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Production of Bioenzymes with Banana Peels by some Selected Fungi Isolated from Poultry Droppings in Sokoto, North Eastern Nigeria

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ABSTRACT

A total of five fungal species were isolated from poultry droppings for their ability to hydrolyze starch and produce amylase. Different culture conditions were optimized viz; innoculum size (1,2,3,4,and 5%,), substrate concentration (1,2,3,4,and 5%,), temperature,(35,45,55,65,75 oC),incubation period (24,48,72,96,and 120hrs), and pH (3,5,7,9,and 11). All the isolates show a high yield of amylase enzyme at the required conditions, the incubation period was higher at 120hrs (0.99mg/ml) by A. The terrus and 48th hrs (0.71mg/ml for A .niger. innoculum size recorded higher yield at 1% (0.65mg/ml)at pH 11 A.niger recorded highest enzyme production. The maximum temperature was at 75 oC by A. The Terrus with the activity of 2.02mg/ml these shows that the enzyme produced in this study can be applied in the paper, detergent and other industries that required amylase for their production.

Key words: Amylase, Enzymes, Banana Peels, Fungi.

1. INTRODUCTION

Amylases are among the most important enzymes and are of great significance in present day biotechnolo

gy taking approximately 25% of the enzyme market (Rao *et al*., 1998). New amylases could be potentially useful in pharmaceutical and fine chemical industries if enzymes with suitable properties could be identified (P. Nigam and D. Singh, 1995). With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many other fields, such as clinical, mechanical and analytical chemistry (Pandey *et al*., 2000). Industrially, alpha amylase (*EC 3.2.1.1*) is used particularly in starch liquefaction, brewing, textile, pharmaceuticals, paper, detergents, drugs, toxic wastes removal and oil drilling (Oyeleke *et al*., 2010; Oyeleke and Oduwale, 2008; Ajayi and Fagade, 2003).
Microbial enzymes derived from microorganisms include fungal and bacterial amylases, and diastases. Enzymes are sold primarily on activity basis that is a quoted cost for a specified activity, secondary feature such as degree of purity, extent of modifications and microbiological specifications can modify this cost of enzymes for analytical and medical purposes these are often in a state of medium to high purity, and are sold in terms of numbers of enzyme unit per lots, whilst those for industrial processing are quoted on a unit weight basis for a standardized products of guaranteed activity per unit weight this applies to most conventionally produced solid and liquid products (Maps enzyme, 2010).

Enzymes carry out a rapid conversion of their substrate at moderate temperature and near neutral pH, usually with high specificity both in selection of substrate from a complex mixture, and in the chemical conversion actually affected. Food stuff is often the substrate, as for the well-known enzyme produced in the stomach or intestine of animals. The activity of the enzyme is due to its catalytic nature. An enzyme carries out its activity without being consumed in the reaction, while the reaction occurs at a much higher rate when the enzyme is present. Enzymes are highly specific and function only on certain types of compound, the

substrate; some enzymes require additional factors termed cofactors that can be metal ion nucleotides etc. Enzymes technology is associated with the application of enzymes as the tool of industry, agriculture and medicine.

2. PHYSICAL AND CHEMICAL PROPERTIES OF ENZYMES

Enzymes molecules are exceedingly different in accelerating the transformation of substrate to end products. A single enzyme molecule can affect the change of as many as 10.000 to 1million molecules of substrate per minute, they are vulnerable to various environmental factors their activity may be diminished or destroyed by a variety of physical or chemical conditions though great f differences exist among them as some may become inactivated by very minor alterations in the environment. The destructions of enzymes by physical or chemical agent result in a loss to the cell of the functions performed by them (Oyeleke *et al*., 2010a, b).

General properties of enzymes

Specificity

Enzymes are very specific in their action i.e. one enzyme will only act on only one specific substrate to give a particular result.

pH

Each enzyme act on a particular range of pH both the optimum operating value determined under analytical condition and the actual ability of the proposed industrial system to adjust from the possibly unsuitable pH in relation to the enzyme stage must be considered.

Temperature

When considering enzyme process, the general rule is that the temperature quotient is between 1.8-2.0.The reaction rates generally increases or decreases by this order of the shift of 10^{o}C. By using high temperature the reaction may be of short duration and hygienic conditions may be maintained more easily. The use of much greater heat for thermo labile enzymes, (enzyme sensible to heat) will be necessary to activate the enzymes to a significant shift which may be necessary to activate without a rise in temperature.

