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Production of Amylase Enzyme by *Aspergillus* and *Fusarium* Species using Sugar Cane Bagasse

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Abstract

Amylases are hydrolytic enzymes produced by some microorganisms and utilized in the hydrolysis of starch. This study aimed to determine the ability of certain fungal isolates isolated from wastes to synthesize the amylase enzyme utilizing synthetic soluble starch and sugar cane bagasse as substrates. *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium spp.*, previously identified to have amylolytic activity, were used for the research. The submerged fermentation process was used to produce amylase, with basal medium and sugar cane bagasse as substrates. The incubation time, substrate and inoculum concentrations, pH, and temperature were all optimized. The dinitrosalicylic acid reagent (DNS) technique was used to determine the activity of the amylase generated. Initial production of amylase using soluble starch (20 g (w/v)) as substrate at room temperature and pH 7.0 shows that all the isolates produce amylase better when their concentration (3%) is high but have different incubation periods of 96 hr for *Aspergillus niger* (8.65 ± 0.21 U/mL/minutes) and *Fusarium spp* (7.15 ± 0.07 U/mL/minutes), while *Aspergillus flavus* (7.30 ± 0.14 U/mL/minutes) requires an extended incubation period of 144 hr to produce the product. Further production using sugar cane bagasse and optimization of production parameters of the isolates reveals that *Aspergillus niger* (4.35 ± 0.07 U/mL/minutes) has an optimum incubation period of 120 hours, an inoculum concentration and substrate concentration of 2% each, and a pH of 6, *Aspergillus flavus* (6.40 ± 0.28 U/mL/minutes) has an optimum incubation period of 144 hours, with inoculum and substrate concentration of 3% each at neutral pH, *Fusarium spp* (6.80 ± 0.28 U/mL/minutes) has an optimum incubation period of 168hr., inoculum concentration of 3%, substrate concentration of 2% and a neutral pH-7 and all the isolates have their best yield at temperature of 30°C. In conclusion, using cheap and readily available sugar cane bagasse can be a more cost-effective option for expensive synthetic starch substrates used in amylase synthesis. The study suggests further investigation into the isolates' identities and the extracted enzyme's industrial application.

Keywords: Amylase, optimization, Parameters, Sugar cane bagasse, synthetic starch.

INTRODUCTION

Enzymes are biological catalysts capable of changing biological reactions. They are generated from Animals, Plants, and, most importantly, Microorganisms. Fungi and bacteria primarily produce industrially stable metabolites such as the Amylase enzyme (Hormoznejad *et al.*, 2022). Amylases are hydrolytic enzymes that disrupt bonds between neighbouring glucose units, resulting in the formation of tiny units or monomers of glucose. (Singh & Kumari 2016). They function through the chemical disintegration of molecules that involves bond cleavage and the addition of

hydrogen cation and the hydroxide anion of water (Arekemase *et al.*, 2020).

Microorganisms are the primary biological source of enzymes, with filamentous fungi accounting for 60% of the total industrial production of enzymes and bacteria and yeasts accounting for the remainder (Orlandelli *et al.*, 2012). These enzymes dominate global industrial output because they are widely used in a variety of industries (Benassi *et al.*, 2012; Pasin *et al.*, 2020). Amylases are an enzyme class with applications in the food, brewing, textile, detergent, and pharmaceutical sectors.

Although numerous microbes produce this enzyme, several publications show that *Bacillus* spp., *Aspergillus* spp., and *Fusarium* spp. are among the most widely employed for industrial processing. (Makut *et al.*, 2021 and Ahmad *et al.*, 2022). The major advantage of using microorganisms for the production of amylase is their large production capacity and ease of manipulation to obtain enzymes of desired characteristics that meet specific industrial demands (Aiyer, 2005 & Onoter *et al.*, 2011). The primary substrate for amylase production is synthetic carbon or media, but its high price in the market is affecting the economies of developing nations (like Nigeria). As a result, researchers are looking into the potential of other carbon sources like agro-industrial wastes or crop biomass that are produced in millions of tons annually as "waste" causing environmental pollution when burned (Singh, 2012). In line with the reports of Pessôa *et al.* (2017) that emphasized the use of Agricultural waste as fermentation substrates, many researchers agree that agricultural waste products containing starch can serve as cost-effective substrates for the production of hydrolytic enzymes (Shinde *et al.*, 2014 and Arekemase *et al.*, 2020).

Although metabolite production is a naturally driven process in microorganisms, controlling physical and chemical production conditions is paramount to developing effective and economical fermentation processes (Rosa *et al.*, 2017). Since the medium's composition and fermentation conditions majorly impact the organism's growth and enzyme synthesis capabilities, optimizing these parameters is crucial to a microbe's ability to produce the product (Ahmad *et al.*, 2022). Thus, optimization is required to identify the ideal microbe growth and manufacturing conditions. However, due to the high cost of production, it is necessary to optimize the culture of these organisms using less expensive carbon sources such as agro-industrial waste in search of better amylolytic producers (Amorim *et al.*, 2020). Therefore, one of the most important steps in constructing an efficient and cost-effective process is the isolates' selection of adequate carbon and optimal production parameters. Thus, this study aims to produce amylase using *Aspergillus* and *Fusarium* species using sugar cane bagasse substrate.

MATERIALS AND METHODS

Inoculum Preparation

Aspergillus niger, *Aspergillus flavus*, and *Fusarium* spp, already isolated from fruit residues and screened to have amylolytic activity, were re-identified and maintained on

potato dextrose agar. Nine (9) ml solution of 9 g/L NaCl was prepared and added to a fully grown fungal plate on Potato Dextrose Agar (PDA). An inoculating loop was gently used to scrape the spores under aseptic conditions. According to Benabda *et al.* (2019) and Ahmad *et al.* (2022), the scraped suspension of spores was stored and used as inoculum throughout the fermentation processes.

Agro-Industrial waste

Sugar cane bagasse was prepared by washing with tap water, pulverized into minute pieces, and dried. Three hundred (300g) was measured and kept for later use. An appropriate amount was used for each fermentation in 100ml of other media constituents.

