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Optimization of Fermentation Conditions for Enhanced Amylase Production by Soil-Derived Fungal Isolates

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Abstract

The increasing demand for industrial enzymes, particularly amylases, is driven by their extensive applications in the food, pharmaceutical, textile, and biofuel industries. Microorganisms, especially fungi, represent important sources of industrial enzymes because of their high production yields, cost-effectiveness, and ease of cultivation and manipulation. This study aimed to isolate and identify amylase-producing soil fungi and optimize key fermentation parameters for enhanced amylase production. Soil samples were baited with potato, serially diluted, and cultured on Potato Dextrose Agar (PDA). Morphological and microscopic characterization identified four fungal isolates: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus flavus*. Preliminary screening on starch agar demonstrated variations in amylolytic activity among the isolates, with *A. niger* exhibiting the highest activity, characterized by a 55 mm zone of clearance. Consequently, *A. niger* was selected for amylase production using submerged fermentation (SmF). The effects of key fermentation parameters, including pH, temperature, and incubation time, were systematically evaluated to determine conditions that maximized enzyme production. Maximum amylase activity of 0.852 U/mL was achieved at an optimum pH of 5.0, temperature of 30 °C, and incubation period of 72 h. These optimized conditions were subsequently employed for enzyme production. The findings demonstrate the potential of indigenous soil-derived *A. niger* as a sustainable source of industrial amylase and emphasize the importance of fermentation-process optimization for improving microbial enzyme yields. The study provides useful baseline information for developing efficient and cost-effective biotechnological processes for industrial amylase production.

Keywords
Amylase,
Soil Fungi,
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Fermentation Optimization,
Enzyme Production,
Biotechnology,
Microbial Enzymes,
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1. Introduction

Amylases (EC 3.2.1.1) are a group of enzymes that catalyze the hydrolysis of starch molecules into simpler sugars like glucose, maltose, and dextrins. These enzymes are ubiquitous in nature, found in plants, animals, and microorganisms, with microbial sources—particularly fungi—being the most industrially exploited due to their high yield, stability, and ease of genetic manipulation (Pandey et al., 2000; Sivaramakrishnan et al., 2006).

Industrial applications of amylases are vast, encompassing food processing (e.g., bread, brewing), pharmaceuticals (e.g., lactose hydrolysis), textiles (e.g., starch removal), and biofuel production (e.g., ethanol from starch). The global enzyme market is projected to grow significantly, with microbial amylases accounting for approximately 30-35% of the total enzymatic products (Nyugen et al., 2002; Van der marrel et al., 2002).

Microorganisms such as *Aspergillus* spp., *Bacillus* spp., and *Rhodothermus* spp. are prominent producers of amylases. Among these, *Aspergillus niger* is particularly esteemed due to its high enzyme yield, safety profile, and adaptability to various fermentation conditions (Holker et al., 2004; Hernandez et al., 2006).

Soil is a rich reservoir of microbial diversity, including fungi capable of secreting industrial enzymes. Previous studies have demonstrated that indigenous soil fungi can be harnessed for enzyme production, especially when fermentation conditions are optimized (Acharya et al., 2014).

This study aims to isolate fungi from soil samples, identify the potent amylase producers, and optimize key fermentation parameters—pH, temperature, and incubation time—to maximize amylase yield. The findings are intended to contribute to sustainable enzyme production methods suitable for local industries in Nigeria and similar developing regions.

3.0 MATERIALS AND METHODS

3.1 STERILIZATION OF GLASS WARE AND EQUIPMENT

Glassware were properly washed with detergent and sterilized in hot air oven at 160^{o}C for 2hours and left in the oven until required.

3.2 SAMPLE COLLECTION

Soil sample was collected by baiting. A piece of potato was cut and buried deep in the soil for 8days in order to attract microorganisms (especially fungi). Soil sample was collected in a sterile polythene bag using a sterilized spatula collect the soil around the decomposing potato, this was transported to Biology/Microbiology laboratory Federal Polytechnic Bauchi.

3.3 SAMPLE PREPARATION

One gramme of the soil sample was suspended in 10mls of sterile distilled water and 10-fold serial dilutions were made up to 10^{-6} dilution.

3.3 MEDIA PREPARATION

The media used in this study were Potato Dextrose Agar (PDA), soluble starch, Agar-Agar, glucose, fructose, peptone, carbon sources, nitrogen sources etc. The media were prepared according to the manufacturer's instructions. Potato dextrose agar was used for isolation, subculturing and identification of the fungal strain, while starch agar was used for screening of amylolytic fungi. A 3.9g of the PDA was dissolved in 120mls of sterile distilled water; this was autoclaved at 121^{o}C per

15minutes after cooling for about 45°C the media was fortified with 3.0g of tetracycline in order to inhibit the growth of bacteria, 20mls of the media was poured into 6 sterile petri-dishes and left undisturbed until the agar solidified.

