Activity of α-amylase Produced by Aspergillus niger at Different pH, Temperature and Incubation Time Using Solid-state Fermentation Process of Corn and Wheat Wastes

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors MSM, MB and SPW designed the study. Authors SPW and MSM performed the statistical analysis. Authors MB, SPW and MSM wrote the protocol and wrote the first draft of the manuscript. Authors MB and SPW managed the analyses of the study. Authors HUA and KVB managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT
In Nigeria, agro by-products have not been fully utilized by many and often discarded at the dumping site. This anthropogenic activity is contributing to an increase in pollution and is a threat to public health. Environmental sustainability requires the wise use of resources that include agro by-products. Therefore, there is a need to utilize the agro by-product for the production of enzymes such as α-amylase. α-amylase is one of the important extracellular enzymes with several uses. The development of suitable technology to produce enzymes at a very lower cost is significant. The solid-state fermentation (SSF) process using corn and wheat wastes as a substrate have been utilized. In this study, Aspergillus niger from abattoir effluent was identified, isolated and used for
1. INTRODUCTION

Enzymes are biocatalyst for a large number of biochemical reactions, α-amylase is one of the most important industrial enzymes use in brewing, textile, and pharmaceutical industries [1]. Amylase can be obtained from plants, animals and microorganisms [2]. Many industries prefer to use amylase from microbial sources [3]. Because is consider organic since is been produce by microorganism. Different types of microorganisms, including fungi, have been utilized in the production of an enzyme, for example, amylase [4]. The use of fungi for the production of enzymes is economical because can be manipulated easily in the residues of agro by-products [5]. Also, fungi are preferred over bacteria for enzyme production because of their filamentous nature, which aids in the penetration of the solid substrate of agro-industrial residues [6]. In Nigeria, staple cereals food such as maize, millet and sorghum are been used as sources of amylase [7]. The complete use of staple food crop as an alternative to microorganism for the production of enzymes such as amylase may have a negative impact on food security. Also, enzymes in commercial quantity from staple crop food when stored for a longer period tend to lose their stability [8]. The use of solid support such as solid-state fermentation (SSF) holds tremendous potential for the production of enzymes [9]. The microorganism uses the carbon source of the crude substrate in the absence or near absence of free water in the interior of a solid matrix [10,11]. The commonly used substrates in SSF are cereal grains waste, for example, corn, wheat and barley [12]. There is also a need to reduced environmental pollution that is emanating from the negative activity of man such as dumping of agro by-product [13]. However, most of the substrates from the agro by-products are characterised by polymeric and insoluble compounds with a high amount of nutrients for microbial growth [12]. In this study, corn and wheat agro by-products were used to build solid-state fermentation and Aspergillus niger were incubated in the solid support matrices for the production of amylase. Therefore, the objectives of this study were to isolate and characterize fungi from abattoir wastewater effluent in Kano, Nigeria. And to evaluate the effect of temperature, pH and incubation time on the amylase activities produced using solid-state fermentation of corn and wheat wastes.

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing of Corn and Wheat Residue

Corn and wheat were purchased from Fagge local market in the Fagge Local Government Council, Kano State, Nigeria. And were ground, sieved and the residue was stored at room temperature. The residue was used in this study.

2.2 Sample Collect and Culturing

Abattoir wastewater effluent was aseptically collected from the gutter of Fagge abattoir, Kano state, Nigeria. The abattoir wastewater effluent sample collected was transported to the microbiology laboratory of Bayero University, Kano, Nigeria. Exactly 10 mL of the wastewater was diluted in 90 mL of sterile distilled water, followed by serial dilution. Then, the serial diluent was aseptically inoculated onto a different plate of sterile Potato Dextrose Agar (PDA). Subculturing was carried out until the pure culture of Aspergillus niger was obtained.

2.3 Microscopic Observation and Isolation of Aspergillus niger

A small portion of the mycelia growth was carefully picked using a sterilized inoculating needle and placed in a drop of lactophenol cotton blue on a microscope slide and covered with a coverslip. The slide was examined under the microscope, first closer view under (x10) and then with (x40) magnification of the objective lens. The isolates were characterized based on the detailed morphology of aerial and substrate.
hyphae, type of hyphae, and type of asexual spores. The morphological examination was compared with the description of Cheesbrough [14]; Oyeleke et al. [15]; Domsch and Gams [16].