Mechanism of enzyme action

An enzyme has on its surface an active site, or catalytic site, site typically is a relatively crevice is the critical site to which a substrate act by weak forces binding of the substrate bind by weak forces .the binding of the substrate to the active site causes the shape of the flexible enzyme to change slightly. This mutual interactions or induced fit, results in a temporary intermediate called enzyme substrate complex. The substrate is held within this complex in a specific orientation so that the activation energy for a given reaction is lowered allowing the products to be formed. The products are then released, leaving the enzymes unchanged and free to combine with new substrate molecules. Enzyme may catalyse reactions that join two substrates to create one product. Theoretically, all enzyme catalysed reactions are reversible the free energy changed of certain reactions makes them effectively non-reversible. The interaction of an enzyme with its substrate is very specific, the substrate fit into the active site like a hand in a glove (Nester *et al.*, 2009).

Conditions affecting enzymes activity

Temperature

An enzyme will work best at a particular temperature and pH called its optimum conditions. Enzymes usually work best in warm conditions (around 40^{o}C) unlike chemical catalyst which often work best when they are very hot (above 45^{o}C) at which most enzymes get denatured and stop working. At this Condition the shape of the enzyme change and it is said to denature. The temperature of a system is to some extent a measure of the kinetic energy of the molecules in the system. Thus, the lower the kinetic energy, the lower the temperature of the system and, likewise, the higher the kinetic energy, the greater the temperature of the system. Increase in the temperature of a system results from increase in the kinetic energy of the system. This has several effects on the rate of reactions;

More energetic collisions, when molecules collide, the kinetic energy of the molecules can be converted into chemical potential energy of the molecules. If the chemical potential energy of the molecules becomes great enough, the activation energy of an exergonic reaction can be achieved and a change in chemical state will result. Thus the greater the kinetic energy of the molecules in a system, the greater is the resulting chemical potential energy when two molecules collide. As the temperature of a system is

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increased it is possible that more molecules per unit time will reach the activation energy. Thus the rate of the reaction may increase. (http//www.ublcorp/files/biological Enzymespdf, 2009).

The number of collisions per unit time will increase.

In order to convert substrate into product, enzymes must collide with and bind to the substrate at the active site.. Increasing the temperature of a system will increase the number of collisions of enzyme and substrate per unit time. Thus, within limits, the rate of the reaction will increase.

The heat of the molecules in the system will increase.

As the temperature of the system is increased, the internal energy of the molecules in the system will increase. The internal energy of the molecules may include the translational energy, vibrational energy and rotational energy of the molecules, the energy involved in chemical bonding of the molecules as well as the energy involved in nonbonding interactions. Some of this heat may be converted into chemical potential energy. If this chemical potential energy increase is great enough some of the weak bonds that determine the three dimensional shape of the active proteins may be broken. This could lead to a thermal denaturation of the protein and thus inactivate the protein. Thus too much heat can cause the rate of an enzyme catalyzed reaction to decrease because the enzyme or substrate becomes denatured and inactive.

Given the above considerations, each enzyme has a temperature are range in which a maximal rate of reaction is achieved. This maximum is known as the temperature optimum of the enzyme. There is an optimum relation between the concentration of enzymes and substrate for Maximum activities. if the amount of enzymes is kept constant and the substrate y of concentration is then gradually increased the velocity of the reaction will increase until it reaches a maximum. Any further increases in substrate concentration will not increase the reaction velocity.

Also for every enzyme there is an optimum particular pH and temperature for its optimal activity. Extreme variation in pH can result in a phenomenon referred to as denaturation (loss of enzyme activity).The same occur with extreme temperature (Wiley *et al*., 2008; Pelzer *et al*., 1986).

Like most chemical reactions the rate of an enzyme catalysed reaction increases as the temperature is raised A ten degree rise in temperature will increase the activity of most enzymes 50 to100% variation in reactions temperature as small as 1 or 2 degrees may induce change of 10-20% in the results .In the case of enzymatic reaction, this is complicated by the fact that many enzymes are adversely affected by high temperatures. Most animal enzymes become denatured at temperature above 40^{o}C. Most enzyme determination is carried out below that temperature. Over a period of time enzymes will be deactivated at even moderate temperature storage of enzymes at 5 ^{o}C or below is generally the most suitable. Some enzymes lost their activity when frozen (http//www.ublecorp.com/files biologicalenzyme.pdf. 2009).