Starch agar was prepared for screening of the isolates, 5.3g of soluble starch was amended with 8g of potato dextrose agar and 4g of agar-agar 17.3g of starch agar was dissolved in 100ml of distilled water, boiled on a Bunsen burner f or 30minutes, allowed to cool at room temperature (25°C) poured in sterile petri-dishes and allowed to solidify. (Pandey, 1999, Heidelbargh and Springer2006).

3.4 ISOLATION OF FUNGAL STRAIN

About 1ml of the solution was spread on the prepared PDA plates (using a sterile bent glass spreader) and incubated at room temperature for 5days for maximum growth of isolates. In order to obtain pure cultures, the young fungal colonies were asepticaly transferred into fresh PDA plates. The isolates were allowed to stay for five days at an abient temperature of 25±3°C after which it was observed both macroscopically and microscopically. (Diba *et al.*, 2007, Mohammadi *et al.*, 2009)

3.5. IDENTIFICATION OF FUNGAL ISOLATES

The fungal isolates were identified based on their morphological characteristics according to taxonomic key. Both macroscopic and microscopic characteristics were observed. The morphological and cultural characteristics such as colonies, colour and texture of the isolates were observed macroscopically on the PDA plates, while, for microscopic identification, a drop of lactophenol cotton blue solution was placed on a clean glass slide and a small tuft of fungus with spore bearing structure was transferred onto the drop of the lactophenol cotton blue and observed under low and high-power objectives of the microscopes. Royal Hurti culture Mini color Chart, 2005).

3.5.1 Screening for Amylolytic Fungi

Screening was done by starch agar plate method. The isolated fungi were inoculated on PDA plates and amended with 2% starch with 1.5% of agar. The plates were inoculated at 25±2°C for 5days, the plates were then flooded with iodine solution (iodine 0.2g), potassium iodide 0.4g, distilled water 100ml) the plate appeared to be dark blue and clear zone around the colonies were observed. Based on the measurement of clear zone observed around the colonies, which was carried out using meter rule, the best isolate, *Aspergillus niger* with a zone of clearance of 5.5cm was utilized for further study (Aneja, 2000).

3.5.2 Growth medium and inoculum preparation

The growth medium was prepared in a 500ml Erlenmeyer's flask and it contained the following composition: soluble starch 2.5g, KH_2PO_4 1.4g, NH_4NO_3 1.0g, KCl 0.5g, $MgSO_4.7H_20$ 0.1g, $FeSO_4.7H_2O$ 0.0014g, and peptone 2g. this were dissolved into the flask containing the sterile distilled water, 200ml of the growth medium was transferred into a 250ml conical flask and sterilized in an autoclave at 121°C for 15minutes, it was allowed to cool at room temperature and a lop full of the *Aspergillus species* from PDA slant was transferred into the growth medium under sterilized condition in laminar air flow. The inoculated flask was placed on an orbital shaker at 121rpm at 30° for 5days which allowed homogenous mixing of the inocula with the growth medium and maintained at pH 4.0 which is the minimal salt medium for the

growth medium (Pandey *et al.*,1999, Heidelbargh and Springer,2006)

3.5.3 Optimization of Fermentation Conditions

Various physical parameters influencing production such as pH (4.0, 4.5, 5.0, 5.5, 6.0), temperature (25°C, 30°C, 35°C, 40°C, 45°C) and incubation time (24, 48, 72, 96 120hours). The strategy followed was to optimize each physical parameter independent of the other and optimal conditions were employed in all the experiments.

3.5.4 Optimization of pH

In order to determine the optimum pH value for amylase production, the fermentation medium was prepared by transferring 200ml of the production medium into a 250ml conical flask and inoculated with the fungus. The effect of the pH was studied by varying the pH of the medium at different ranges of (4.0, 4.5, 5.0, 5.5, 6.0pH) for 24hours at 25°C. The temperature and time were kept constant at each pH value range. The pH of the medium was first adjusted to 4.0 using sodium acetate buffer pH 6.0, and the medium was inoculated with the fungus and incubated at 25°C for 24hours. Incubation was done at 30°C for 2days, 35°C for 3days, 40°C for 4days and 45°C for 5days at the same pH, the same was repeated for pH 4.5, 5.0, 5.5 and 6.0 respectively. The optimum pH for the fermentation of the was fixed for subsequent experiments.

