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# XYLANOLYTIC ABILITY OF BACTERIA ISOLATED FROM CORN COBS COLLECTED FROM DUMP SITES IN OYE EKITI, SOUTH WEST, NIGERIA

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## Abstract

*Lignocellulosic materials are potential sources of various microorganisms of industrial importance. Though the uses of these materials have been on the increase, little attention has been paid to potentiality of the bacteria from them. The aim of this work was to investigate the ability of bacteria from corn cob in the production of xylanase. Bacteria were isolated from corn cob collected from dump sites in Oye-Ekiti, Nigeria. The isolates were characterized using standard microbiological techniques. Five xylanase producing bacteria were isolated. Three of them are Pseudomonas aeruginosa (XA1, XA2 and NA2), one Pseudomonas putida (NA1) and one Bacillus sp.(XA3). The isolates (XA1, XA2 and NA2) with substantial xylanase production were scrutinized to quantify the amount of the enzyme produced, using different concentrations of xylan, carbon sources, nitrogen sources and pHs. Maximum amount of xylanase (14.5 mg/mL) was produced by Pseudomonas sp. NA2 in the presence of sucrose. Effect of diferent agro-industrial wastes and incubation time were also investigated on the production of xylanase. Corn cob elicited the highest enzyme production (1.168mg/mL) after 48hr of incubation of isolate NA2 while XA1 gave the least value (0.016mg/mL) of the enzyme after 72hr of incubation. These isolates can be of tremendous advantage in the production of xylanase and in the treatment of agro-industrial wastes.*

## 1.0 Introduction

The main constituents of Lignocellulosic material are cellulose, hemicellulose, and lignin [1]. Hemicellulose is a heteropolysaccharide composed of different hexoses, pentoses, and glucoronic acid. Xylan is the most common hemicellulose component of grasses and woods [2]. Biodegradation of xylan, a major component of plant, requires hydrolysis by the activity of several enzymes [3]; [4]. Wheat bran and corn cob are a rich source of xylan and xylose. Therefore, these are attractive substrates for production of xylanase and ß-xylosidase enzyme [5]. Large amount of Agro-industrial wastes generated across the globe can be employed in producing cost effective xylanases, the use of these low cost agro-industrial wastes as substrates will also contribute to reduction in environmental pollution associated with the improper disposal of these wastes. Enzymes have shown to be of great importance in various industries as they are able to carry out reactions under ambient conditions which make their use eco-friendly and preferred above the polluting chemical technologies [6]. Polysaccharides degrading enzymes are widespread and are naturally found in plants, animals and microorganisms [7]. However, due to their structural stability, easy access to them and the ability to manipulate their gene to produce desirable traits, microorganisms are considered to be the most effective source of industrial enzymes [8]. Enzymes such as xylanase have immense industrial applicability. Thus, they have been used in imperative industrial processes such as biofuels like bioethanol production [9] agricultural, plant waste management and textile industry [5, 9, 10]. The wide range application of xylanase in industries and the risk of environmental pollution associated with the use of chemicals in such applications have led to an increase in the demand for the enzyme. In a bid to meet up with this demand, there is a need to find means for producing a cost effective xylanase on a large scale. Therefore, the motive of this study was to isolate xylanolytic bacteria from corn cob and use them to produce xylanase of industrial importance by using various agro-industrial wastes as substrates.

## 2.0 Materials and Method

**2.1 Sample collection:** Corn cobs were collected from dump sites in Oye-Ekiti, Nigeria, brought to laboratory and ground into fine particle.

**2.2 Isolation of xylanolytic bacteria:** Nutrient agar and modified Natalie medium composed of 0.1% yeast extract, 1.2% agar, 0.05% $K_2HPO_4$, 1% xylan and 1% tryptone were used for the isolation of xylanolytic bacteria. One gram of ground corn cob samples was suspended in 10ml of sterile distilled water and serially diluted. 1 ml from the diluted sample was used to inoculate the nutrient and modified Natalie agar using pour plate techniques, the plates were then incubated at 35 $^oC$ for 24 hr. Distinct colonies were severally transferred to obtain pure cultures.

