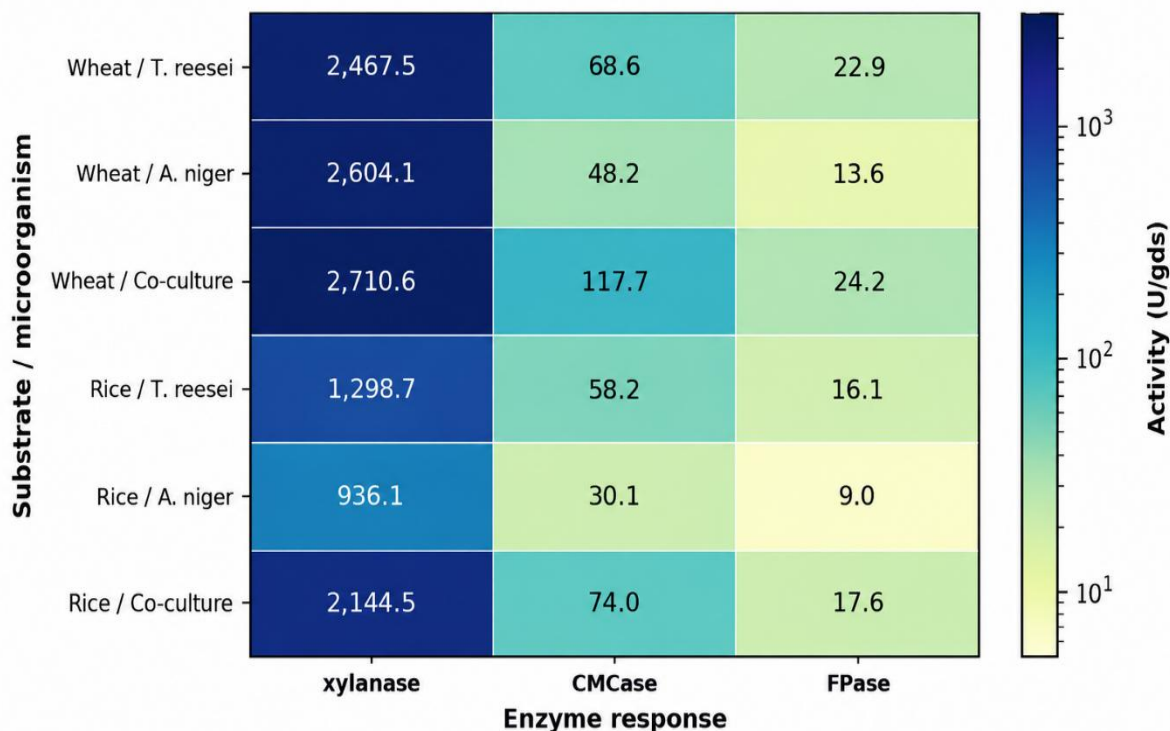


Figure 4. Heatmaps showing the enzyme-production profiles of fungal monocultures and co-cultures on wheat bran and rice straw. (a) Xylanase, (b) CMCase, and (c) FPase. Darker shading represents higher reported activity within each enzyme-specific panel.

No statistically significant substrate–microorganism interactions have been defined by the comparisons. In the selected experimental group, co-cultivation was correlated with higher enzyme measurements, as indicated by the activity patterns. These interaction effects were not consistently available to be formally confirmed by repeated measurements made independently under identical conditions, however.

3.5 Fermentation Duration and Its Association with Enzyme Production

Of the 458 records, for 94.0% of all enzyme observations, numerical incubation durations had been provided. The mean duration of incubation was 162.76 hours $\pm$ 101.46 hours and the median was 144 hours. The interquartile range for incubation period was 96 to 168 hours, and recorded incubation period ranged from 14 to 720 hours. Pearson correlation analysis of 57 xylanase records with compatible U/gds and incubation times showed a weak, negative relationship between enzyme activity and incubation time ($r = -0.223$). The descriptive characteristics are shown in Table 5 and the incubation-time distribution and its linkage with the production of xylanase are represented on Figure 5(a–b).