RE-Confirmation of Amylolytic Activity

Starch medium (Prepared by dissolving 0.5g, 0.15g, 0.15g, 0.5g, 1g and 2g of peptone, beef extract, yeast extract, sodium chloride (NaCl), starch, and agar in 100mL of distilled water, respectively) was used to reconfirm the activity and viability of the test isolates. The isolates were reconfirmed by streaking on a prepared starch agar plate and incubated at room temperature for 3 days. After the incubation, drops of iodine solution (Iodine - 0.2%, Potassium Iodide -0.4%, Distilled water - 100mL) were sprayed on the starch agar plates using a dropper. Blue colour was observed, and its presence after 30 seconds indicated negative results, while clear zones of hydrolysis around the growth indicated positive results.

Production of Amylase by the isolates using soluble starch as substrate

Basal medium containing (g/L) of NaNO₃- 1.0g, MgSO₄.7H₂O- 0.5 g, FeSO₄-0.01g, soluble starch 20.0 g was prepared. One hundred millilitres (100 ml) of the prepared basal medium was dispensed into a 250 mL conical flask and sterilized by autoclaving. The preparation was inoculated with 1 ml (1% of 100 ml) of each of the three selected fungal inoculum prepared. The inoculated medium was adjusted to neutral pH and kept at room temperature on a shaking incubator for 72, 96, 120, 144 and 168 hours for different fermentation periods, at the end of each fermentation period. The culture medium was filtered using Whatman filter paper and centrifuged at 5000 rpm for 20 minutes. The supernatant obtained is the crude extract of the enzyme and was further assayed for amylase activity using the Dinitrosalicylic reagent method (DNS) and UV Spectrophotometric method. The effect of inoculum concentration was further ascertained by optimizing inoculum concentration to 2 and 3% (Malik *et al.*, 2017).

Initial production of Amylase by the isolates using sugar cane bagasse substrate

In a 250 mL Erlenmeyer flask, a basal medium and 1g (1% of 100 mL) of sugar cane bagasse were used as the production medium. The production medium was sterilized by autoclaving using standard procedure. The production media was inoculated with 1% (1ml) inoculum of each of the isolates (*Aspergillus niger*, *Aspergillus flavus*, and *Fusarium spp*). For 72 hours, the fermentation was adjusted to neutral pH and kept at room temperature in a shaking incubator to represent the first or initial fermentation.

Effect of physical and chemical parameters

In a similar way used by [Chimata et al. \(2010\)](#) in their experiments and little modifications. Systematic Optimization of substrate concentration (2 and 3 %), inoculum concentration (2 and 3%), Incubation Period

$$\text{Amylase Activity (U/ml/min)} = \frac{\text{Maltose released}(\mu\text{g}) \times \text{Total volume of Released media(ml)} \times \text{Dilution factor}}{\text{molecular weight of maltose} \times \text{Enzyme used(ml)} \times \text{Time of Incubation}}$$

(72,96,120,144 and 168 hours), pH (2,3,4,5,6,7,8,9 and 10), and temperature (20°C,30°C,40°C,50°C and 60°C) was carried out using the one - factor - at - a - time optimization process.

Assay of Amylase activity

Using a similar procedure reported by [Hasan et al. \(2017\)](#), The activity of all the enzymes extracted in this research was determined using the Dinitrosalicylic reagent method (DNS) and UV Spectrophotometric method. A UV-visible spectrophotometer (Model no. 721-VIS) was used to estimate the amount of reducing sugar released during the reaction of the mixture by taking the absorbance of the Solution at 540nm. One unit of amylase activity denotes the enzyme required to release 1g of reducing sugar (maltose) per minute under assay conditions. The activity of the enzyme was finally calculated using the below relationship;

RESULTS

Amylolytic Activity of the Re-confirmed Isolates

Growing the isolates on a starch medium to reconfirm the viability shows that all isolates are still active and have amylase-producing potentials, as shown in Figure 1 below. It

indicates that on a 20mm petri-dish plate, all the Isolates break down starch to produce a clear zone of 63.6 mm, 53.2mm, and 50.4 mm for *Fusarium spp*, *Aspergillus niger* and *Aspergillus flavus* respectively. This indicates that all isolates can produce amylase enzyme using starch as substrate.

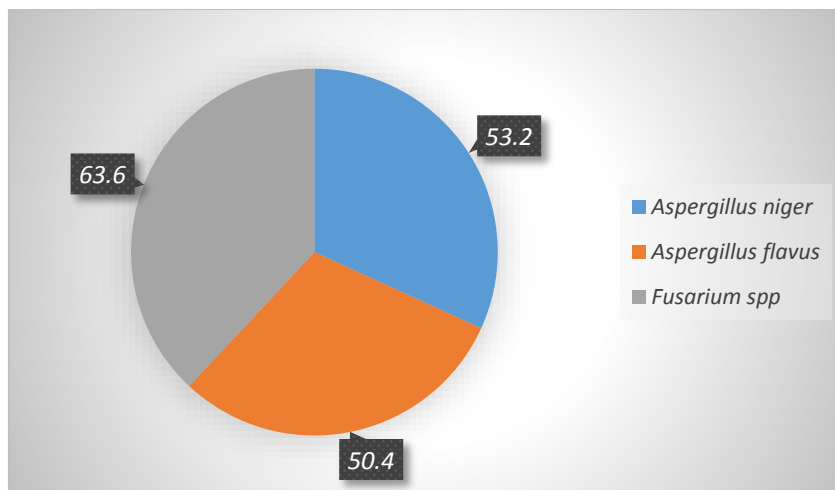


Figure 1: Amylolytic activity of the re-confirmed isolates based on their Zone of Hydrolysis

Production of Amylase by the isolates using Soluble Starch production medium

The production of amylase using a medium containing all the chemical requirements in the right proportion at room temperature and neutral pH with varying incubation periods and inoculum concentration showed that *Aspergillus niger* (7.30 U/mL/minutes), *Aspergillus flavus* (7.45 U/mL/minutes) and *Fusarium spp* (7.05

U/mL/minutes) had highest amylase activity when the incubation period was 96 hours, 144 hours and 96 hours respectively as shown in Figure 2. This indicates that when the inoculum concentration was 1%, the organisms utilized the medium and produced amylase with the highest activity during the aforementioned incubation hours.