**2.3 Identification and screening of the Isolates**

To identify the bacteria, gram staining and biochemical tests; catalase test, MR-VP test, Triple sugar iron test, sugar fermentation tests, Oxidase, Motility, $H_2S$, Urase, Gelatinase, pigment production, Arginine dihydrolase, $NO_3$ reduction tests and growth at $42^0C$ for *Pseudomonas* spp.) were carried out on the isolates as described according to [12]**.** Xylanolytic activity of bacteria was detected using Congo red [13]. Pure colonies grown on the modified Natalie

media were flooded with 1% aqueous Congo red, followed by flooding with 1M NaCl after 20 minutes and later with 1M HCl. The plates were thereafter examined for appearance of halo clearing zone of hydrolysis. Congo red is known to interact with (1,3- and 1,4-) β-D-glucans. The bacteria with the wider clearing zone were selected for xylanase production. Pure cultures of the isolates were maintained on nutrient agar slant stored at 4 $^0$C for further studies, but subculture every week.

**2.4 Inoculum preparation**

The isolates were used to inoculate the modified Natalie medium (14) broth medium with composition (w/v), 1% xylan, 1%tryptone, 0.5% yeast extract, 0.1% $KH_2PO_4$ and incubated for 24hrs at 35$^0$C. 24hrs old cultures were used to inoculate the enzyme production medium.

**2.5 Xylanase production medium**

The medium was prepared in 10ml McCarthy bottles with the medium composition (w/v),1% xylan, 1%tryptone, 0.5% yeast extract, 0.1% $KH_2PO_4$. Medium was inoculated with 0.5ml of 24hrs broth cultures of the isolates adjusted to O.D. 0.3 at 540nm, it was then incubated at 35$^0$ C for 24hrs.Uninoculated bottles served as control.

**2.6 Xylanase assay**

Enzyme assay was carried out as done by Mahenthran [15]. The medium was centrifuged at 4000rpm for 30minutes to separate bacterial cells from crude enzyme, the supernatant was collected and used as crude enzyme for the assay. Half millilitre (0.5 mL) of crude enzyme was transferred into test tubes and added to 0.5 ml of 1% xylan for enzyme assay. The preparation was incubated at 50 $^o$C for 30 minutes. 1ml of Dinitrosalicylic acid (DNS) reagent was added to the solution to stop the reaction. The preparation was then heated in boiling water for 5 minutes and cooled immediately. The reaction mixture was read with spectrophotometer (pul-model) absorbance at 540nm. The control was treated the same way. Xylanase activity was expressed as 1mg of reducing sugar (xylose equivalent) per milliliter of enzyme solution. Xylanase activity was determined from the calibration curve constructed with varying concentrations of D-xylose (16). D-xylose was varied from 0.2 mg/ml to 2.0mg/ml, this was used to prepare a standard curve.

**2.7 Optimization of xylanase production**

The effect of different concentrations of xylan was studied at 0.5%, 1%, 1.5%, 2.0% and 2.5% of xylan in the preparation of the medium. Glucose, sucrose, galactose, maltose, fructose and lactose were used to replaced xylan in the medium for studying effect of carbon sources;. Yeast extract and tryptone in the production medium was substituted with yeast extract only, tryptone only, ammonium sulphate, urea and peptone, to determine their effects on enzyme production. Effects of different pHs (5, 6, 7, 8 and 9) on the enzyme production were also studied.

**2.8 Effect of different agro industrial wastes and incubation period on xylanase production**

Agro industrial wastes (corn cob, groundnut shell, sawdust and sugarcane bagasses) were ground into smaller particles and passed through sieve to obtain finer particles. The modified Natalie different media (14) (in which carbon source was replaced with agro industrial wastes) were prepared in 10ml McCarthy bottles with the medium composition (w/v) 1% agro industrial waste at the optimum conditions for each isolate.

Media were autoclaved at $121^0$C for 15 minutes, the media were then inoculated with 0.5ml of 24 hr old broth cultures of the isolates adjusted to O.D. 0.3 at 540nm, it was incubated at $35^0$ C and assayed for xylanase production at 24 hr interval for three days. Samples were withdrawn every 24 hr for three days and assayed to determine the quantity of enzyme produced.

## 3.0 Results and Discussion

### 3.1 Isolation and identification of organisms

Five xylanase producing bacteria were isolated from corn cob and identified as *Pseudomonas aeruginosa* (XA1, XA2 and NA2), *Pseudomonas putida* (NA1) and *Bacillus* sp. ( XA3) using morphological and biochemical tests. The zone of hydrolysis of the isolates in this study range between 0.45-1.1 cm which indicate their potential for industrial applicability. Other studies had shown hydrolysis zones of 0.8 and 1.6 cm on xylanase producing medium, though for fungal isolates [17].