2.4 Preparation of Inoculum

Spore suspension of selected Aspergillus niger isolates was prepared by scraping off fungal spores within a 1cm cork borer with 40 mL of distilled water. This was made up to a 60 mL mark. Two (2) mL of this suspension was used to inoculate the substrate.

2.5 Solid-State Fermentation for α-amylase Activity

The medium was prepared as described by (Sethi and Gupta, 2015). Five grams each of corn and wheat bran were separately weighed into a 250 ml Erlenmeyer flask each and moistened with 5 ml of the following fermentation medium composition (1.25g of KCl, 0.35g of KH₂PO₄, 0.025 g of MgSO₄.7H₂O, 2.5g of NH₄NO₃, 0.0025g of FeSO₄.7H₂O, 5 soluble starch, 100 ml of distilled water at pH 6.5). The substrate and fermentation medium were mixed thoroughly, heated on a hot plate to homogenized and then sterilized in an autoclave at 121°C for 15 min. Each Erlenmeyer flask contained substrate and was inoculated with 2 ml of the spore suspension of A. niger.

2.6 Enzyme Extraction

Fifty (50) mL of 0.1 M phosphate buffer and 0.1 M and acetate buffer for (pH 6 above) and (pH 5 below) respectively were poured on each substrate bed and agitated for 30 min at 250 rpm using a rotary shaker. The solution was filtered using a cheesecloth and the filtrate was centrifuged at 2000 rpm for 5 min. The decanted supernatant was used as the crude enzyme extract [17,18].

2.7 Screening for Amylolytic Activity

The amylolytic activity of the test isolates was determined by using the starch agar plate method as described by Fossi et al. [19], by inoculating the test organism individually into Potatoes Dextrose Agar medium which was supplemented with 1 g (1%) of starch. The agar plates were then incubated at 30°C for 5 days. After the incubation period, Lugol's iodine solution was added to the culture plate to identify the zones around the cultures. The diameter formed after the addition of iodine solution was measured to represent the amylolytic activity.

2.8 Determination of α-amylase Activity

The α-amylase activity was determined by measuring the reducing sugars released as a result of the action of crude enzymes on starch. Amylase activity was determined using the method described by Sindiri et al. [20]. The reaction mixture consists of 0.5 mL of the crude enzyme, 0.5 mL of 1% soluble starch in 0.02 M citrate phosphate buffer with 0.06M NaCl, pH 6.5. The mixture was incubated for 3 min at room temperature, the reducing sugars liberated were estimated using the 3, 5-dinitrosalicylic acid (DNS) method [21]. Colour development was read at 540 nm with a UV – mini spectrophotometer against a blank, prepared by substituting the hydrolyze sample with distilled water. The reducing sugar content was subsequently determined by referring to a standard curve of known glucose concentration.

2.9 Protein Determination

The protein concentration of the enzyme extracts was determined following the method of Lowry et al. (1970) with Bovine Serum Albumin (BSA) as standard and 0.2ml of protein extract was measured into tubes and 0.8 ml distilled water was added to it. Distilled water was used as blank while BSA standard curve was equally set up (5, 10, 15, 20, 25, 30 mg/ml), 5.0ml of the alkaline solution was added into 10 ml of all the tubes, mixed thoroughly and allowed to stand for 10 mins, The absorbance was read at 540nm in a spectrophotometer.

2.10 Effect of pH, Temperature and Incubation Time on α-amylase Activity

Using the solid-state fermentation (SSF) the effect of pH on α-amylase activity in the different substrates (corn and wheat wasters) was investigated by adjusting the pH of basal salt solutions to 3.5, 4.0, 5.0, 6.0, 6.5, and 8.0. The substrates were then incubated for 5 days at room temperature. In another different experiment, the effect of temperature on α-amylase activity was examined using SSF in different substrates and incubated at 30, 40, 50, 60 and 70°C at pH 5.5 for 4 days. Also, the effect of the incubation period on α-amylase activity
was studied by evaluating the enzyme activity on 24, 48, 72, 96 and 120hrs of incubation period in the different solid substrates at pH 5.5 and room temperature.

2.11 Determination of Specific Activity

The specific activity of an enzyme gives the measurement of the activity of the enzyme (expressed in units/mg).

\[
\text{Specific activity} = \frac{\text{Enzyme activity} (\text{Unit/ml})}{\text{Protein Concentration} (\text{mg/ml})}
\]

2.12 Optimization of pH, Temperature and Incubation Period on Amylase Activity

The optimum pH value, temperature and incubation period for α-amylase activity on the corn and wheat wastes by isolated Aspergillus niger under solid-state fermentation were studied. For the pH value, 0.1 M acetate buffer was used for the pH range of 3.0-5.5 while 0.1 M phosphate buffer was used for the pH range of 6.0-8.0. For the determination of optimum temperature, the reaction mixtures were incubated at the various temperatures of 30-70°C at constant pH and incubation time [17].

