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Comparative study of amylase production from bacteria and fungi

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Abstract : *In the present study amylase was produced by Bacillus and Aspergillus utilizing potato extract in a submerged fermentation. The activity of the crude enzyme from potato was determined using Caraway-Somogyi iodine/ potassium iodide (IKI) method. The effects of varied pH and incubation time were also investigated. Amylase activity of the crude extract was measured by monitoring the amount of starch hydrolyzed by the crude extract over time. The results showed the presence of amylase activity in the extract, depicted by its ability to gradually decrease the concentration of the starch solution used as substrate. The optimum pH of the crude enzyme obtained from fungus was at about 6 and that for bacteria was 7. The enzyme activity in case of fungus decreases as the pH rises form 6 to8, comparing to bacterial enzymatic activity it was found to increase with the increase in pH. This study demonstrated that the abundant potato could be exploited for production of amylase.*

Key Words: *Amylase, Bacillus, Aspergillus, fermentation, submerged, potato extract*

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Introduction :

Enzymes are substances present in the cells of living organisms in minute amounts and are capable of speeding up chemical reactions (associated with life processes), without themselves being altered after the reaction. Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Pandey et al., 2000). Fungal sources are confined to terrestrial isolates, viz. *Aspergillus sp., Rhizopus sp.* and to only one species of *Penicillium*, *P.*

brunneum (Haska and Ohta, 1994; Pandey et al., 2000). Many strains used in food industry are isolated from fermented food media. Industrial conversion of starch with raw starch saccharifying amylase has been reported to represent an economically superior alternative to the conventional process which uses regelatinised starch as substrate based on energy utilization and process simplicity. Only few micro-organism including *Aspergillus* species have been reported to posses ability to produce raw starch degrading amylase. Members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment. There are various factors that influence the nature of their metabolic processes and enzymes produced (Ajavi and Fagade, 2006). A great deal of attention is being given to thermophilic and extremely thermophilic microorganisms and their enzymes (Ajavi and Fagade 2006). *Bacillus* species produce a large variety of extra cellular enzymes, such as amylases, which have significant industrial importance (Cordeiro et al., 2003). In the same vein, bacterial enzymes are known to posses more thermostability than fungal amylases (Eke and Oguntimehin, 1992). Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile to paper industries (Lin et al., 1997; Pandey et al., 2000). The amylases can be derived from several sources such as plants, animals and microbes. The microbial amylases meet industrial demands; a large number of them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry (Pandey et al., 2000). The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics (Lonsane and Ramesh, 1990).

Materials and Methods :

Soil samples : 10gm of soil samples collected from Patna women's college campus and was used for the isolation of bacteria.

Water samples : Stagnant water from Patna women's college campus was chosen as the source for the isolation of fungus. About 10ml of the stagnant water sample was collected in vial & was taken to the laboratory for analysis.

Preparation of media : Media were prepared according to the standard methods. Potato dextrose agar medium was prepared for the isolation of fungus & Nutrient agar supplemented with 1% starch was prepared for the isolation of bacteria.

Isolation and Identification of *Aspergillus* isolates : One ml (1ml) of the pond water was vortexed into 9ml of sterile saline water. Serial dilution was prepared upto 10^{-6} dilution & 0.1ml of the dilution was added, using the pour plate method to the potato dextrose agar medium. Identification was based on morphological characteristics of the culture in petri plates. Prepared samples were observed under the microscope & identified (Aneja 2004).

Isolation and Identification of *Bacillus* isolates : 0.1g of the soil sample was weighed into 9.9ml of saline. Serial dilution upto 10^{-7} dilution was prepared and 0.1ml of the dilution was added using the pour plate method to the nutrient agar fortified with 1% starch. The agar plates were incubated at 37^{0}C for 24-48hrs. *Bacillus* isolates, based on colony characteristics were purified by subculturing on fresh nutrient agar slants to obtain pure cultures.

The bacterial isolates were characterized and identified based on following morphological and biochemical tests, Gram staining, Catalase test, Sugar fermentation, Starch hydrolysis & Citrate utilization (Cheesbrough, 2003; Oyeleke & Manga, 2008).

Preparation of crude enzyme :

From Fungus : The medium composition for amylase production contained 50g/L boiled potato and 2.0g/L ammonium sulphate as nitrogen source, adjusted to the different pH 6, 7, and 8 sterilized by autoclaving. Fermentation was carried out in 500ml flasks with a working volume of 250ml at 30^0C in a rotator shaker at 100rpm for 72hrs. The fermentation was started with pure culture. Periodical analysis of the samples taken was carried out for amylase activity. Enzyme recovery from the fermentation broth was by filteration and the filtrate served as crude enzyme.

From Bacteria : The selected *Bacillus* isolate was inoculated in bacterial amylase production medium which contain 6g/L bacteriological peptone, 0.5g/L $MgSO_4.7H_2O$, 0.5g/L Kcl & 1g/L starch. The flask was incubated in shaker incubator, operated at 120rpm & at 30^0C. After the incubation period, the resultant broth was centrifuged at 10,000rpm for 15min & supernatant was collected as source of crude enzyme.