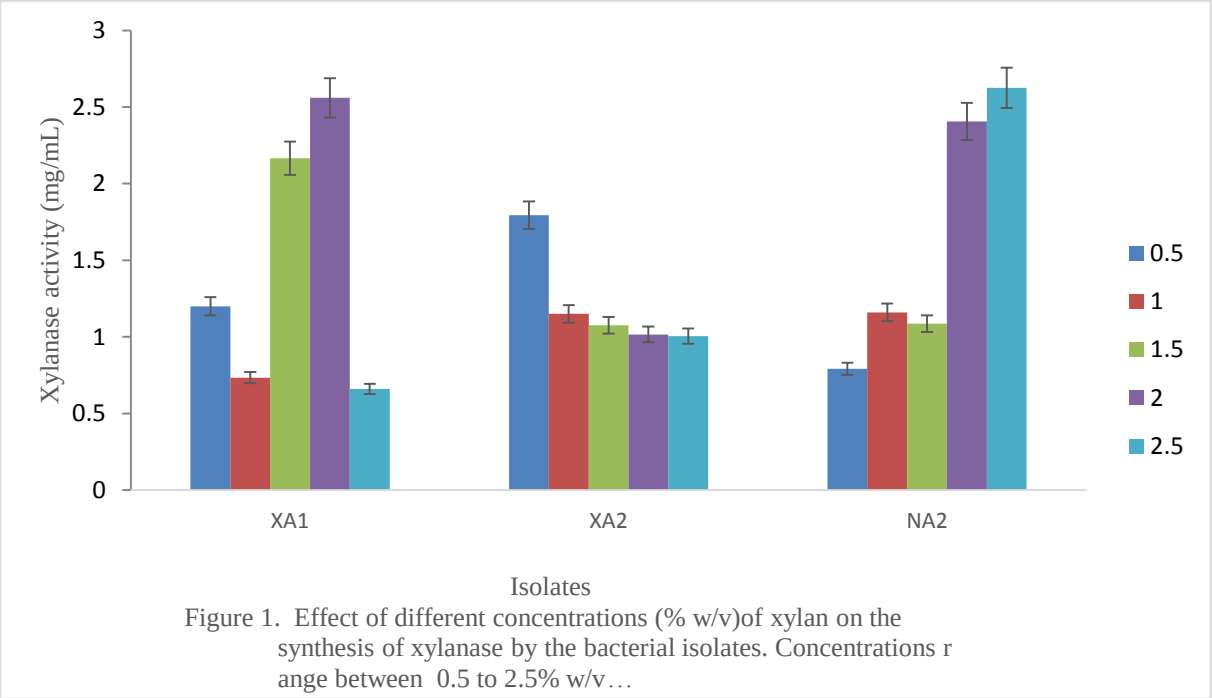


Figure 1. Effect of different concentrations (% w/v)of xylan on the synthesis of xylanase by the bacterial isolates. Concentrations r ange between 0.5 to 2.5% w/v…