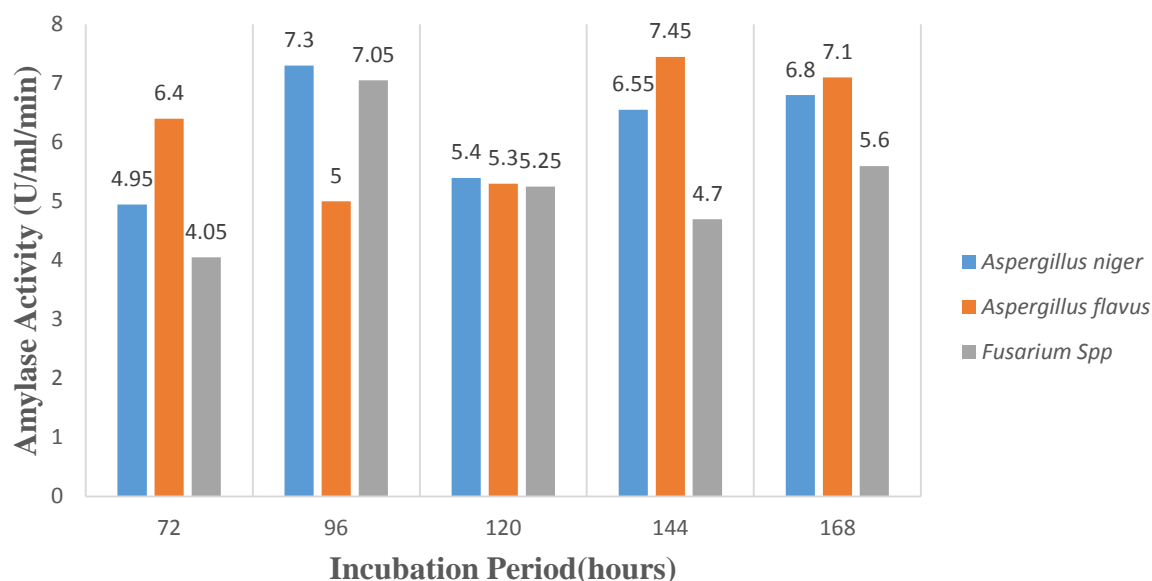


Figure 2: Effect of incubation period on the activity of amylase produced by the isolates

Effect of inoculum concentration on the activity of amylase produced by the isolates using soluble starch production medium.

Systematically, maintaining the fermentation parameters (Rtm, neutral pH and substrate concentration) and best incubation period for each of the isolates. The results obtained show that *Aspergillus niger* (8.65 U/mL/minutes),

Aspergillus flavus (7.3 U/mL/minutes), and *Fusarium spp* (7.15 U/mL/minutes) produced the enzyme with the highest activity when the inoculum concentration was 3% (3mL) for all the isolates as shown in Table 1. Thus, the higher the concentration of the inoculum, the higher the production of amylase (activity) by the isolates.

Table 1: Activity of Amylase Enzyme Produced by the isolates at varying Inoculum Concentrations using soluble starch production medium

Isolates	Amylase Activity (U/mL/minutes) (±S.D)	
	Inoculum concentration	
	2mL (2%)	3mL (3%)
<i>Aspergillus niger</i>	3.5±0.14	8.65±0.21
<i>Aspergillus flavus</i>	5.85±0.07	7.3±0.14
<i>Fusarium spp</i>	5.95±0.21	7.15±0.07

NB: Average (MEAN)±Standard Deviation (SD)

Optimum activity of Amylase Enzyme produced by the isolates using soluble starch production medium

The result obtained shows that *Aspergillus niger* produces amylase with the best activity when the incubation period was 96 hours and inoculum concentration was 3%, *Aspergillus*

flavus produce amylase with the best activity when the incubation period was 144 hours and inoculum concentration was 3% and *Fusarium spp* produces amylase with best activity when incubation period was 96 hours and inoculum concentration was 3%, as shown in Table 2.

Table 2: Optimum Amylase activity produced by the isolates using soluble starch production medium

Isolates	Optimum Parameters					Amylase Activity (U/mL/minutes) (±S.D)
	Incubation period(hr.)	Inoculum Concentration (%)/100mL	Substrate concentration(w/v)	Temperature (°C)	pH	
<i>Aspergillus niger</i>	96	3	20	Ambient	7	8.65±0.21
<i>Aspergillus flavus</i>	144	3	20	Ambient	7	7.30±0.14
<i>Fusarium spp</i>	96	3	20	Ambient	7	7.15±0.07

NB: Average (MEAN)±Standard Deviation (SD)

Production of Amylase Enzyme by the isolates under submerged fermentation technique using Sugar cane bagasse

At the end of initial fermentation using 1mL (1%) of *Aspergillus niger*, *Aspergillus flavus* and *Fusarium Spp* as inoculum, the activity of the enzyme

recorded was 5.25 U/mL/minutes, 5.80 U/mL/minutes and 7.30 U/mL/minutes using sugar cane bagasse substrate respectively as shown in Figure 3. Thus, *Fusarium Spp* was found to produce a better yield of amylase enzyme by utilizing sugar cane bagasse better than other isolates.