As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme catalytic activity is at its greatest. This optimum temperature is usually around human body temperature (37^{o}C) for the enzyme in human cells. Above this temperature the enzymes structure begins to break down (denature) since at higher temperature intra and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy. Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intermolecular bonds, changing the shape of the enzyme and, therefore its effectiveness. The rate of an enzyme catalysed reaction depends on the concentration of the enzyme and substrate. As the concentration of the either is increased the rate of reactions increases. For a given enzyme concentration, the rate of reactions increases with increasing substrate concentration up to a point. Above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzymes/substrate complex has to dissociate before the active sites are free to accommodate more substrate ,provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration .(http//www.ublcorp/files/biological Enzymespdf., 2009).

PRODUCTION OF ENZYMES

Micro organisms such as fungi, bacteria, or yeast are living entities which are referred to as ‘cultures’. They are isolated from nature and chosen for their desired characteristics, or they are modified using genetic engineering. The organism must then be preserved. Which is typically done by freezing , various chemicals can be used to protect the organism .for production of the enzyme , the organism is inoculated into a nutrient medium that allows the organism to propagate and develop the enzyme

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sufficiently, since these organism are living cells, they must be fed a diet that is conducive to their well being, this propagation may consist of growing the organism in flask of nutrient or broth or on agar surface containing nutrients or broth when appropriate growth has been achieved, the culture is then added to a large fermented where it is allowed to grow and produce the enzymes. There are two of such enzymes productions intracellular and extra cellular. Intracellular means that the organism produce the enzyme within its cell wall, which must then be lysed (broken open) to harvest the enzyme. Extracellular production means that the organism secrets the enzyme trough its cell wall as part of its metabolism, and is covered from the media in which it grows. At the end of the fermentation, certain chemical and filtering process are used to separate the enzyme from the dead organism and other waste material. And the enzyme concentrate is then blended with chemical preservatives to make a finished product (Mathescsharf, 2009).

Enzymes are produced by cellular anabolism the naturally occurring biological process are making more complex molecules from simpler ones. Source organism for food processing includes bacteria, fungi, higher plants and animals (White and White, 1997). Enzymes may be extracted from a given source organism by a number of different methods (Nielson *et al*., 1991). Most of the organism that produce commercial enzymes include moulds such as *Aspergillus niger, Rhizopus oryzae, Rhizormucor meihei*, and yeast such as *Candida* specie and *Sachcharomyces* spp. A considerable amount of research has been conducted on genetically modifying fungi and their strain to increase the yields and consistencies of the enzyme, many of the prospective donor organisms are pathogenic and are being screened for genetic sequences to be inserted into non pathogenic host, improvement of production method is possible without the use of recombinant DNA techniques.

Amylase is an enzyme that breaks down starch to reducing sugar. Amylase is present in human saliva, where it begins chemical process of digestion. The pancreas also makes amylase (alpha amylase) to hydrolyse dietary starch into di and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. All amylases are glycoside hydrolyser and act on alpha- 1, 4 glycosidic bonds. It will start to denature at around 60˚C (Maton *et al.,* 1993). Alpha amylase (EC 3.2.1.1) is used particularly in starch liquefaction, brewing, textile, pharmaceuticals, paper, detergents, drugs, toxic wastes removal and oil drilling. The enzyme production is largely dependent on the type of strain, medium composition, cell growth, initial pH and thermo stability (Qirang, and Zhao, 1994; Haq 2002a, Haq *et al* 2005a).

Sources of Amylases

Amylases can be derived from several sources, such as plants, animals and micro organisms. Because of their short growth period, the enzymes from microbial source generally meet industrial demand (Pandey *et al.*, 2000). At present, *Bacillus*, *Aspergillus* and *Rhizopus* species are considered to be the most important sources of industrial amylases (Gupta *et al.*, 2003). Growth conditions and nutrients promote high yield of microbial amylases. However, carbon sources such as dextrin, fructose, lactose, maltose, glucose and starch are very expensive for commercial production of these enzymes (Haq *et al.*, 2005b). Agricultural wastes are being used for both liquid and solid state fermentation to reduce the cost of fermentation medium. In Nigeria, most of these products are used as source of food for humans and livestock. It is necessary to search for other substrates which when exploited will not have any negative economic impact on humans or livestock but rather enhance its maximum utilization, and also create wealth for the people.