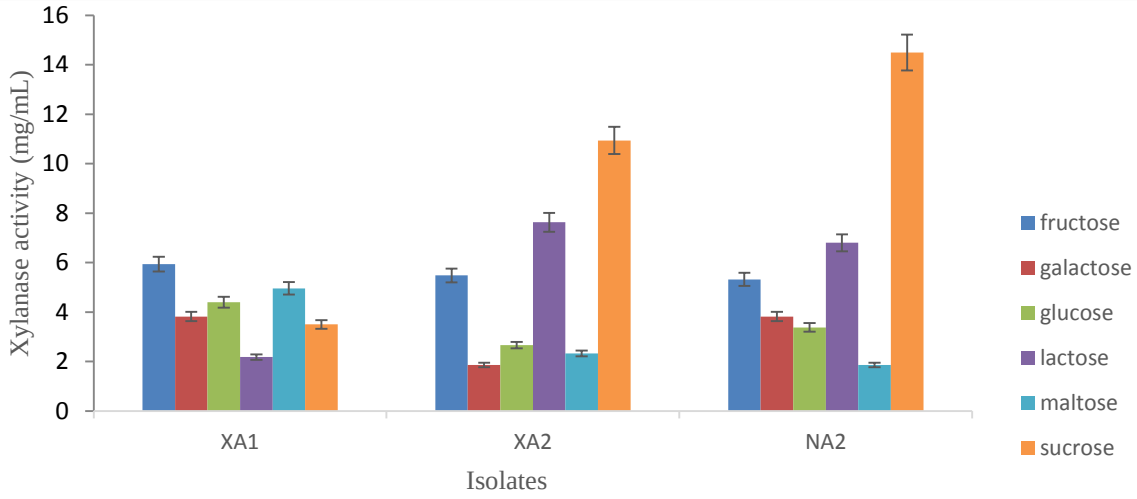


Figure 2 Effect of different carbon sources on the xylanase synthesis by the bacterial isolates.

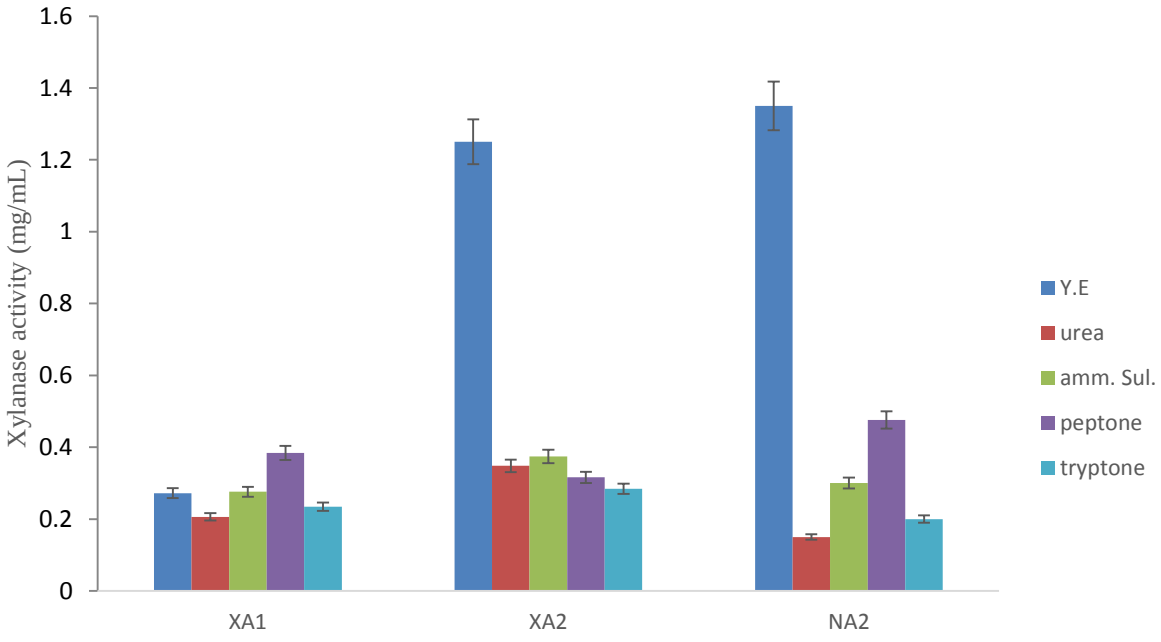


Figure 3: Effect of different nitrogen sourses on the synthesis of xylanase by the bacterial isolates.

Legends: Y.E = Yeast extract, amm. Sul =Ammonium sulphate

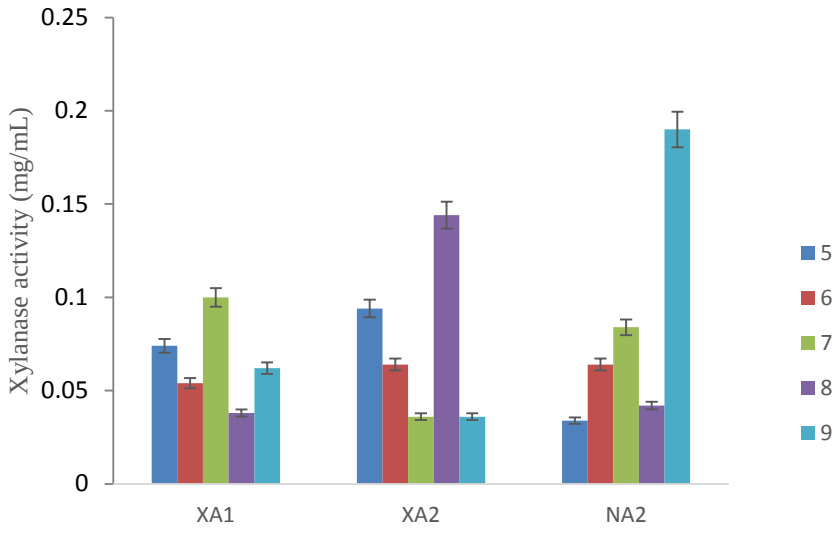


Figure 4 : Effect of different pH ranges on the xylanase synthesis by the bacterial Isolates .