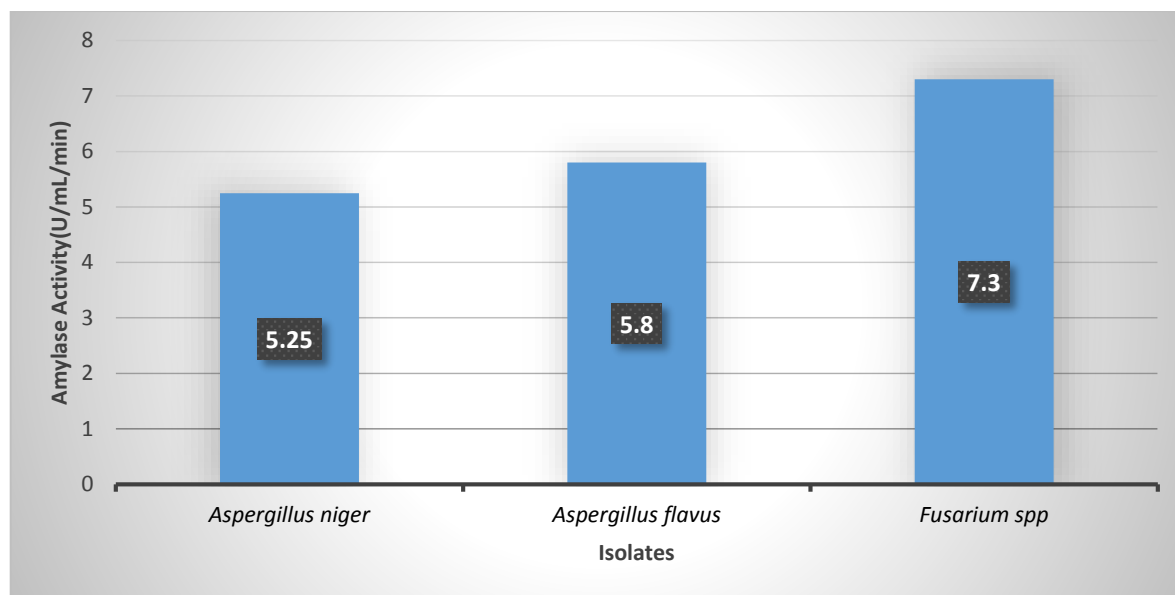


Figure 3: Activity of Amylase enzyme produced by the isolates using sugar cane bagasse as substrate

Production of Amylase Enzyme at different Incubation Periods by the isolates using sugar cane bagasse as substrate

Results obtained after varying the incubation period show that *Aspergillus niger* (15.20 u/mL/minutes) has a better yield of the enzyme during the 5th day (120 hours of

incubation), followed by *Aspergillus flavus* (14.25 u/mL/minutes) and then *Fusarium Spp* (13.05 u/mL/minutes) at 144hr and 168hr respectively as shown in Figure 4 below. This indicates that *Aspergillus niger* utilizes sugar cane bagasse in a shorter time with better yields than the later isolates.

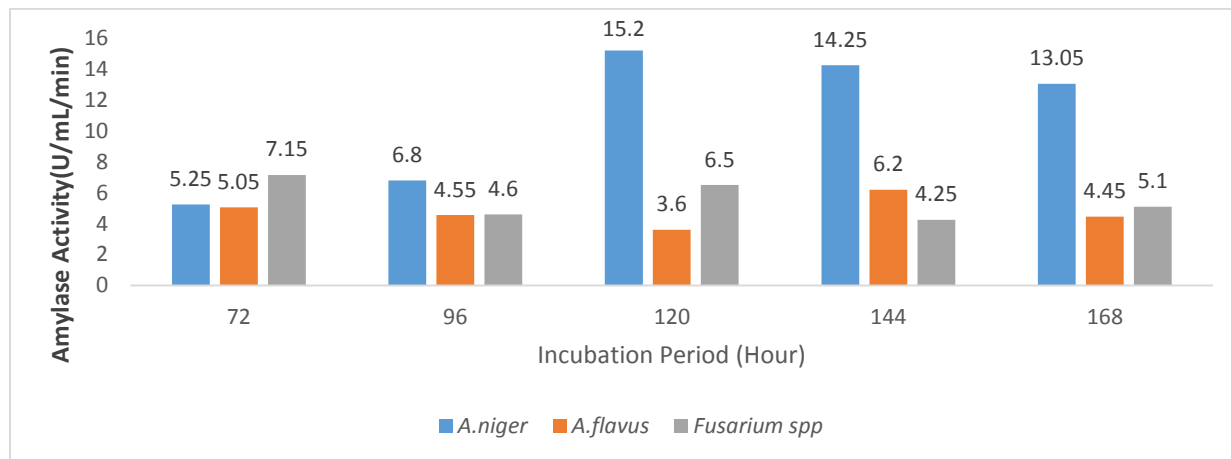


Figure 4: Activity of Amylase enzyme produced by the isolates using sugar cane bagasse as substrate at different incubation Periods

Production of Amylase Enzyme at different Temperature by the isolates using sugar cane bagasse as substrate

Systematically maintaining the optimized Parameter, Production at different temperatures shows that all the isolates have a better yield when the temperature is 30°C as

shown in Figure 5. Although they have a similar yield at the same temperature, *Fusarium spp* (8.95 u/mL/minutes) shows a slightly better yield than *Aspergillus flavus* (8.75 u/mL/minutes) and *Aspergillus niger* (6.35 u/mL/minutes) with the least production yield.

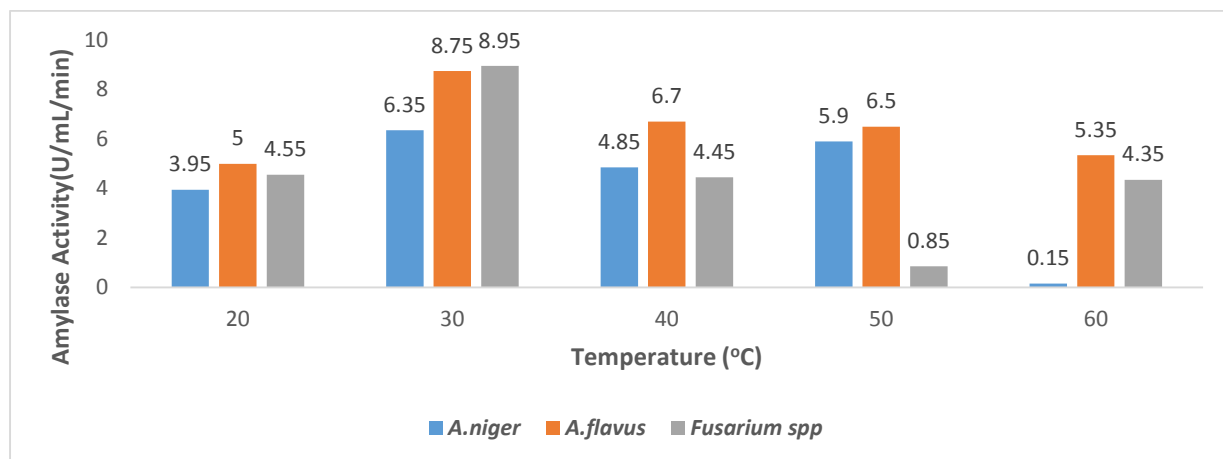


Figure 5: Activity of Amylase enzyme produced by the isolates using sugar cane bagasse as substrate at different Temperature