- **Amylase assay**

Amylase activity was measured by the 3, 5-dinitrosalicylic acid (DNS) method Miller. (1991); Oyeleke and Oduwole (2009), by monitoring the amount of reducing sugars liberated from starch. Amylase was assayed by adding 1ml of enzyme (crude extract/fermented broth supernatant) to 0.5 ml of 1% soluble starch and incubated for 30 min at 37^{o}C in water bath. The reaction was stopped by adding 1 ml of 3, 5 dinitrosalicyclic acid, followed by boiling for 10 min. The final volume was made to 5 ml with distilled water and the absorbance measured at 540 nm with a spectrophotometer (Jenway 6100).

One amylase unit (U) was defined as the amount of enzyme per millilitre culture filtrate that released 1 microgram glucose per minute.

Preparathion of medium for production of amylase by fungal isolates

The media were prepared by weighing the following medium composition in grams per litre, soluble starch 2.0, $MgSO_4$. $7H_2O$, 0.1, KH_2PO_4, 1.4, KCL, 0.5, $FESO_4.7H_2O$ 0.01, and NH_4NO_3 1.0, Banana peel 20.0 g. This was dissolved in 1000ml of distilled water and heated to homogenized and 50ml was dispense into 100ml capacity conical flask sterilized at 121^{o}C for 15min after which they were allowed to cool at room temperature before inoculation and were incubated at 35^{o}C for 5 days.

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Determination of optimum pH for amylase production by fungal isolates

The reaction mixtures of 120hrs containing enzymes from different strains of fungal isolates were prepared, optimum pH for enzyme amylase activity was examined by running the assay activity between the ranges of 3.0, 5.0, 7.0, 9.0 and 11 the enzyme activity for each pH was determined using the method of Betrand *et al*. (2004) .

Effect of incubation period on amylase production by fungal isolates

This research was carried out at different incubation period of 1, 2, 3, 4 5 6, 7, 8, 9 and 10 after which assay was determined by Dinitrosalicyclic acid method (Betrand *et al*., 2004).

Determination of optimum temperature amylase production by fungal isolates

Optimal temperature for amylase activity was determined by assaying the activity of the enzymes at diffrent temperature ranges of 35 ^{o}C, 45 ^{o}C, 55 ^{o}C, 65 ^{o}C and 75 ^{o} C.

Determination of innoculum size on amylase production by fungal isolates

These activities was determined by assaying the enzymes at different sizes of 1%, 2%, 3%, 4% and 5% the enzyme solution was maintained with different sizes of the inoculum.

Effect of substrate concentration on amylase production by fungal isolates

The effect of substrate concentration was determined by assaying the activity of the enzymes of each with different substrate concentration of 1, 2, 3, 4, and 5%.

RESULTS

Table 1: Morphological and identification of fungal isolates from poultry droppings

Isolates	Macroscopy	Microscopy	Organisms
A1	Black and powdery	Smooth walled and non septate conidiospore	*Aspergillus niger*
A2	Dull yellow to brown	Hyphae were septate and hyaline, conidiospore long and smooth	*Aspergillus terreus*
B1	Velvety and powdery	Erect and septate, conidia walled and curved	*Curvularia sp*
B2	Black and powdery	Smooth walled and non septate conidiospore	*Aspergillus niger*
C	Black and powdery	Smooth walled and non septate conidiospore	*Aspergillus niger*

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Effect of substrate concentration on the activity of amylase produced by the fungal isolates