Table 5. Descriptive Characteristics of Incubation Duration and Xylanase Activity

Parameter	Result
Available incubation measurements	458 (94.0%)
Mean incubation duration	162.76 h
Standard deviation	101.46 h

Median incubation duration	144 h
First quartile (Q1)	96 h
Third quartile (Q3)	168 h
Minimum duration	14 h
Maximum duration	720 h
Xylanase correlation observations	57
Pearson correlation coefficient	-0.223

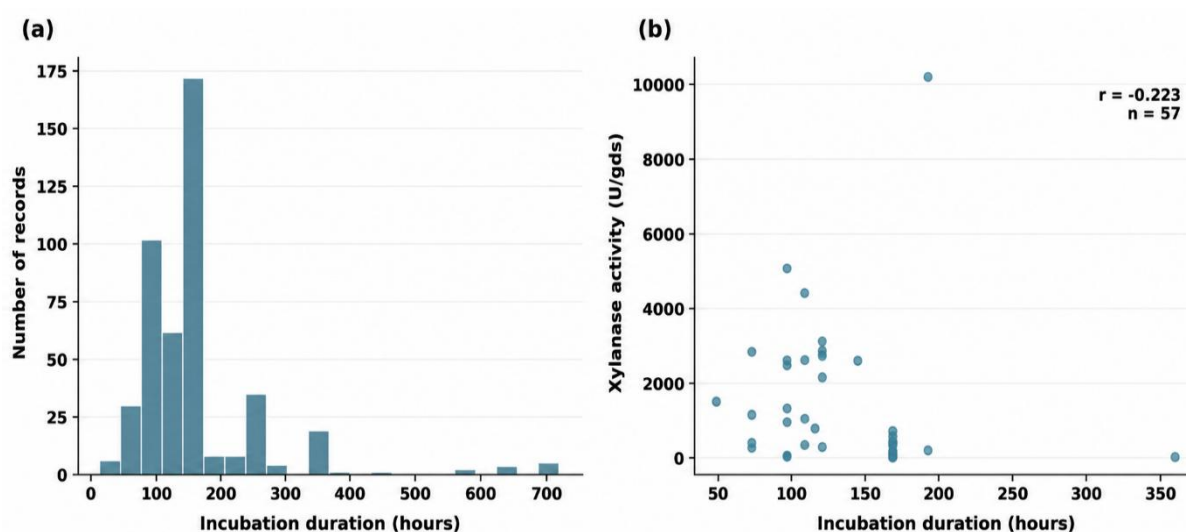


Figure 5. Incubation characteristics of cereal-based solid-state fermentation records. (a) Distribution of incubation duration across 458 observations and (b) relationship between incubation duration and xylanase activity among 57 compatible observations.

The weak negative correlation is descriptive, and has not been corrected for differences in substrate, microorganisms, or studies. Significant differences in fermentation time were found in the original studies when analyzed. The correlation between the incubation period and enzyme production was weak and negative, so it does not prove that incubation over a longer period decreased the production of enzymes; but this is probably because the incubation conditions, fungal strains and substrate characteristics varied in the source experiments.

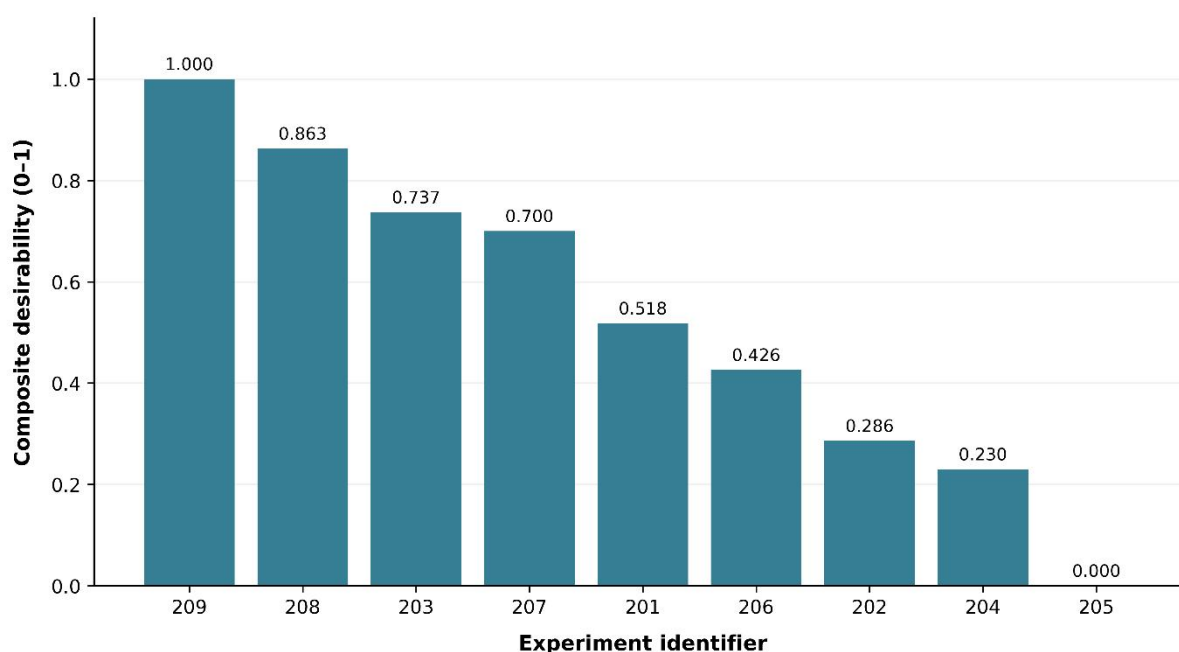
3.6 Multi-Response Optimization of Enzyme Production