3.5.5 Optimization of Temperature

To determine the optimum temperature for amylase production, the fermentation medium was prepared and 200ml of the medium was transferred aseptically into a conical flask, the medium was adjusted to the determined optimum pH and remained so. The effect of the temperature was studied by varying the temperature at different range of 25°C, 30°C, 35°C, 40°C, 45°C. The medium was inoculated with the fungus and incubated at 25°C for 1day. At the same time incubations was carried out for 2,3,4 and 5 days at the predetermined optimum pH and the optimum temperature for fermentation of the substrate was now fixed for subsequent experiment. (Alva et al., 2007, Varalashikmi *et al.*, 2009, George and Mike, 2012).

3.5.6 Optimization of incubation time

The optimum incubation time for amylase production was carried out using the same fermentation medium in which 200ml of the medium was transferred into a sterile conical flask. The effect of incubation time was studied by varying the time at different ranges of 1,2,3,4, and 5 days at the determined optimum pH and Temperature (Alva, *et al.* 2007, Varalashimi *et al,* 2009., George and Mike 2012).

3.5.7 Production of Amylase (using the Optimized pH, Temperature and Time)

The medium used for optimization was also used for the production of amylase. This was also carried out in 250ml Erlenmeyer flask in which 200ml of the production medium containing the required salt was transferred into the flask, additional growth sources such as glucose, fructose, sucrose, maltose, peptone, soluble starch, urea and yeast extract each 2g were added into the growth medium, also 15g of treated corn stock was added as substrate the pH of the medium was adjusted to the determine optimum pH. 5ml of the homogenous suspension was inoculated into the growth medium. After the inoculation, the fermentation was carried out at the determined optimum temperature and time

(Heidelbargh and Springer, 2006, Mishra and Behera 2008).

3.5.8 Enzyme Extraction

Amylase enzyme was extracted by adding 50ml of citrate buffer at pH 6.0 and the flask was agitated on a shaker at 180rpm for one hour,the mixture was filtered through Watman filter paper No 1 and centriguge at 100rpm for 5minutes, the supernatant was collected and treated as crude enzyme. (Pandey *et al.*, 2006).

3.5.9 Assay for Amylase

Amylase activity was determined using the modified method of Xiao *et al.*, (2006). The reaction mixtures consisted of 2ml of 0.1%(w/v) starch in 0.2ml citrate phosphate buffer pH 6.0 and 0.5ml of emzyme. These were the experimental, the control consisted of only 2ml of the prepared substrate, the contents of both experimental and control tubes were incubated at 35^0C for 30 minutes, the reactions were terminated with 3ml of 1N HCL,2mililiter of the mixture from each of the sets of experimental and controls was transferred into new sets of clean test tubes.3mililiter of 0.1N HCL were added into the contents of each test tube after which 0.1ml of iodine solution was added. Optical density reading was taken spectrophotometrically at 620nm.Enzyme activity was defined in units, as one unit of enzyme which produced 0.1% reduction in the blue colour of the starch iodine complex

4.0 RESULT

The fungi isolated and identified based on culture and morphological characteristics were *Aspergillus fumigates, A. flavus, A. flavus and A. niger* as presented in the table 1. four fungi belonging to the same genus *Apergillus* were identified. The results of testing the amylolytic activity of the isolated fungi species were as presented in table 2 as can be seen *Aspregillus niger* showed the highest amylase activity of 55mm.

The results obtained from the optimization of the fermentation conditions for production of amylase enzyme using the isolated fungi in a submerged fermentation were as presented in table 3, 4 & 5 as can be seen, the optimum pH, temperature and time were pH 5.0, with 0.645u/ml, Temperature $30^o C$ with 0.841u/ml and incubation 72h with 0.852u/ml respectively.

Table 1: Appearance of Colony and Morphological Characteristics of Isolated Fungi

Appearance of Colony on PDA	Morphology of Hyphae	Suspected Organisms
Yellowish-green compact colony on PDA plate	Septate hyphae from which conidiophores arose	*Aspergillus fumigatus*
Light-yellowish compact green colony on PDA plate	Septate hyphae arising from vegetative hyphae	*Aspergillus flavus*
Yellowish-green compact colony on PDA plate	Septate hyphae arising from vegetative hyphae	*Aspergillus flavus*
Dark brown compact colony with more intense colouration at the centre	Septate hyphae with crude branching arising from vegetative hyphae	*Aspergillus niger*

Source: Laboratory Observation (2026)

Table 2: Screening of Fungal Isolates for Amylolytic Activity

S/N	Suspected Isolate	Iodine Test on Starch	Diameter of Zone of Clearance (mm)
1	*Aspergillus fumigatus*	Positive (+)	45
2	*Aspergillus flavus*	Positive (+)	50
3	*Aspergillus fumigatus*	Positive (+)	50
4	*Aspergillus niger*	Positive (+)	55

Source: Laboratory Analysis (2026).