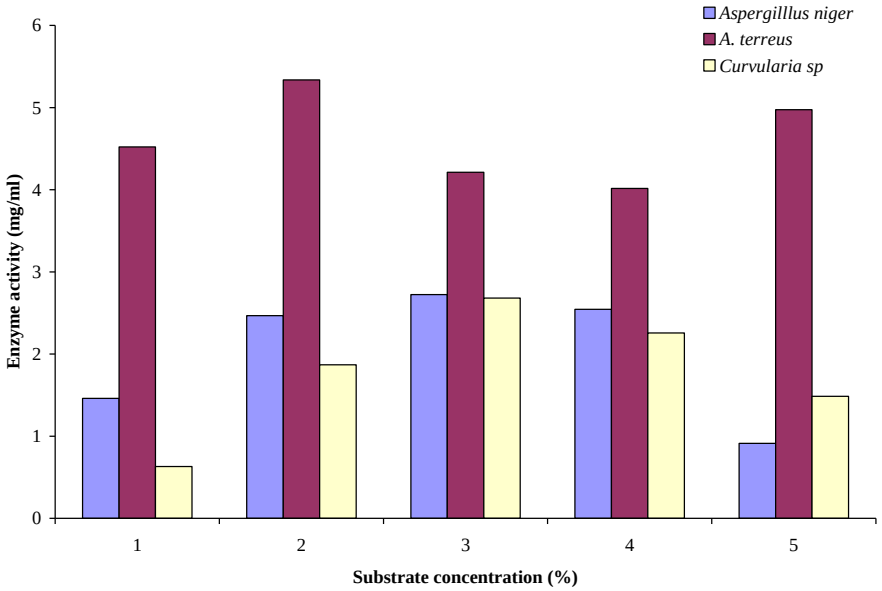

Fig 2: Effect of innoculum size on the activity of amylase produced by the fungal isolates

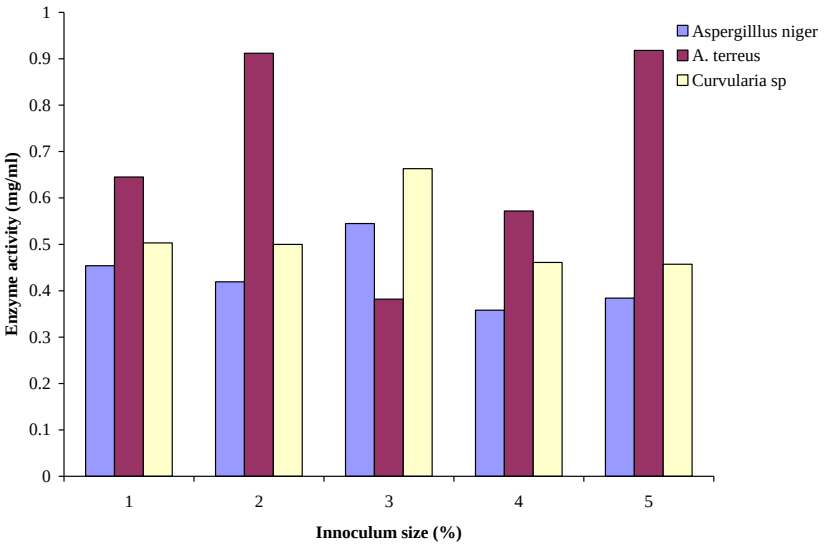

Fig 3: Effect of incubation period on activity of amylase produce by fungal isolates

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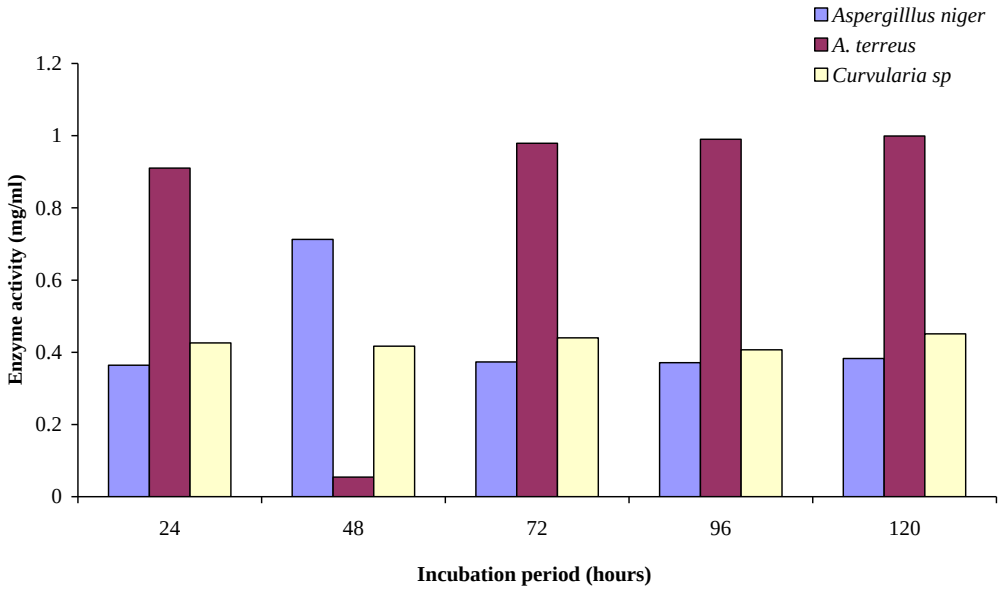