Production of Amylase Enzyme at different pH by the isolates using sugar cane bagasse as substrate

Systematically maintaining the optimized Parameter, Production at different pH indicated that *Fusarium Spp* (6.8

u/mL/minutes) and *Aspergillus flavus* (6.4 u/mL/minutes) have a better yield at neutral (pH-7) while *Aspergillus niger* (4.35 u/mL/minutes) require a more acidic condition (Ph-6) for a better yield as shown in Figure 6.

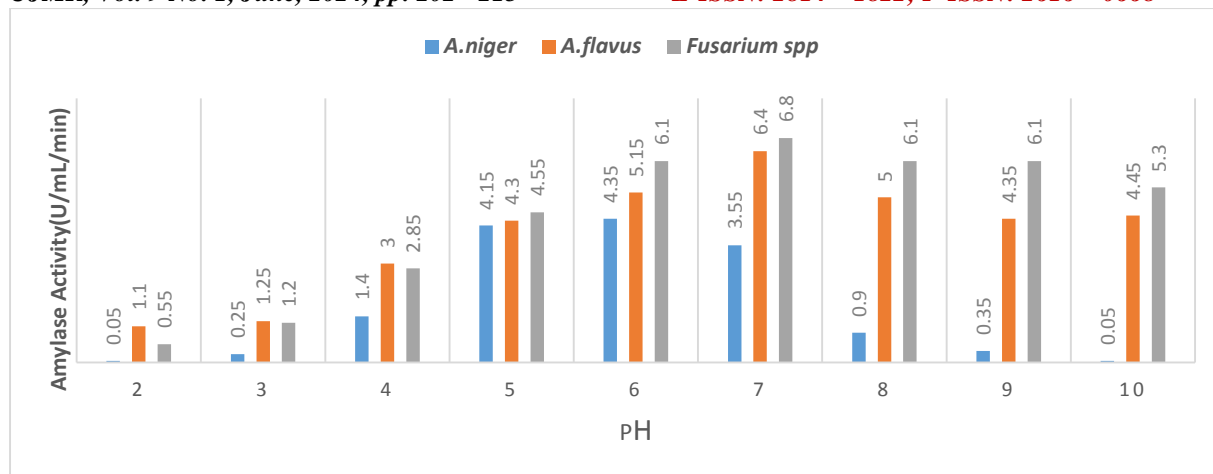


Figure 6: Activity of Amylase enzyme produced by the isolates using sugar cane bagasse as substrate at different pH

Production of Amylase Enzyme at varying inoculum concentrations by the isolates using sugar cane bagasse as substrate

Systematically maintaining the optimized Parameter, Varying the Inoculum concentration indicated that *Fusarium Spp* (5.10±0.28

u/mL/minutes) and *Aspergillus flavus* (3.90 ±0.14 u/mL/minutes) have better yield when their concentration is high (3%) while *Aspergillus niger* (5.70 ±0.14 u/mL/minutes) has a better yield at a lower concentration of 2% as shown in Table 3.

Table 3: Effect of inoculum concentration on amylase activity produced by the isolates using sugar cane bagasse as substrates.

Substrate	Amylase Activity (U/mL/minutes) (±S.D)	
	Inoculum concentration	
	2mL (2%)	3mL (3%)
<i>Aspergillus niger</i>	5.70±0.14	3.75±0.07
<i>Aspergillus flavus</i>	3.75±0.07	3.90±0.14
<i>Fusarium spp</i>	1.50±0.02	5.10±0.28

NB: Average (MEAN)±Standard Deviation (SD)

Production of Amylase Enzyme at varying substrate concentrations by the isolates using sugar cane bagasse as substrate

Systematically maintaining the optimized Parameter, Varying the Inoculum concentration indicated that *Fusarium Spp* (4.00±0.14

u/mL/minutes) and *Aspergillus niger* (12.95 ±0.21 u/mL/minutes) have better yield of amylase at lower concentration (2%) while *Aspergillus flavus* (7.50 ±0.42 u/mL/minutes) has a better yield at a higher concentration of 3% as shown in Table 4.

Table 4: Effect of Substrate concentration on amylase activity produced by the isolates using sugar cane bagasse as substrates.

Isolates	Amylase Activity (U/mL/minutes) (±S.D)	
	Substrate concentration	
	2mL (2%)	3mL (3%)
<i>Aspergillus niger</i>	12.95±0.21	10.95±0.21
<i>Aspergillus flavus</i>	5.95±0.21	7.50±0.42
<i>Fusarium spp</i>	4.00±0.14	3.75±0.07

NB: Average (MEAN)±Standard Deviation (SD)

Optimum activity of Amylase Enzyme produced by the isolates using Sugar cane bagasse as substrate

The result obtained shows that *Aspergillus niger* produces amylase with the least activity at an optimum incubation period of 120hr., inoculum concentration and substrate concentration of 2%, and a pH of 6. *Aspergillus flavus* produced amylase with the best activity

when the incubation period was 144 hours and inoculum and substrate concentration was 3% at a neutral pH. *Fusarium spp* produces amylase with the best activity when the incubation period was 168 hours, inoculum concentration was 3%, and substrate concentration was 2% and a neutral pH-7. All the isolates have their best yield at a temperature of 30 °C, as shown in Table 5.