A multi-response optimization was performed to compare fermentation systems to those of available measurements of xylanase, CMCase, and FPase. All three responses reported in U/gds were included in 13 experimental identifiers, nine of which from the same source article were chosen for use as the principal comparison. The enzyme activities were min-max scaled and then a composite desirability score (on a 0–1 scale) was used and was calculated as an equally weighted geometric mean. The scores obtained are shown in Table 6 and Figure 6.

For the same culture system, the highest composite desirability score (1.000) was obtained for Experiment 209 (rice straw–wheat bran) with the co-cultivation of *T. reesei* RUT C-30 and *A. niger* BC-1. The xylanase, CMCase and FPase activities reported were 3,106.34, 131.34 and 35.80 U/gds respectively. Experiment 208 showed the second highest desirability score (0.863) followed by the experiment 203 with a desirability score (0.737). The four experiments that performed the best were the fungal co-cultivation experiments and experiment 205 performed the worst with composite performance.

Table 6. Multi-Response Optimization of Selected Solid-State Fermentation Experiments

Experiment	Xylanase (U/gds)	CMCase (U/gds)	FPase (U/gds)	Desirability
209	3,106.34	131.34	35.80	1.000
208	2,859.60	119.51	30.99	0.863
203	2,710.62	117.71	24.17	0.737
207	2,757.20	99.53	24.98	0.700
201	2,467.53	68.57	22.89	0.518
206	2,144.52	74.00	17.58	0.426
202	2,604.06	48.22	13.57	0.286
204	1,298.70	58.16	16.05	0.230
205	936.06	30.06	9.02	0.000

**Figure 6. Composite Desirability Scores for Multi-Response Enzyme Production**

The co-culture fermentation system was identified as one of the promising systems for multiple production of lignocellulolytic enzymes by optimization analysis. However, the desirability scores were obtained from measurements compiled in a retrospective manner and should only be interpreted as relative rankings and not as results of an experimental optimization.

4. Discussion

The present study showed the great differences in enzyme production in several different substrates based on cereals which indicates the effect of substrate composition on solid state fermentation (SSF). The xylanase activity of wheat bran was higher with median value of 2467.53 U/gds than in the case of rice straw. The differences could be attributed to disparity in substrate accessibility, structural polysaccharides and/or carbohydrate availability. Historically, the possibilities of rice straw as a raw material for producing valuable biological products have been pointed out, with the application of rice straw as a fermentation material (Ali et al., 2024a). The xylanase activity was relatively high with wheat bran which is supported by the results of Ali et al. (2024b) that wheat bran is suitable for production of fungal xylanase. However, caution should be used in interpreting the differences observed since there were differences in the substrate composition and cultivation conditions between the original studies. Analogous studies have shown that wheat and rice residues can be used for cellulase and xylanase production (Devi et al., 2024). The other factor that may account for the difference in enzyme activity is the microbial compatibility of the substrate (Sharma & Bajaj, 2018).