2.13 Statistical Analysis

The experiment was carried out with at least triplicate where necessary. The data obtained were analyzed using Design expert (6.0.6 software). The analysis of variance (ANOVA) and regression analysis was performed on the data obtained. The results obtained from the central composite design (CCD) were used to fit a second-order polynomial equation.

\[
Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC \quad (1)
\]

Where \( Y \) = Predicted α-amylase response

\( \beta_0, \beta_1, \beta_2, \beta_3 \) = intercept; \( \beta_{11}, \beta_{22}, \beta_{33} \) = coefficient of linear effect; \( \beta_{12}, \beta_{13}, \beta_{23} \) = coefficient of variable squared effects; \( \beta_{11}, \beta_{22}, \beta_{33} \) = coefficient interaction effect of variables and A, B, C with the corresponding effects of variable A, B, C respectively. Fischer’s test was used to determine the significance variable, and the coefficient of determining of the \( R^2 \) value was used to explain the proportion of variance by the model.

3. RESULTS

3.1 Isolation, Identification and Screening

The culture of Aspergillus sp. was isolated from abattoir wastewater effluent. Lactophenol cotton blue stain of the culture was observed. And the culture-confirmed as Aspergillus niger. The culture was tested for starch hydrolysis. When starch agar medium was inoculated with the organism and subsequently flooded with iodine solution, the zone of clearance around the microbial growth indicated the production of α-amylase. Aspergillus niger a higher greater area of clearance was selected for further studies on amylase activity (data not shown).

3.2 Effect of pH

The result in Fig. 1 shows the effect of pH on α-amylase activity produced by Aspergillus niger. The activity of α-amylase produced at pH 6.5 was the highest in both the corn and wheat SSF with 82.45 Unit/mL and 119.36 Unit/mL, respectively.

3.3 Effect of Temperature

Fig. 2 shows the effect of different temperature on α-amylase activity in corn and wheat residue using Aspergillus niger. α-amylase produced shows to be thermostable. The optimum temperature for α-amylase activity in both the substrate was 50°C with the corresponding amylase activity of 163.71 unit/mL and 144.48 unit/mL of corn and wheat residues.

3.4 Effect of Incubation Period

Fig. 3 shows the effect of incubation time on α-amylase activity in corn and wheat residue using Aspergillus niger. A high yield of α-amylase activity in corn and wheat wastes were noticed after 4 days of incubation (amylase activities from corn and wheat wastes are 90.61 Unit/mL and 87.34 Unit/mL, respectively). The production of amylose by Aspergillus niger under solid-state fermentation in both the residues of corn and wheat increases with time while the highest activity was recorded after 4 days of incubation.

3.5 Interaction Effect of pH and Temperatures (AB); pH and Incubation Period (AC), and Temperature and Incubation period (BC)

In Fig. 4 the relationship effects between temperature and pH while the incubation time
was kept at the centre level of 10 ml solution of the fermentation media. Low and high-temperature conditions did not produce higher α-amylase activity. While the higher amount of α-amylase activity was recorded between 45-50.50°C. The pH 5-7 showed a slight positive effect in the production of α-amylase activity. Fig. 5 shows the interaction between an incubation time and initial pH while the temperature was held at the centre level 10 ml solution of the fermentation media. The highest activity was recorded in the middle levels (90-108 h) of the incubation period at pH (5.8-6.5). Fig. 6 shows the interaction between an incubation time and temperature, while pH was kept at the centre level 10 ml solution of the fermentation media. The highest activity was recorded in the middle levels of both factors, that is, 90-108 h and 45-50.50°C for incubation time and temperature, respectively.

Fig. 1. Effect of pH on α-amylase activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes

![Fig. 1. Effect of pH on α-amylase activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes](image1)

Fig. 2. Effect of Temperature on α-amylase activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes

![Fig. 2. Effect of Temperature on α-amylase activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes](image2)
Fig. 3. Effect of pH on α-amylase activity produced by Aspergillus niger using solid-state fermentation of corn and wheat wastes

![Graph showing effect of pH on α-amylase activity.]