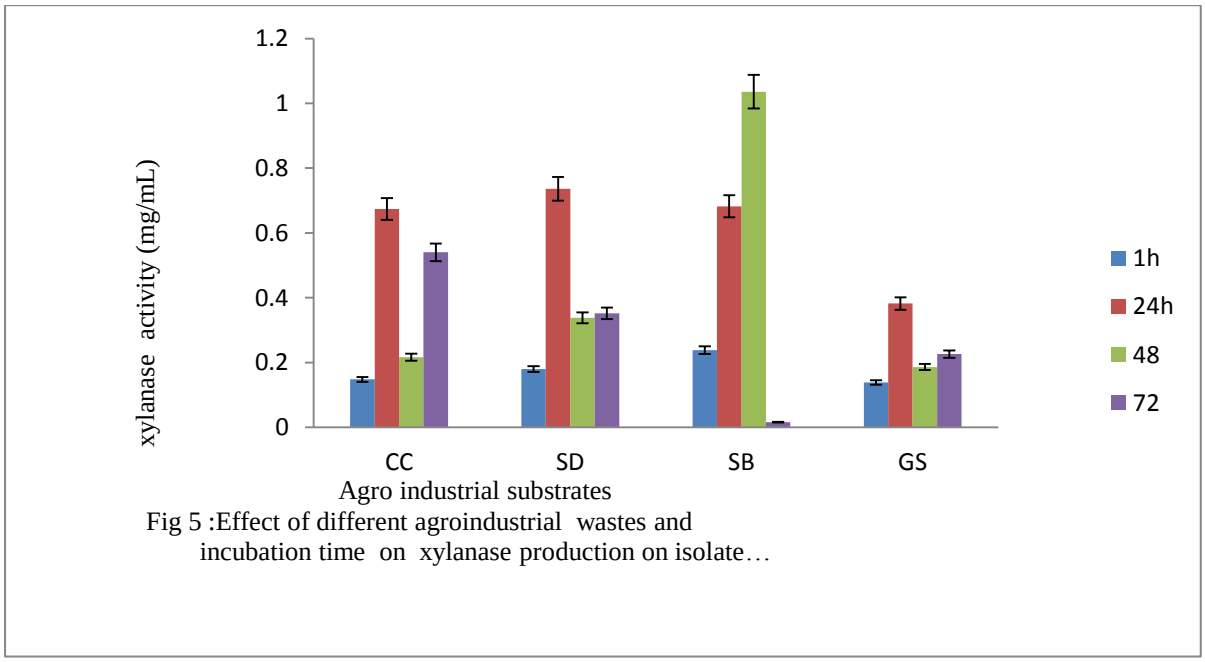


Fig 5 :Effect of different agroindustrial wastes and incubation time on xylanase production on isolate…