Fig 4: Effect of temperature on activity of fungal isolates producing amylase

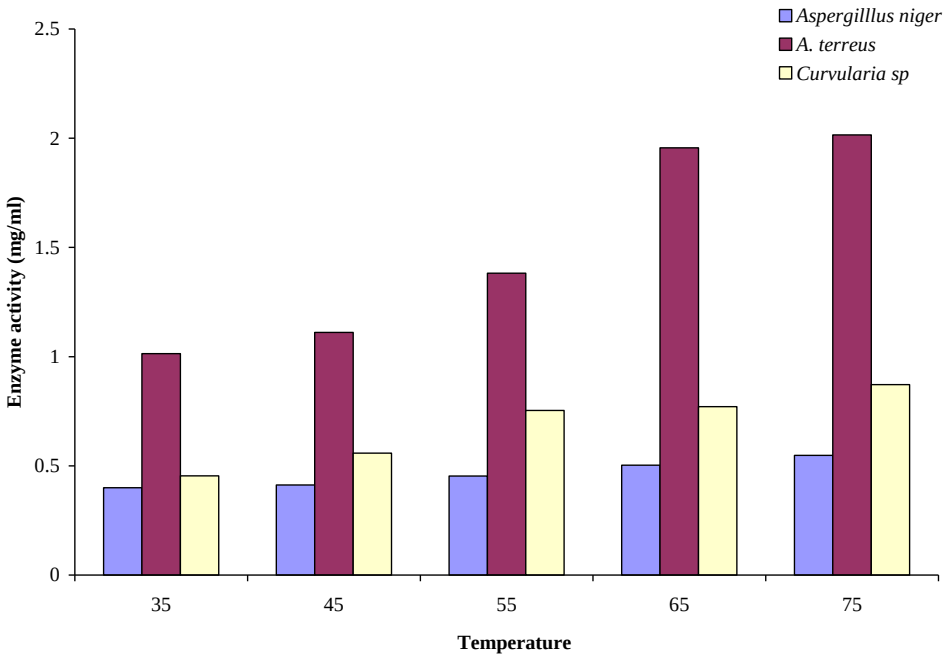

Fig 5: Effect of pH on the activity of amylase produced by the fungal isolates

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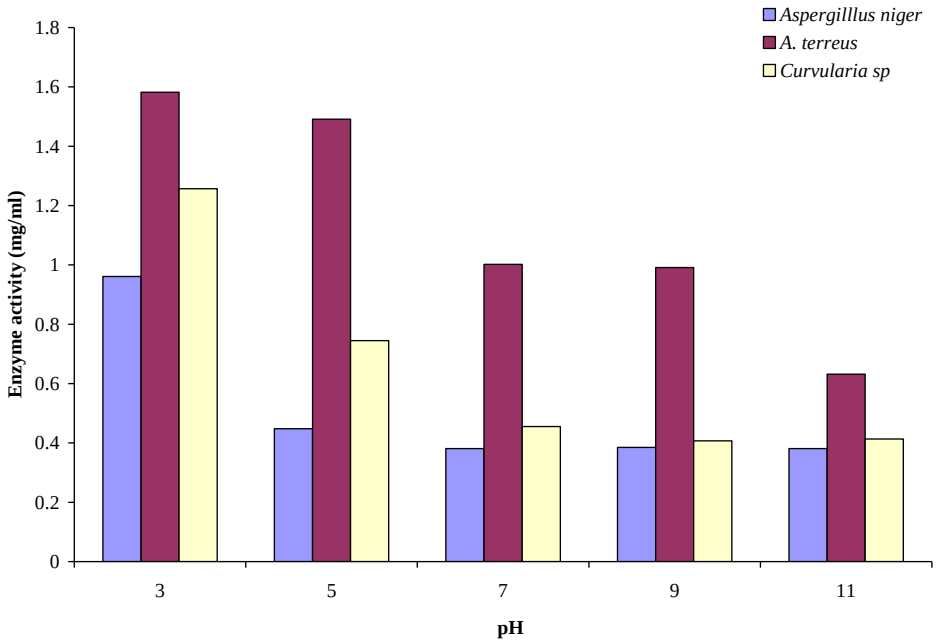