Table 5: Optimum Amylase activity produced by the isolates using sugar cane bagasse as substrate

Isolates	Optimum Parameters					Amylase Activity (U/mL/minutes) (±S.D)
	Incubation period(hr.)	Inoculum Concentration mL %/mL	Substrate concentration(w/v)	Temperature (°C)	pH	
<i>Aspergillus niger</i>	120	2	2	30	6	4.35±0.07
<i>Aspergillus flavus</i>	144	3	3	30	7	6.40±0.28
<i>Fusarium spp</i>	168	3	2	30	7	6.80±0.28

NB: Average (MEAN)±Standard Deviation (SD)

Optimum Amylase Activity produced by the isolates using Soluble Starch as Substrate and sugar can bagasse substrates.

Table 6 compares the overall activity of amylase produced by the isolates using different fermentation media. It shows the amylase activity produced by the isolates when the best fermentation conditions (Tables 2 & 6) were

employed using a soluble starch production medium and the best activity produced by the isolates utilizing the best substrate among the three used substrates. There is no significant statistical difference between the activity of amylase produced by the isolates using soluble starch and sugarcane bagasse as substrates ($p = .273$).

Table 6: Amylase activity produced by the isolates using soluble starch fermentation medium and sugar cane bagasse substrate

Isolates	Amylase Activity(U/mL/minutes) (±S.D)	
	Soluble starch Medium	Substrates
<i>Aspergillus niger</i>	8.65±0.21	4.35±0.07
<i>Aspergillus flavus</i>	7.30±0.14	6.40±0.28
<i>Fusarium spp</i>	7.15±0.07	6.80±0.28

NB: Average (MEAN) ±Standard Deviation (SD); P = .273

DISCUSSION

The result obtained in this research reveals that all three isolates screened for amylolytic activities have a high potential for amylase activity. The results show that *Fusarium spp* (63.6 mm) has the highest production potential, followed by *Aspergillus niger* (53.2) and *Aspergillus flavus* (50.4). This result is similar to the results of [Ahmad et al. \(2022\)](#) and [Abouamama et al. \(2023\)](#), who report a high amylolytic activity (> 50mm) by *Aspergillus spp* and *Fusarium spp* using the same methods of starch hydrolysis. Their high amylolytic potentials might be attributed to their non-fastidious growth requirements and ability to easily utilize simple carbon sources ([Khokhar et al., 2011](#)).

Synthetic starch is expensive, thus the need for cheaper and easily accessible sources of carbon in the production of amylase enzyme. Hence, the use of sugarcane bagasse as substrate. The need to optimise production parameters cannot be overemphasised for the successful and economical production of these important industrial enzymes.

The results obtained show that *Aspergillus niger* and *Fusarium spp* have their best production at 96th hr. of incubation, while *Aspergillus flavus* reached its peak at 144th hr. of incubation. Thus, optimum amylase production by the isolates using soluble starch as a main carbon source at ambient temperature and a pH of 7.0. [Varalakshmi et al. \(2009\)](#) also reported room temperature to be the best amylase production temperature for *Aspergillus niger*.

Asrat and Girma (2018) reported amylase production by *Aspergillus niger* on the 96th and 144th hr. of incubation. Oyewale (2013) also reported similar optimum conditions of pH-7.0, a higher concentration of inoculum as well as soluble starch as substrate, similar to the results obtained in this work. These results showed that the activity of the amylase enzyme produced by all three isolates increases as the incubation period increases with a slight decrease at some points, this might be attributed to the preferential use of the substrate by the isolates leading to two or more log phase on growth curve. Moreover, the production of higher activity when the inoculum concentrations are high might be attributed to the higher number of spores to utilize available substrate and produce more products.

On the other hand, the production of amylase enzyme using sugar cane bagasse as substrate reveals that there is no significant statistical difference in the production capacities of the isolates using the two substrates when optimum conditions are employed. The result showed that all three isolates have their optimum production at a temperature of 30°C, as the report of Amorim *et al.* (2020), who reported fourteen fungi used in their research to grow at a temperature of 30°C. However, contrary to the results of Ominyi (2013) that report a higher temperature of 45°C is optimum for *Aspergillus* spp, and results of Hormoznejad *et al.* (2022) that conclude 25°C to be the optimum temperature for amylase production fungi in their systematic review of experimental studies. *Aspergillus niger* (4.35±0.07) produces amylase with the least activity at 120hr., and 2% inoculum and substrate concentration, respectively and a pH of 6 contrary to 104hr reported as the optimum but similar to pH 5.95 as reported by Kwatia *et al.*, (2017). The observed results are consistent with those published by Hormoznejad *et al.* (2022) and Ominyi (2013). *Aspergillus flavus* (6.40±0.28) produced amylase with the highest activity after 144 hours. Similar to the findings of Ali *et al.* (2017), who observed that *Aspergillus flavus* produces amylase enzyme after 96-120 hours, with a peak on the 96th hr. This is also in line with the results of Fadahunsi and Garuba (2012) and Oyewale (2013), who also reported 6 days to be optimum for *Aspergillus* spp to produce alpha-amylase in their study.

Furthermore, the results are consistent with those of Kareem *et al.* (2009), who reported alpha-amylase production by *Aspergillus oryzae* on cowpea waste after 3 days, differ from the findings of Balkan and Ertan (2010), who

reported alpha-amylase production after 168 hr with *Penicillium brevicompactum*. All of the findings in this investigation utilizing *Aspergillus flavus* differ from those of Arunsasi *et al.* (2010), who found that 18 days was the optimal incubation period for the same isolate using *Cocos nucifera* meal as a substrate to manufacture alpha-amylase. Repression caused by the accumulation of glucose in the medium could be why amylase activity decreases beyond its optimum parameter requirements. *Fusarium* spp (6.80±0.28) produced amylase with the best activity when the incubation period was 168hr and inoculum concentration was 3% while the substrate concentration of 2% and a neutral pH-7 Contrary to the results of Makut *et al.* (2021) that reported 72hr and pH of 5 as optimum for *Aspergillus* and *Fusarium* spp. The result of substrate concentration obtained is similar to the report of Oyewale (2013), who reported 2% substrate concentration of agricultural raw materials to produce a better yield than other concentrations. The high yield obtained when the concentration is higher agrees with Ramachandran *et al.* (2004), that explain increase in the number of cells leads to an increase in yield. The results of higher concentration (3%) as optimum for *Aspergillus flavus* and *Fusarium* spp utilize sugar cane bagasse obtained in this research are contrary to the result of Adnan *et al.* (2019) that used 5×10⁷ spores/100mL but similar to results by Ramachandran *et al.* (2004) that reported an increase in the number of inoculum lead to increase in amylase activity, they also reported that concentration above 2% similar to concentration used in this study lead to decrease in alpha-amylase activity, thus, limitation of nutrient as the concentration increase may lead to the decrease in activity (Haq *et al.*, 2012). The results obtained also agree with the reports of Aisien and Igbinosa (2019) where higher concentrations of cassava peel substrate yield alpha-amylase with higher activity than lower substrate concentrations. Ali *et al.* (2017) also agree with the results of this study, where 3g of mandarin peel was reported to produce maximum alpha-amylase activity among the varied concentrations. More so, a lower concentration of 2g (w/v) of sugar cane bagasse using *Aspergillus niger* and *Fusarium* spp were found to yield higher amylase activity than higher concentrations similar to the results reported by Oyewale (2013), who reports 2% substrate concentration of different agricultural raw materials to produced maximum alpha-amylase activity.