Fig. 4. Three dimensional (3D) presentation of the effect of temperature and pH on α-amylase activity produced by Aspergillus niger using solid-state fermentation of corn and wheat wastes

![3D graph showing the effect of temperature and pH on α-amylase activity.]

| Incubation period (hrs.) | α-amylase activity (Unit/ml) |
|-------------------------|------------------------------|
| 24                      | 2.40                         |
| 48                      | 4.15                         |
| 72                      | 5.20                         |
| 96                      | 6.45                         |
| 120                     | 7.20                         |

| Enzyme activity (Unit/mL) | Incubation period (hrs.) |
|--------------------------|--------------------------|
| Corn                     | Wheat                    |
| 12.60                    | 14.15                    |
| 14.30                    | 17.75                    |
| 16.45                    | 18.85                    |
| 20.00                    | 22.25                    |

A: pH
B: Temperature (°C)
Fig. 5. Three dimensional (3D) presentation of the pH and incubation period (AC) on α-amylose activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes when Temperature was kept at the centre level.

Fig. 6. Three dimensional (3D) presentation of the effect of temperature and incubation (BC) on α-amylose activity produced by *Aspergillus niger* using solid-state fermentation of corn and wheat wastes when pH was kept at the centre level.


4. DISCUSSION

In this study, solid-state fermentation (SSF) as a solid support system for the production of an important biomolecule such as α-amylase by Aspergillus niger has been examined. Studies revealed that Aspergillus japonicus [22,23,4], Aspergillus penicilloides [24] and Aspergillus oryzae [25] have shown potential in the production of α-amylase. In Nigeria, the use of isolates of fungi, for example, Aspergillus niger from local abattoir wastewater or amylase activity has not been fully investigated.

In this study, isolates of Aspergillus niger from the abattoir wastewater tested for the production of hydrolytic enzymes showed the highest zone of clearance of 23 mm. The selected isolates were inoculated into the substrate matrix of corn and wheat wastes for the production of α-amylase. Ohimain et al. [26] demonstrated the existence of amylase activity by Pseudomonas, Bacillus, Micrococcus, Candida, Aspergillus, Fumigatus, Penicillium, Mucur and Fusarium isolated from palm oil mill effluent. Also, amylase was produced by Fusarium species isolated from fermented mineral salt supplemented with 25% corn starch [27]. In another different study, cassava peels inoculated with bacterial isolates have shown to produce amylase [28]. Amylase production by microorganisms is caused by several factors among which are the availability of carbohydrates, nitrogen compounds and other minerals Day et al. [29]. Also, favourable surrounding conditions is important for proper growth of microbes and production of enzymes.

In this study, the pH, temperature, and incubation time were important factors that determine the production of amylase activity by Aspergillus niger using corn and wheat waste substrates. Amylase was produced from both corn and wheat substrates, although more amylase activity was recorded (49%) in corn substrates than that of a wheat substrate (44%). The difference in α-amylase activity produced from the two substrates could be due to the degree of complexity of the chemical substrate structure utilized by Aspergillus niger [30]. Substrate composition has been reported to significantly influence enzyme production and activity [31]. In this study, the pH with a higher amount of amylase activity produced was found to be 6.5 for corn and wheat substrates. Ali et al. [17], recorded a comparable result of pH 6.5 for the highest amylase activity from fungi. Okolo et al. [32] also observed similar values of pH 6.0 to 6.5 produced by Aspergillus niger.

In this study, the gradual increase in amylase activity correlates with the temperature regime of 30-50°C for corn and wheat wastes substrates. This suggests that enzymes production can attend a thermostable state [33]. In another similar study, higher enzyme activity corresponds to a temperature of 45°C [34]. The findings by Oyeleke et al. [15] was different where the lower temperature of 30°C produced high amylase activity by A. flavus and A. fumigatus strains. Although, it has been postulated that at lower temperature or more extreme temperature low enzyme activity may be recorded due to the inactivation or thermal denaturation of enzyme protein [35]. Oyeleke et al. [15] also reported that an increase in temperature led to a decrease in amylase activity.

Here in this study, a high yield of α-amylase activity produced by Aspergillus niger was observed in both corn and wheat waste after 4 days of incubation. But after 4 days of incubation, there was an instant decrease in the activity of α-amylase which might be due to the reduction of nutrient or buildup of the toxic end product or loss of total moisture or even change might have occurred due to temperature and pH of the fermentation medium. In this present study, pH 6.5 contributed to the highest α-amylase activity produced. While temperature 50°C showed higher produced activity of α-amylase for both corn and wheat waste.