Table 3: Optimization of pH at 4.0, 4.5, 5.0, 5.5 and 6.0 at Varying Temperature and Incubation Time

pH	Time (h)	Temperature (°C)	Enzyme Activity (U/mL)
4.0	24	25	0.070
4.0	48	30	0.082
4.0	72	35	0.061
4.0	96	40	0.053
4.0	120	45	0.050
4.5	24	25	0.072
4.5	48	30	0.079
4.5	72	35	0.076
4.5	96	40	0.069
4.5	120	45	0.065
5.0	24	25	0.423
5.0	48	30	0.645
5.0	72	35	0.563
5.0	96	40	0.245
5.0	120	45	0.202
5.5	24	25	0.402
5.5	48	30	0.532
5.5	72	35	0.425
5.5	96	40	0.385
5.5	120	45	0.340
6.0	24	25	0.352
6.0	48	30	0.421
6.0	72	35	0.410
6.0	96	40	0.333
6.0	120	45	0.302

Source: Laboratory Analysis (2026).

Table 4: Optimization of Temperature at 25, 30, 35, 40 and 45°C at pH 5.0

S/No.	Temperature (°C)	pH	Time (h)	Enzyme Activity (U/mL)
1	25	5.0	24	0.061
2	25	5.0	48	0.069
3	25	5.0	72	0.072
4	25	5.0	96	0.029
5	25	5.0	120	0.025
6	30	5.0	24	0.611
7	30	5.0	48	0.691
8	30	5.0	72	0.841
9	30	5.0	96	0.811
10	30	5.0	120	0.794
11	35	5.0	24	0.551
12	35	5.0	48	0.712
13	35	5.0	72	0.745
14	35	5.0	96	0.652
15	35	5.0	120	0.620
16	40	5.0	24	0.057
17	40	5.0	48	0.054
18	40	5.0	72	0.049
19	40	5.0	96	0.045
20	40	5.0	120	0.042
21	45	5.0	24	0.049
22	45	5.0	48	0.044
23	45	5.0	72	0.042
24	45	5.0	96	0.039
25	45	5.0	120	0.032

Source: Laboratory Analysis (2026).

Table 5: Optimization of Incubation Time at Optimized pH and Temperature

S/No.	Incubation Period (h)	pH	Temperature (°C)	Enzyme Activity (U/mL)
1	24	5.0	30	0.652
2	48	5.0	30	0.691
3	72	5.0	30	0.852
4	96	5.0	30	0.711
5	120	5.0	30	0.541

Source: Laboratory Analysis (2026).

5.1. DISCUSSION

The optimization of fermentation conditions for the production Amylase

Emzyme by soil fungi in a submerged fermentation condition was successfully carried out. Four fungal species were isolated, identified and screened for amylolytic activity. *Aspergillus niger* had the highest amylolytic activity and was used to optimized the fermentation conditions for the production of amylase and produced amylase Several species of different fungi particularly *Aspergillus species* have been isolated from the soil and used to produce amylase based on the highest producer of amylolytic activity. Acharya *et al.,* (2014) carried out a similar work and out of seventeen different strains of fungi isolated four species gave good amylolytic activity which was identified as *Aspergillus oryzae*, *A.niger*,*Sporotrichum spp* and *Aspergillus spp.*

The conditions optimized in this study were temperature, pH and incubation time, (tables 3,4 and 5) The effect of varying incubation temperature between $25^{O}C$-45^{O}, checked on the production of amylase using *A.niger* were as shown in table **3**. The result showed that maximum amylase production was observed at $30^{o}C$.Increase of enzyme production was observed at lower temperatures while at higher temperatures there was decrease. Acharya, (2014) observed $45^{O}C$ with *Aspergilus oryzae* and reported the same trend of increase and decrease in enzyme activity at lower and higher activities respectively. In the same vein, Krishna (2005) reported that both cell growth and production of enzyme and other metabolites are usually sensitive to temperatures, Also, Muhammad *et al.,* (2012) observed that amylase production by fungi is related to the growth which sequentially depends upon the incubation temperature hence the optimum temperature depends on whether the culture is a mesophylic or thermophylic (Sivaramakrishnan *et al.*, 2006).