CONCLUSION

Conclusively, the *Aspergillus* and *Fusarium* spp studied are good sources of amylase enzyme. However, the use of sugar cane bagasse (Agro-industrial waste product) can be an alternative to expensive soluble synthetic starch in the production of Amylase Enzyme since there is no significant statistical difference ($P = .273$) between the activity of the enzyme produced using soluble starch and sugar cane bagasse substrates.

REFERENCES

- Abouamama. S., Anis. B., Abir. S., Maroua.H and, Sirine. B. (2023). Amylolytic and antibacterial activity of filamentous fungi isolated from the rhizosphere of different plants grown in the Tamanghasset region. *Heliyon* 9, [Crossref]
- Adnan, W.D., Hassan, S.A and Yusra, M.B.M. (2019). Production of Amylase Enzyme by local isolation of *Aspergillus* production of amylase enzyme by local isolation of *Aspergillus niger*. *Biochem. Cell. Arch.* 19 (2):3301-3305. [Crossref]
- Ahmad M, A and Shamsuddeen, U and Yahaya, S. (2022). Production of amylase enzyme by *Aspergillus niger* and *Fusarium spp* using wheat bran and groundnut shell as substrate. Book Of Proceedings, International Ankara Multidisciplinary Studies Congress: 29-31 July 2022: 1203-1214. www.izdas.org
- Aisien, E. T. and Igbinosa, I. H.(2019). Production, purification, and characterization of alpha-amylase from *Aspergillus niger*, *Aspergillus flavus* and *Penicillium expansum* using cassava peels as substrate. *Nigerian Journal of Biotechnology*, 36 (2): 114-126. [Crossref].
- Aiyer P.V.(2005). Amylases and their applications. *African Journal of Biotechnology*. 2005; 4(13): 1525-1529. www.ajol.info
- Ali, E. H., El-nagdy, M. A., Al-Garni, S. M., Ahmed, M. S., and Rawaa, A. M. (2017). Enhancement of alpha-amylase production by *Aspergillus flavus* AUMC 11685 on mandarin (*Citrus reticulata*) peel using submerged fermentation. *European Journal of Biological Study*,7 (3), 154-164. [Crossref]
- Amorim, I.C.S., Marinho, G.O., de Oliveira, T. M.F.S., Roa, J.P.B., dos Reis, A.B., Nelson, D.L., Pasin, T.M. and Benassi, V.M. (2020) Isolation of Filamentous

RECOMMENDATION

The research recommends more in-depth studies of other substrates, the molecular identity of the isolates, the activity of the produced amylase as well as its industrial applicability and scale production.

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- Fungi from the Caatinga Region and Production of Amylolytic Enzymes of Great Industrial Interest. *Journal of Biosciences and Medicines*, 8, 152-164. [Crossref]
- Arekemase M.O., Alfa O.P., Agbabiaka T.O., Ajide-Bamigboye N.T., Aderoboye O.Y., Orogu J.O. and Ahmed T. (2020). Optimization of amylase produced from bacteria isolated from cassava peel dumpsite using submerged fermentation. *Science World Journal* Vol. 15(No 1). www.scienceworldjournal.org
- Arunsasi., ManthiriKani. S, Jegadeesh. G. and Ravikumar, M. (2010). Submerged Fermentation of Amylase Enzyme by *Aspergillus flavus* using Cocos Nucifera Meal. *Kathmandu University Journal of Science, Engineering and Technology*, 6 (2), 75-87. [Crossref]
- Asrat, B., and Girma, A. (2018). Isolation, production and characterization of amylase enzyme using the isolate *Aspergillus niger* FAB-211. *International Journal of Biotechnology and Molecular Biology Study*, 9 (2), 7-14. [Crossref]
- Balkan, B and Ertan, F. (2010). The production of a new fungal alpha-amylase degraded the raw starch by means of solid-state fermentation. *Prep Biochem Biotechnology*. 40 (3): 213-228. [Crossref]
- Benabda, O., Sana, M., Kasmi, M., Mnif, W., & Hamdi, M. (2019). Optimization of Protease and Amylase Production by *Rhizopus oryzae* Cultivated on Bread Waste Using Solid-State Fermentation. *Journal of Chemistry*.1-9. [Crossref]
- Benassi, V.M., Lucas, R.C., Michelin, M., Jorge, J.A., Terenzi, H.F. and Polizeli, M.L.T.M. (2012) Production and Action of an *Aspergillus phoenicis* Enzymatic Pool Using Different Carbon Sources. *Brazilian Journal of Food Technology*, 15, 253-260. [Crossref]