Also in this study, the interaction effect of environmental conditions of the media such as pH, temperature and incubation time were evaluated and results presented using 3-dimensional graphs. The result shows that pH of 5-6.5, temperature of 45-52.50°C and incubation time 90-108 h produced a higher amount of amylase activities in both corn and wheat wastes. Furthermore, the values of these factors analyzed shows the optimum of each predicted optimized values of pH (6.25), temperature (49.53°C) and incubation period 104 h with a and predicted amylase activity of 17.95 U/mL. Among the different factors studied, temperature was the most significant parameter that influenced the production of amylase activity.

5. CONCLUSION

Aspergillus niger was isolated and characterized from an abattoir effluent, and use for the
production of the α-amylase activity in a solid-state fermentation process of corn and wheat wastes. Isolates of Aspergillus niger showed a zone clearance of 23 mm in diameter. While pH 6.5 has the highest α-amylase activity in corn and wheat wastes. Changed in the temperature to 50ºC produced a higher amount of α-amylase activity while 4 days of incubation time also produced higher α-amylase activity.

The relationship of the interaction effect of the factors (pH, temperature and incubation time) on the amylase production activity by Aspergillus niger in solid-state fermentation media, were evaluated and the result showed the optimal environmental conditions on 3-dimensional effect.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johnson FS, Obeng AK, Asirifi I. Amylase production by fungi isolated from cassava processing site. Journal of Microbiology and Biotechnology Research. 2014;4(4):23-30.
2. Saranraj P, Stella D. Fungal amylase—a review. International Journal Microbiology Resource. 2013;4(2):203-211.
3. Xu H, Sun L, Zhao D, Zhang B, Shi Y, Wu Y. Production of α-amylase by Aspergillus oryzae As 3951 in solid-state fermentation using spent brewing grains as substrate. Journal of the Science of Food and Agriculture. 2008;88(3):529-535.
4. Krishna PR, Srivastava AK, Ramaswamy NK, Suprasanna P, D’souza SF. Banana peel as a substrate for α-amylase production using Aspergillus niger NCIM 616 and process optimization. Indian Journal of Biotechnology. 2012;11(3):314-319.
5. Aiyer PV. Amylases and their applications. African Journal of Biotechnology. 2005;4(13):1525-1529.
6. Ramachandran S, Patel AK, Namoothiri KM, Francis F, Nagy V, Szakacs G, Pandey A. Coconut oil cake—a potential raw material for the production of α-amylase. Bioresource Technology. 2004;93(2):169-174.
7. Egwim E, Amanabo M, Yahaya A, & Bello M. Nigerian indigenous fermented foods: processes and prospects. Mycotoxin and Food Safety in Developing Countries. 2013;153.
8. Dzogbefia VP, Amoke E, Oldham JH, Ellis WO. Production and use of yeast pectolytic enzymes to aid pineapple juice extraction. Food Biotechnology. 2001;15(1):25-34.
9. Kwalia S, Dzogbefia VP, Ofosu IW. Optimization of amylase production by Aspergillus niger cultivated on yam peels in solid-state fermentation using response surface methodology. African Journal of Biochemistry Research. 2017;11(7):34-42.
10. Bhargav S, Panda BP, Ali M, Javed S. Solid-state fermentation: an overview. Chemical and Biochemical Engineering Quarterly. 2008;22(1):49-70.
11. Nyamful A, Moses E, Ankudey EG, Woode MY. Solid-State Fermentation of Aspergillus niger MENA1E and Rhizopus MENAC011A for glucoamylase production on agricultural residues. International Journal of Scientific and Research Publication. 2014;s4(6):5-8.
12. Sadh PK, Duhan S, Duhan JS. Agro-industrial wastes and their utilization using solid-state fermentation: A review. Bioresources and Bioprocessing. 2018;5(1):1-15.
13. Wante SP, Anoliefo GO. Impact of developmental needs of the people of Mubi-North local government area of Adamawa State, Nigeria on Environmental Sustainability; 2014.
14. Cheesbrough M. Medical laboratory manual for tropical countries. M. Cheesbrough, 14 Bevills Close, Doddington, Cambridge Shire, England. 1981;1:20-33.
15. Oyeleke SB, Auta SH, Egwim EC. Production and characterization of amylase produced by Bacillus megaterium isolated from a local yam peel dumpsite in Minna, Niger State. Journal of Microbiology and Antimicrobials. 2010;2(7):88-92.
16. Domsch KH, Gams W. Fungi from arable soils. Fungi from Arable Soils. 1970;222.
17. Alli AI, Ogbonna CIC, Rahman ATMF. Hydrolysis of certain Nigeria: Cereal starches using crude fundal amylase. Nigerian Journal of Biotechnology. 1998; 9(1):24-36.