Fig 5:Add figure captions

DISCUSSION

A total of five organisms were isolated and identified from poultry waste. A. *niger*, *Aspergillus terrus*, and *Curvularia* sp as shown in table1.All the isolates were hydrolyzers of starch and showed a wide zone of clearance. Primary screening of the isolates was carried out by starch hydrolysis method. All isolates showed good clearance haloes on the plates. For the fungal isolates *Aspergillus niger* had amylolytic 12.27mg/ml, This was followed by *Aspergillus terrus* which had an amylolytic activity of 10.00mg/ml, *Curvularia sp* had an amylolytic activity 10.97mg/ml. The variation in amylolytic, produced by the different isolates may probably be due to their genetic make-up. Similar observation was made by Omemu *et al*., (2005). Olajuyigbe and Ajele (2005), *Alva et al*, (2007) and Oyeleke and Oduwale (2009) from the amylolytic and proteolytic haloes produced by different species of *Bacillus* and *Aspergillus spp* isolated from the soil.

Optimizing the physical parameters plays an important role in enzyme production. Fig 1: Shows the effect of substrate concentration on the activity of *Aspergillus niger*, *Aspergillus terrus*, and *curvularia sp* is shown in Fig 1. *Aspergillus niger* increase in activity from 1.46 mg/ml of 1% substrate concentration to 2.46 mg/ml of 2% substrate concentration, further increase was recorded in 3% concentration 2.54 mg/ml which was followed by a sharp decrease in activity to 0.91 mg/ml. *Aspergillus terrus* had an amylase activity of 4.52 mg/ml of 1% substrate concentration followed by a higher increase of 5.33 mg/ml of 2% substrate concentration a slight decline was observed in 3% substrate concentration 4.21 mg/ml and in 4% substrate concentration 4.01 mg/ml there was a slight increase in 5% substrate concentration of 4.97 mg/ml. From the figure *Curvularia sp* recorded an activity of 0.63 mg/ml in 1% substrate concentration, then there was increase in activity as the concentration of the substrate increases to 2% substrate concentration (1.87 mg/ml), 3% 2.68 mg/ml and a slight decrease was observed in 4% conc. 2.26 mg/ml, then follow a sharp decrease in the 5% conc. of the substrate to 1.49 mg/ml .*Aspergillus terrus* recorded the highest amylase production activity of 5.34 mg/ml among the three fungal isolates. Fig 2: Shows the effect of innoculum size on the activity of amylase produced by fungal isolates. *Aspergillus niger* had an amylase activity of amylase production 0.45 mg/ml in 1% innoculum size, followed by a decrease in the activity of the production to 0.42 mg/ml ,it was then followed by a slight increase in the activity of 0.55 mg/ml in 3% innoculum size then further decreases to 0.36 mg/ml in 4% innoculum size and a slight increase at 5% to 0.38 mg/ml. *Aspergillus terrus* had its activity recorded in 1% innoculum size to be 0.65 mg/ml , it then increases to 0.91 mg/ml as the conc. increases to 2% innoculum size there was a sharp decrease at 3% innoculum size of 0.38 mg/ml then further increases to 0.57 mg/ml at 4% innoculum size, its reaches a peak of activity in the production 0.92 mg/ml at 5% innoculum size . *Curvularia sp* recorded its activity in the production of amylase in 1% as 0.51 mg/ml and it decreases slightly to 0.50 mg/ml of 2% conc. there was a slight increase to 0.66 mg/ml of 3% conc and further began to decrease as the concentration increases to 4% 0.46 mg/ml and 0.45 mg/ml at 5%. It was observed that *Aspergillus terrus* has the highest amylase activity among the three fungal isolates. Fig 3: The effect of incubation period on the activity of amylase produced by fungal isolates shows that *Aspergillus Terrus* has the highest amylase activity at 120 hrs with amylase activity of 0.99 mg/ml, this agrees with the report of Sujeeta kamla (2017) who reported higher amylase activity after 120hrs of incubation this was followed by A*spergillus niger* which had maximum activity of 0.71 mg/ml after 48 hrs of incubation, and disagrees with the findings of Bijay