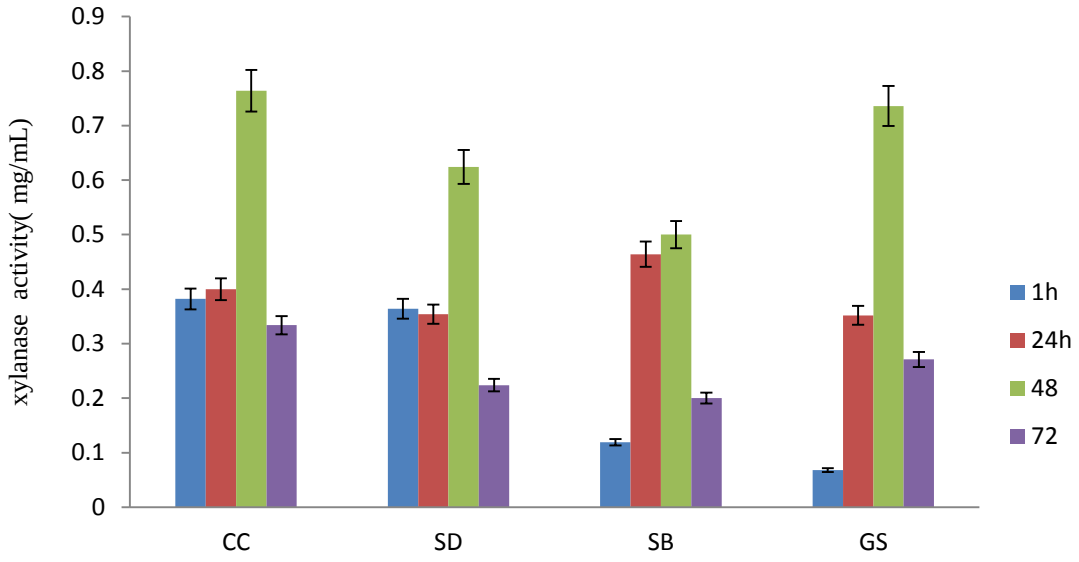


Fig 6 : Effect of different agroindustrial wastes and incubation time on xylanase production on isolate XA2.

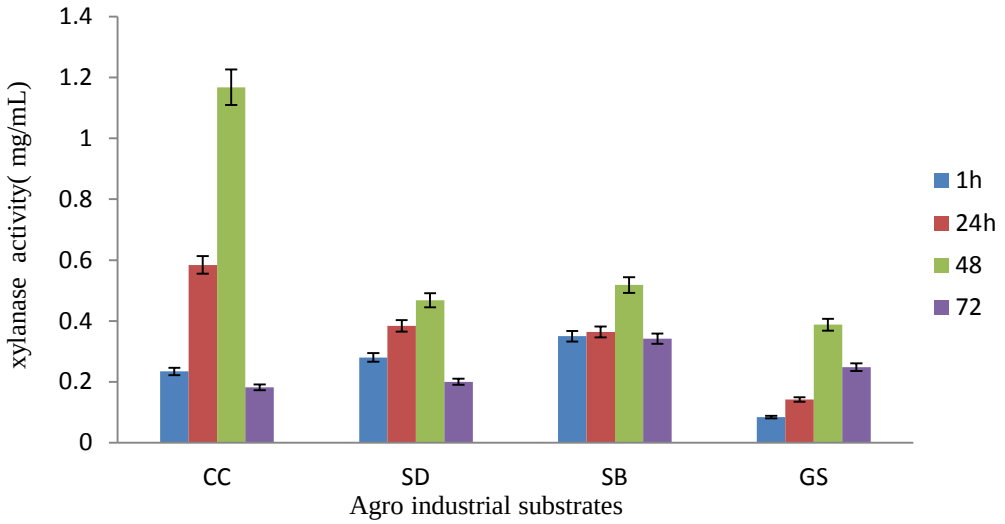


Fig 7 : Effect of different agroindustrial wastes and incubation time on xylanase production on isolate NA2.

CC=Corn cob, SD= Sawdust, SB= Sugracane bagasse, GS= Groundnut shell…

Different xylan concentrations (0.5%, 1%, 1.5%, 2% and 2.5%) were used in production of xylanase. Isolates XA1, XA2 and NA2 gave the best yield of xylanase at different substrate concentrations of 2%, 0.5% and 2.5% respectively. 1.5%, 1% and 2% gave higher yield next to the best substrates concentration in isolates XA1, XA2 and NA2 respectively (Fig. 1). Effect of different concentrations of xylans was reported [18] where it was observed that 0.5% xylan concentration was most effective for xylanase production on *Streptomyces*. This was also reported [19] where 0.5% xylan produced high yield of xylanase. Similar result was observed for isolate XA2 in this study. These two studies together with reports from [20] where 1.5% seems to best the optimum substrate concentration for xylanase however contradicts the highest production of xylanase recorded for XA1
and NA2 at xylan concentration of 2% and 2.5%. The differences observed might be due to the ability of individual microorganism in the utilization of the substrates Mohamed et al. (21).