18. Oyeleke SB, Ibrahim AD, Manga SB, Rabah AB, Auta H, Ladan F. Production of bacterial amylase by Bacillus species isolated from rice husk dumpsites in Sokoto metropolis, Nigeria. International Journal of Biological and Chemical Sciences. 2011;5(1).

19. Fossi BT, Tavea F, Ndouenkeu R. Production and partial characterization of a thermostable amylase from ascomycetes yeast strain isolated from starchy soils. African Journal of Biotechnology. 2005; 4(1):14-18.

20. Sindiri MK, Machavarapu M, Vangalapati M. Alfa-amylase production and purification using fermented orange peel in solid state fermentation by Aspergillus niger. Indian Journal Apply Resource, 2013;3(8):49-5.

21. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959;31(3): 426-428.

22. Adeajuwon AO, Oluduro AO, Agboola FK, Ajayi AA, Olutiola PO, Burkhardt BA, Segal SJ. Expression of α-amylase by a tropical strain of Aspergillus niger: Effect of carbon source of growth. Nature and Science. 2015;13(8):66-69.

23. Khan JA, Yadav SK. Production of alpha amylases by Aspergillus niger using cheaper substrates employing solid state fermentation. International Journal of Plant, Animal and Environmental Sciences. 2011;1(3):100-108.

24. Ali I, Akbar A, Anwar M, Prasongsuk S, Lotrakul P, Punnapayak H. Purification and characterization of a polyextremophilic α-amylase from an obligate halophilic Aspergillus penicilloides isolate and its potential for sewage with detergents. BioMed Research International; 2015.

25. Ichinose S, Tanaka M, Shintani T, Gomi K. Improved α-amylase production by Aspergillus oryzae after a double deletion of genes involved in carbon catabolite repression. Applied Microbiology and Biotechnology. 2014;98(1):335-343.

26. Ohimain El, Izah SC, Jenakumo N. Physicochemical and microbial screening of palm oil mill effluents for amylase production. Greener Journal of Biological and Chemical Sciences. 2013;3(8):307-318.

27. Nwagu TN, Okolo BN. Extracellular amylase production of a thermotolerant Fusarium sp: isolated from Eastern Nigerian soil. Brazilian Archives of Biology and Technology. 2011;54(4):649-658.

28. Brisibe EA, Bankong H. Biotechnological potential of alpha-amylase production by Bacillus subtilis using cassava peel powder as a substrate. Biotechnology International Journal. 2014;1201-1211.

29. Day N, Soni R, Soni SK. A novel thermostable alpha-amylase from thermophilic Bacillus sp. SN-1 and its application in the liquefaction of sorghum starch for ethanol fermentation. Asian Journal of Microbiology Biotechnology and Environmental Sciences. 2002;4(1):159-164.

30. Sodhi HK, Sharma K, Gupta JK, Soni SK. Production of a thermostable α-amylase from Bacillus sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochemistry. 2005; 40(2):525-534.

31. Ueda S, Saha BC. Behaviour of Endomycopsis fibuligera glucoamylase towards raw starch. Enzyme and Microbial Technology. 1983;5(3):196-198.

32. Okolo BN, Ire FS, Ezeogu LI, Anyanwu C, U, Odibo FJC. Purification and some properties of a novel raw starch-digesting amylase from Aspergillus carbonarius. Journal of the Science of Food and Agriculture. 2001;81(3):329-336.

33. Goyal N, Gupta JK, Soni SK. A novel raw starch digesting thermostable α-amylase from Bacillus sp. I-3 and its use in the direct hydrolysis of raw potato starch. Enzyme and Microbial Technology. 2005; 37(7):723-734.

34. Hashemi M, Mousavi SM, Razavi SH, Shojaosadati SA. Comparison of submerged and solid state fermentation systems effects on the catalytic activity of Bacillus sp. KR-8104 α-amylase at different pH and temperatures. Industrial Crops and Products. 2013; 43:661-667.
35. Carninci P, Nishiyama Y, Westover A, Itoh, M, Nagaoka S, Sasaki N, Hayashizaki Y. Thermostabilization and thermoactivation of thermolabile enzymes by trehalose and its application for the synthesis of full-length cDNA. Proceedings of the National Academy of Sciences. 1998;95(2):520-524.

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