The decrease in enzyme activity was observed at higher temperature because of the change in membrane composition and cause protein catabolism as well as inhibition of fungal growth. The result also indicated that the enzyme production corresponds closely to the growth of fungus. Similar types of observations were recorded by Kheng and Uma (2012). The effect of different pH (4-6) checked using *A.niger* was 5.0 as shown in Table 3, this was the same as the lower limit of the range of the pH values reported by Moller *et al* (2004) for *Aspergillus species* such as *A.oryzae, A.fuccum* and *A.niger,*but lower than pH 7 was reported by Acharya (2014).

Lower and higher pH have been reported to affect the stability of extracellular enzyme values and cause rapid denaturation (Kaira and Sandhu, 1986). The initial pH of the medium affects the synthesis and secretion of amylase (Muhammed *et al.*, 2012) furthermore, Ellerial *et al* (2002) reported that amylase production by microbial strains strongly depends the extra cellular pH as it influences many enzymatic reactions as well as the transport of various component across the cell membrane.

The optimum incubation time was found to be 72 hours for *A. niger* in submerged state fermentation, further increase in incubation time decreases enzyme production. The same 72hours was reported by Acharya (2014) as incubation time for *A.oryzae* the decrease in enzyme production with increase in incubation time might be due to the deficiency of nutrient and accumulation of toxic substances.

(Chamber *et al.*, 1999 and Shafique *et al.*, 2009).

5.2 CONCLUSION

This study demonstrated the potential of an indigenous *Aspergillus* strain for amylase production under submerged fermentation (SmF) conditions. The optimization of key physical parameters, particularly pH, temperature, and incubation time, resulted in improved amylase production, confirming that fermentation conditions significantly influence fungal growth, metabolic activity, and extracellular enzyme yield. The findings further demonstrate that appropriate selection of a high-performing fungal strain, coupled with optimization of the fermentation environment, is essential for achieving desirable levels of amylase production.

The ability of the selected *Aspergillus* strain to produce amylase with appreciable starch-degrading activity highlights its potential for various biotechnological and industrial applications. The observed enzyme stability and efficient starch conversion further enhance its suitability for processes in which effective hydrolysis of starch-containing substrates is required. These characteristics are particularly important for industries such as food processing, pharmaceuticals, textiles, brewing, starch processing, and biofuel production.

The utilization of locally isolated fungi for enzyme production could also provide significant economic benefits, particularly in Nigeria, where dependence on imported industrial enzymes and relatively expensive synthetic or conventional processing methods may increase production costs. Developing microbial enzyme-production systems based on indigenous fungal strains and locally available substrates could therefore contribute to more sustainable and cost-effective industrial biotechnology.

Overall, the study establishes that optimization of fermentation parameters and appropriate selection of microorganisms are critical determinants of amylase yield. The results provide a useful basis for further optimization, scale-up, purification, characterization, and potential industrial application of fungal amylase.

5.3 RECOMMENDATIONS

Based on the findings and limitations of this study, further investigations are recommended to improve amylase production and establish the industrial potential of the selected fungal isolate. Since the present study focused primarily on optimizing physical fermentation parameters, future studies should investigate nutritional and process-related factors that may further enhance fungal growth and enzyme productivity.

Particular attention should be given to optimizing different carbon and nitrogen sources, their concentrations, and carbon-to-nitrogen ratios during submerged fermentation. The potential use of inexpensive and locally available agro-industrial residues as alternative substrates should also be investigated to reduce the overall cost of enzyme production and improve the economic feasibility of large-scale fermentation.

Further studies should evaluate additional parameters such as inoculum size, agitation rate, aeration, substrate concentration, moisture conditions, and mineral supplementation. Statistical optimization techniques, including response surface methodology, could also be employed to

determine interactions among fermentation variables and establish optimum conditions for maximum amylase yield.

In addition, the produced amylase should be purified and characterized with respect to its molecular properties, kinetic behaviour, substrate specificity, thermal stability, pH stability, and tolerance to relevant industrial processing conditions. Molecular identification and characterization of the selected *Aspergillus* strain are also recommended to complement morphological identification and confirm its taxonomic identity.

Finally, pilot-scale and subsequently large-scale fermentation studies should be conducted to assess the reproducibility, productivity, stability, economic feasibility, and commercial potential of the amylase-production process. Such investigations would provide the necessary information for translating laboratory-scale findings into sustainable industrial amylase production, particularly within the Nigerian biotechnology sector.

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