### 3.1 Effects of different carbon sources on xylanase production by the bacterial isolates

Different sugars (glucose, sucrose, galactose, maltose, fructose and lactose) were used to replace xylan during xylanase production (Fig. 2). It was observed that NA2 and XA2 produced the best yield of xylanase with sucrose followed by lactose. Lactose and sucrose on the contrary seem to produce the lowest yield in XA1 with the lowest yield observed with lactose. Fructose appeared to be the best carbon source for the production of xylanase in XA1.Galactose gave the lowest yield of xylanase in isolate XA2 followed by maltose while maltose gave the lowest yield of xylanase in NA2 and galactose was observed to produce least next to maltose. Sucrose when used as sole carbon source for xylanase production was observed to stimulate the highest production of xylanase with isolates XA2 and NA2, this is in accordance with the study conducted by [21] where sucrose stimulated the highest production of xylanase in *Aspergillus* under submerged fermentation. Jampala [22] also reported that sucrose was the best carbon source for xylanase production. The high xylanase activity observed with sucrose has been attributed to easy assimilation of the sugar by microorganisms [21]. Fructose when used as carbon source produced high yield of xylanase with XA1 as against the observation of Karni et al. [23] who reported repressiveness in the yield of xylanase when fructose was used as supplementary carbon. However, Badhan *et al*. [24] reported the expression of additional xylanase production when fructose was used.

### 3.2 Effect of different nitrogen sources on xylanase production by the bacterial isolates

Different nitrogen sources (yeast extract, tryptone, ammonium sulphate, urea and peptone) were used in xylanase production. Yeast extract was observed to produce the highest yield of xylanase when used as nitrogen source in both NA2 andXA2 and peptone stimulated highest yield of xylanase in XA1 which is in agreement with many scientists who reported that yeast extract and peptone were the best nitrogen sources for xylanase production [21]. Yang et al. [25] also reported that yeast extract elicited the highest production of xylanase using *Paecilomyces hermophilia*. The yield of xylanase after yeast extract was followed by peptone and ammonium sulphate for isolates NA2 and XA2 respectively. Peptone elicited the highest enzyme production with isolate XA1 and this was closely followed by ammonium sulphate. Maximum production of xylanase by *Streptomyces*in the presence of was also

reported [26]. The least xylanase production was observed when urea was used as nitrogen source for NA2 while the least enzyme production for both XA1 and XA2 was observed with tryptone (Fig. 3). Yeast extract and peptone are the best nitrogen sources because they contain different growth factors that can stimulate the growth and replication of microorganism [21]. The authors also reported the least stimulating ability of urea in production of xylanase which was observed for isolates XA2 and NA2 in this study. This might not be unconnected with absence of availability of ready to use amino acids in urea Narumol and Jirapa, (27).

### 3.3 Effect of different pH values on xylanase production by the bacterial isolates

Buffers of different pHs (5, 6, 7, 8 and 9) were used in the preparation of medium for xylanase production (Fig 4). pHs 7,8 and 9 were observed to be the best for xylanase production in XA1, XA2 and NA2 respectively. These were closely followed by pH 5 in both XA2 and XA1 while pH 7 was next to the pH 9 in the amount of xylanase production by isolate NA2. pHs 8, 9 and 5 showed the lowest yield of xylanase in XA1, XA2 and NA2 respectively. The higher xylanae production at high pHs by bacteria in this study may be due to bacterial alkaline pH homeostasis. These include elevated levels of transporters and enzymes that promote proton capture and retention (e.g. the ATP synthase and monovalent cation/proton antiporters), metabolic changes that lead to increased acid production, and changes in the cell surface layers that contribute to cytoplasmic proton retention Etana et al. (28).. That some bacteria are able to function and produce xylanase at high pH as observed in this work was documented by Poorna, and Prema [29]. It has earlier been reported that bacillus of different strains had their optimum pH at pH 8 [30, 31]. This was observed with isolates XA2. The maximum production of xylanase at pH 7 by XA1 also agrees with what had been reported for *Bacillus pulmulus* [32].