- Chimata, M. K., Sasidhar, P., & Challa, S. (2010). Production of extracellular amylase from Agricultural residues by a newly isolated *Aspergillus* species in solid-state fermentation. *African Journal of Biotechnology*, 9 (32), 5162-5169. www.ajol.info
- Fadahunsi, S. and Garuba, O. E. (2012). Amylase Production by *Aspergillus flavus* Associated with the Biodeterioration of Starch-Based Fermented Foods. *New York Science Journal*, 5(1),13-18 <http://www.sciencepub.net/newyork> .
- Haq, I., Hameed, U., Mahmood, Z. and Javed, M.M. (2012). Solid-state fermentation for the production of alpha-amylase by *Paenibacillus amylolyticus*. *Pakistan Journal of Botany*. 44: 341-346. [http://www.pakbs.org/pjbot/PDFs/44\(S11\)/51.pdf](http://www.pakbs.org/pjbot/PDFs/44(S11)/51.pdf)
- Hasan, M. Md., Wal Marzan,L., Asamul Hosna., Al Hakim and Azad.A.(2017). Optimization of some fermentation conditions for the production of extracellular amylases by using *Chryseobacterium* and *Bacillus* isolates from organic kitchen wastes. *Journal of Genetic Engineering and Biotechnology*. 15, 59-68. [[Crossref](#)]
- Hormoznejad. R., Saberi.S., Moridnia. A., Azish.M and Elyasi F.B. (2022). Optimization of the Alpha-Amylase Production from Microbial Source: A Systematic Review of Experimental Studies. *Trends in Medical Science*; 2(2):e129317 : [[Crossref](#)]
- Kareem, S.O., Akpan, I and Oduntan, S.B. (2009). Cowpea waste: a novel substrate for solid-state production of amylase by *Aspergillus oryzae*. *African Journal of Microbiology Study*, 3 (12): 974-977. academicjournals.org
- Khokhar, I., Mukhtar, I and Mushtaq, S. (2011). Isolation and Screening of Amylolytic Filamentous Fungi. *Journal of Applied Science and Environmental Management*. 15 (1), 203-206. [[Crossref](#)]
- Kwatia. S., Dzogbefia.V.P., and Isaac William Ofosu.I.W.(2017). Optimization of amylase production by *Aspergillus niger* cultivated on yam peels in solid-state fermentation using response surface methodology. *African Journal of Biochemistry Research*. Vol. 11(7), pp. 34-42. [[Crossref](#)] .
- E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668
- Makut, M.D., Alfa,F.U , Ekeleme, I.K., Owuna, J.E and Emelogu, N.J. (2021). Production of amylases by some aspergillus and fusarium species isolated from waste corncobs in Keffi, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 16(02), 122-129. DOI: [[Crossref](#)]
- Malik, K., Mehta, S. and Sihag, K. (2017). Isolation and Screening of Amylase Producing Fungi. *International Journal of Current Microbiology and Applied Sciences*. 6 (4), 783-788. [[Crossref](#)]
- Ominyi, M.C.(2013). Optimization of α -amylase and glucoamylase production from three fungal strains isolated from Abakaliki, Ebonyi State. *European Journal of Experimental Biology*, 3(4):26-34. www.pelagiaresearchlibrary.com
- Onoter, SB, Steilmann, P., Beroilni, J, Rotta, D, Ffrencini, AS, Kagimura, Y, Groff, SA, Mazzali, L.(2011). Amylolytic enzymes produced by the fungus *Colletotrichum gloesporioides* in rice semi-solid fermentation. *Journal of Yeast fungi Research*. 2(3): 28-32. [[Crossref](#)]
- Orlandelli, R.C., Specian, V., Felber, A.C. and Pamphile, J.A. (2012) Enzimas de Interesse Industrial: Produção por fungos e aplicações. *SaBios-Revista de Saúde e Biologia*, 7, 97-109. revista2.grupointegrado.br
- Oyewale, M.O. (2013). Optimization of growth and amylase production by *Aspergillus flavus* grown on some Agricultural raw materials in Nigeria. *Pakistan journal of microbiology*. 12 (5):427-432. [[Crossref](#)]
- Pasin, T.M., Moreira, E.A., Lucas, R.C., Benassi, V.M., Ziotti, L.S., Cereia, M. and Polizeli, M.L.T.M. (2020) Novel Amylase-Producing Fungus Hydrolyzing Wheat and Brewing Residues, *Aspergillus carbonarius*, Discovered in Tropical Forest Remnant. *Folia Microbiologica*, 65, 173-184. [[Crossref](#)]
- Pessôa, M. G., Paulino, B. N., Cezar, M., Mano, R., Neri-numa, I. A., Molina, G., & Pastore, G. M. (2017). *Fusarium species – a promising toolbox for industrial biotechnology*. [[Crossref](#)]
- Ramachandran S, Patel AK, Nampootheri KM, Francis F, Nagy V, Szakacs G, Pandey A. (2004). Coconut oil cake—a potential raw material for the production of alpha-amylase. *Bioresources Technology*, 93 (2):169-174. [[Crossref](#)]

- Rosa, T.M.F., Marinho, B.M. and Benassi, V.M. (2017) Produção de celulases e formação de açúcares fermentescíveis pela degradação do bagaço de cana-de-açúcar. *Revista Ciência & Tecnologia*, 9, 38-43. scirp.org
- Shinde, R.N., Dhangar, M.J. and Narwade, R.B. (2014). Amylase production on solid-state fermentation by wild type and mutant *Bacillus licherniformis* and *Aspergillus niger* from agro-wastes. *International Journal of Pharmaceutical Science and Research*, 5 (7): 2703 - 2713. [[Crossref](#)]
- Singh, P. and Kumari, P. (2016). Isolation and characterization of amylase-producing *Bacillus* spp. from selected soil sample. *International Journal of Research in Biosciences*, 5 (2): 24- 29. https://www.ijesm.co.in/uploads/23/1120_pdf.pdf
- Singh, R., Kapoor, V. and Kumar. (2012). Utilization of Agro-Industrial Wastes for the Simultaneous Production of Amylase and Xylanase by Thermophilic Actinomycetes. *Brazilian Journal of Microbiology*,43(4), 1545-1552. [[Crossref](#)]
- Varalakshmi, K. N., Kumudini, B.S. Nandini, B. N., Solomon, J., Suhas, R., Mahesh, B. and Kavitha, A. P. (2009). Production and characterization of alpha-amylase from *Aspergillus niger* JG1 24 Isolated in Bangalore. *Polish Journal of Microbiology*. 58 (1): 29-36. pubmed.ncbi.nlm.nih.gov