The performance of microbial varied among the substrate – microorganism combinations, especially between monoculture and co-culture fermentation systems. The co-cultivation of *Trichoderma reesei* and *Aspergillus niger* resulted in significantly higher xylanase, CMCase and FPase activities among the experimental groups. These differences can be due to complementary enzyme functions and/or better utilization of complex components of substrate. In cellulase production studies, the optimization of fermentation conditions and the selection of fungal strains have been proven to be important (Boondaeng et al., 2024). The research on fungal enzyme cocktails also confirmed the possible benefits of a synergistic effect between complementary activities of the lignocellulolytic enzymes (Mardetko et al., 2021). Moreover, co-cultivation of microorganisms has been explored for enhancing bioproduct formation in solid substrate culture (Prabhu et al., 2022). The current observations, however, do not provide for statistically meaningful microbial interactions as replication of experiments was not available in all cases.

There was considerable variation in enzyme production measurements in the fermentation conditions. The incubation time was in the range of 14 to 720 hours and the correlation between incubation time and xylanase activity was found to be weakly negative ($r = -0.223$). The correlation indicates that there is no consistent increase in enzyme activity with longer fermentation times for all of the records compiled. This variation may be due to variation in growth of microorganisms, stability of enzymes, and nutrients. The importance of fermentation conditions control for microbial production with better efficiency has been highlighted in the past (Kumar et al., 2021). Optimisation of cultivation parameters also has been correlated with enhanced production of extracellular amylase (Premalatha et al., 2023). Similarly, studies on phytase production have demonstrated the importance of selecting appropriate fermentation conditions to improve enzyme activity and process efficiency (Priya et al., 2024). Accessibility of the lignocellulosic components might be further affected by the pretreatment of the substrate, which can also have a significant impact on enzyme production (Wang et al., 2023).

The combination of rice straw–wheat bran mixture and fungal co-cultivation was the most desirous combination observed and obtained highest composite desirability score of 1.000 in the multi-response optimisation. Here we see that consideration of a number of responses from different enzymes, rather than optimizing each enzyme separately, may be important. Response surface methodology is very useful in optimizing fungal enzyme associated processes in previous studies (Abdel-Aty et al., 2022). The same optimization method has been employed in optimization of α -amylase production using the agricultural residues (Abdel-Mageed et al., 2022). Multi-enzyme assessment also serves to highlight the relevance of simultaneous optimization of cellulase, xylanase and ligninase production (Amadi et al., 2020). The value of the systematic fermentation evaluation is also evident from the statistical optimization of fungal xylanase production (Kazeem et al., 2023).

Although these results were achieved, there were several limitations to the study. Secondary data was based on various experimental procedures, with units of activity that were not compatible and with inconsistent documentation of independent replicates. Furthermore, some enzyme responses were tested at various incubation times and thus composite desirability scores were not necessarily interpreted as simultaneous production outcomes. To expand the use of the fermenter in other industrial processes, standardised operating conditions and fermentation performance at larger scales need to be validated (Postigo et al., 2021). Further studies are needed to confirm the identified production patterns and to establish the reproducible optimal fermentation conditions through controlled experiments using different substrates (cereals), strains (fungi) and enzyme combinations.

5. Conclusion

The present study showed that the use of cereal based substrates, fungal microorganisms and fermentation conditions are very important in enzyme production by solid state fermentation (SSF). Secondary experimental data showed that the enzyme activities like xylanase, CMCase and FPase varied to a large extent among the different substrates and microbial combinations. The wheat bran showed relatively good xylanase activity, whereas differences in the xylanase production activity between rice straw, corn stover and other cereal residues indicated the effects of the characteristics of the substrate on the production activity of the enzyme. The enzyme activities of fungal co-cultivation (*Trichoderma reesei* + *Aspergillus niger*) were higher than those of the monocultures under the experimental comparisons selected. The fermentation period was very different, ranging from 12 to 144 hours, and was negatively correlated with xylanase activity, as more activity did not always result in a longer fermentation period. The best combination observed was the rice straw–wheat bran mixture in combination with fungal co-cultivation as seen from the composite desirability score which was calculated as 1.000. The results of these analyses suggest that several enzyme responses could be studied in an effort to discover desirable fermentation

systems for sustainable applications in industry. But, differences in experimental protocols, measurement units, and incubation conditions precluded direct comparisons and causal interpretations. The results of this work should be confirmed by controlled fermentation experiments, by standardizing the enzyme assays and by using other combinations of substrate/microorganism for further optimization strategies that can be repeated and industrialized.

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