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k. Sethi (2016) who reported highest activity of *A . terrus* at 96hrs then there was a decline in growth after 72 hrs and 96 hrs. This could be that after maximum production of amylase the production of other byproducts and depletion of nutrients. The byproducts inhibited the growth of fungi and hence enzyme formation. From the figure C*urvularia sp* recorded the least activity of 0.40 mg/ml after 96 hrs of incubation among the three fungal isolates. This agrees with the findings of Kathiresan *et al.,* 2006 who reported maximum activity in 96 hrs by *penicillinium fellutanum.*

The effect of temperature on amylase production by the fungal isolates is shown in Figure 4. A*spergillus niger* had amylase activity of 0.40 mg/ml/ at 35^{o}C, this was followed by an increase in amylase activity as the temperature increased. Maximum amylase activity of 0.55 mg/ml was recorded at 75^{o}C The optimum temperature for amylase enzyme activity produced by *Aspergillus terrus* was also at 75^{o}C with an enzyme activity of 2.02 mg/ml ,minimum activity was recorded at 35^{o}C with an activity of 1.01 mg/ml. *Curvularia sp* had minimum amylase activity at 35^{o}C (0.45 mg/ml) , maximum activity was recorded at 75^{o}C (0.87 mg/ml). this result is contrary to the report of kandu and Das (1970) and Ray 2004 who reported *A. oryzae, Betryodiplodia theobromae, and rhizophus oryzae* had maximum activity at 30^{o}C Mannivannan 2006 also reported 30^{o}C as optimum for *Penicillium fellutanum* isolated from mangrove rhizosphere soil. The effect of pH on the activity of amylase produced by fungal isolates is shown in Fig 5.From the figure *A niger* had amylase activity of 0.96 mg/ml at pH value of 3,this was followed by an decrease in amylase activity as pH of increase . (0.45 mg/ml) 0.38mg/ml, 0.39 mg/ml and the 0.38mg/ml at pH value 11 the highest enzyme activity was recorded at pH 3 by *Aspergillus niger Aspergillus terrus* recorded its lowest activity at pH of 11 with 0.063 mg/ml. *Curvularia sp* had an amylase activity of 0.41 mg/ml at pH of 11. *A*spergillus *terrus* recorded the highest activity of amylase production with an activity of 1.58 mg/ml at pH 3. This findings does not agree with the report of Pate *et al*.2005 who reported *A.oryzae* had amylase activity at pH 5 and also contradict the findings of Olama and Sabry 1989 where he reported highest amylase activity at pH 7 by *A. flavus* and *A.niger* at pH 5, this study suggest that enzyme require wide range of pH from slightly acidic to alkaline the high pH activity suggest the presence of alpha amylase and glucoamylase Abu *et al.,* 2005 this property makes the enzyme suitable for industrial production of paper and detergents. This study Agrees with findings of Bajpai and Bajpai (1989), who reported higher amylase activity of *A.niger* at pH 10-11.

CONCLUSION

This study revealed that poultry droppings and Banana peel, are examples of domestic and industrial agro-wastes, that can be use to produce large amounts of amylase, enzymes when hydrolyzed by amylolytic, microorganisms instead of being left alone for natural degradation, these enzymes can be utilized effectively under optimum conditions, to produce amylase.

RECOMMENDATION

The results obtained from this study give rise to the following recommendations:-

i. Domestic waste can be exploited for the cultivation of amylolytic enzyme production.
ii. Companies should be encouraged to use local isolates for the production of enzymes instead of using funds for its importation.
iii. Companies should exploit the potentials of using agro waste with the potentials of producing enzymes since these waste are available and at no cost.

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