### 3.4 Effect of different agro industrial and incubation hour on xylanase production by isolates XA1, XA2 and NA2

Agro-industrial wastes are potential source of microorganisms because of their nutritional components. *Pseudomonads* are ubiquitous Gram negative rods with great metabolic potentials which allow for their isolation in various environments [33]. Four of the isolates in this study, were identified as *Pseudomonas sp*. All the wastes investigated for enzyme production in this work supported enzyme production. But the enzyme production was highly influenced by fermentation period, xylan content and the organism (Fig. 5, 6 and 7). Figure 5 showed the ability of isolate XA1 to produce xylanase from corn cob, groundnut shell, sawdust and sugarcane bagasse. Isolate XA1 produced well with all the agro industrial wastes. Sawdust was found to be the most suitable substrate for xylanase production at 24hr this was closely followed by sugarcane bagasse and corn cob respectively while groundnut shell produced the least xylanase activity at that hour. However, the highest amount of xylanase (1.04mg/mL) was produced with sugarcane bagasse substrate at 48 hrs. After 1 hour of incubation corn cob produced the highest yield of xylanase (Figure 6), this was followed by sawdust and groundnut shell was found to be the least xylanase producer of all the substrates. After 24 hours of incubation, sugarcane bagasses was observed to be the best producer of xylanase followed by corn cob and sawdust respectively. The results in Figure 7 depicted relatively low amount of xylanase production by NA2 after 1 hour of incubation with sugarcane bagasse having highest yield of xylanase closely followed by sawdust and

groundnut shell was observed to be the least producer. Increase in xylanase production was observed with increase in incubation time from 1 to 24 hours, xylanase was moderately produced after the first 24 hours of incubation, Corn cob was observed to be the most suitable substrate because of the highest level of xylanase production. This was followed by sawdust, sugarcane bagasse and groundnut shell with 0.384, 0.364 and 0.142 mg/mL respectively. Agro industrial wastes such as corn cobs, sugarcane bagasse, sawdust and groundnut shell have been reported used in enzyme synthesis using various organisms [34, 35, 36]. Corn cobs are rich sources of xylan (28%) and Xylose (23%) and this may be responsible for the ability of corn cob to produce high yield of xylanase. Corn cob in this current study was observed to be the most suitable substrate for the production of xylanase for both isolates NA2 and XA2 with xylanase activities of (1.17mg/mL) and (0.76mg/mL) respectively after 48 hours of incubation. The observation corresponds with a study carried out with *Candida tropicalis Strain Ly* 15 [37] for the production of xylitol and ethanol from xylanase fermentation broth. Another study carried out [38] using local fungal isolates gave similar results with corn cob giving the highest yield of endoxylanase. Although sugarcane bagasse contains high percentage of hemicelluloses and low ash content and has been reported to produce xylanase at high rate when compared to other lignocellulosic wastes [39], the results in this study are in contrary as the two of the three isolates elicited highest xylanase production after 48 hours of incubation in corn cob. This might be as a result of the fact these isolates are obtained from corn cob and are used to the environment from which they were isolated.

Maximum yield of xylanase (0.74mg/mL) was observed with sawdust by isolate XA1 after 24 hours of incubation, similar result has been reported [40] with the study on *Aspergillus niger* in xylanase production. Low yield of xylanase activity was observed for isolate XA2 after 24 hours in the presence of sawdust. This observation has also been reported by Kalpana,and Rajewan [38] where sawdust produced least yield of xylanase next to tomato pomace by *Streptomyces sp* The general low yield of xylanase for all isolates except for XA2 was observed in groundnut shell, this is similar to reports of Kango et al. [42], where it was noted that groundnut shell produced the lowest yield among all other substrates in *Emericella nidulans N162*. The lower yield of xylanase in groundnut shell may be due the low component of hemicellulose in it [30]. Knob et al. [28] had earlier reported the low xylan content of groundnut shell and the level of xylan determines amount of xylanase released.

Nasr *et al*. [43] had reported the production of endoxylanase using *Aureobasidium* where maximum yield of xylanase was obtained after 48 hours of incubation and there was no further increase in xylanase production after 72 hours of incubation. The observation by these authors is in conformity with the results in this study in relation to isolates XA2 and NA2 where the peak of xylanase activity was attained after 48 hours of incubation and drop in xylanase production was obtained after 72 hours of incubation. Ho, [44] gave similar reports and attributed the maximum xylanase activity at 48 hours to the rapid multiplication of culture at the log phase experienced about the 24-48 hours after incubation. They attributed the decline observed from 48- 72 hours to depletion of available nutrients for the isolates. The fluctuations of xylanase activities during the time course of reaction from 1hr to 72hr as witness in the degradation of the lignocelluloses for all the isolates may be

due to catabolic repression [45]. It can be concluded from this study that *Pseudomonas* spp. are good candidates for production of xylanase especially with the use of agro-industrial wastes.

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