Test for amylase : With 1% starch solution & crude enzyme extract obtained from both bacteria and fungus at different pH were tested , iodine was used as indicator, the change in color from blue to purple indicated the presence for amylase.

Enzyme activity assay : Amylase activity of crude extracts was determined using Caraway-Somogyi iodine/potassium iodide (IKI) method. The first step in the assay was the gelatinization/ liquefaction of the soluble commercial starch used as the substrate. This was done by adding 40 ml of 1% soluble starch to 50 ml of gently boiling water in a beaker, while stirring. The gelatinized starch solution was allowed to cool to room temperature, after which the total volume was made up to 100 ml with distilled water. Next, 1.0 ml of the gelatinized starch solution was further diluted to 100 ml with distilled water. This was used as the stock solution (substrate) for the assay.

The activity of the partially purified amylase enzyme of both bacteria and fungus was examined using 5ml of soluble starch as substrate and 3 ml of 0.1M phosphate buffer pH5.6 was added. After incubation 3ml of 10% HCl was added in order to terminate the reaction then 3ml of working indicator (iodine-potassium iodide solution) was added and the absorbance of the reaction was measured on spectrophotometer at a wavelength of 620nm. This was taken to be 0 h incubation time. The procedure was repeated from the termination step at 20 min interval for 60 min. The amount of starch hydrolyzed per unit time was estimated from a standard curve of starch (substrate) concentration against absorbance. Enzyme activity was defined as the amount of soluble starch hydrolyzed by 1ml of enzyme extract in 1min.

Effect of pH on enzyme activity : The effect of pH change on the amylase activity of the crude extract was studied using 0.1 M phosphate buffer solutions of pH ranging from 4.0 to 8.0 in increments of one pH unit.

Five Identical test tubes labeled 4-8 were set-up, with each test tubes representing each of the different pH values studied. About 5.0 ml of the starch stock solution was added to each of the test tubes followed by the addition of 3.0 ml of each of the different pH buffer solutions. About 1.5 ml of

the enzyme extract was added to each test tube to initiate hydrolysis. The reaction was allowed to continue for 15 min by incubating at 37^0C, after which 1.0 ml of the reaction mixture was transferred into another test tubes containing 3.0 ml of 10% HCL to terminate the reaction. About 3.0 ml of the working indicator (iodine Potassium iodide) was then added to 1.0 ml of the assay solution. Finally, the absorbance was read at 620nm. The procedure was repeated 3 times. The rate of starch hydrolysis by the crude enzyme was calculated and plotted against pH.

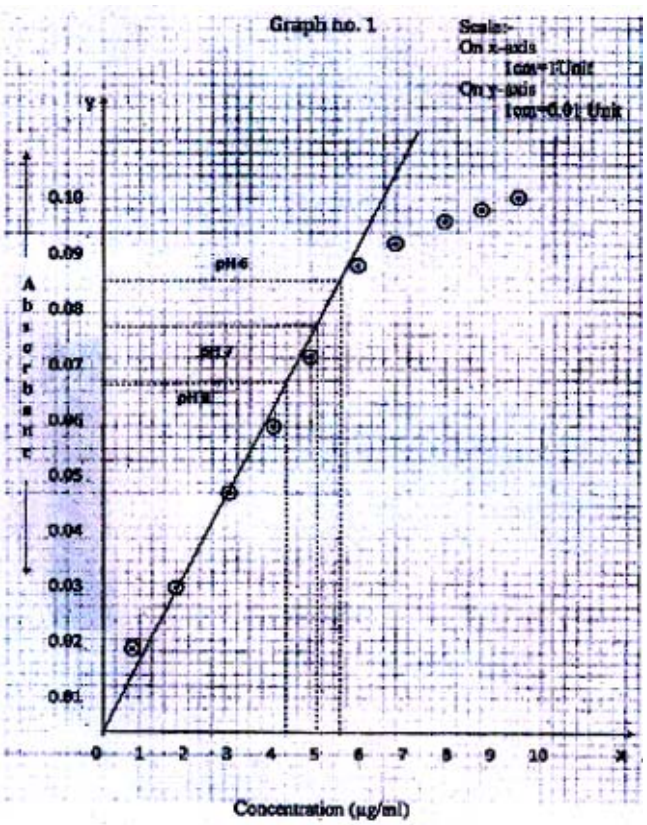
Results and Discussion :

Determination of Enzyme Activity : In order to determine the hydrolytic activity of crude extract from both bacteria and fungus the media for extraction was maintained at different pH i.e. 6, 7, 8. From the graph no.1, it was observed that at pH 6 the crude enzyme extracted from bacteria, the amount of starch hydrolysed was found to be 5.5μg/ml while at pH 7 amount of starch hydrolysed was found to be 5μg/ml & at pH 8 the amount of starch hydrolyzed was found to be 4.4μg/ml. Comparing to graph no.2 the crude enzyme extract obtained from fungus it was found that at pH 6 amount of starch hydrolysed was 5μg/ml, at pH 7 it is 5.6μg/ml & at pH 8 it 6.1μg/ml.

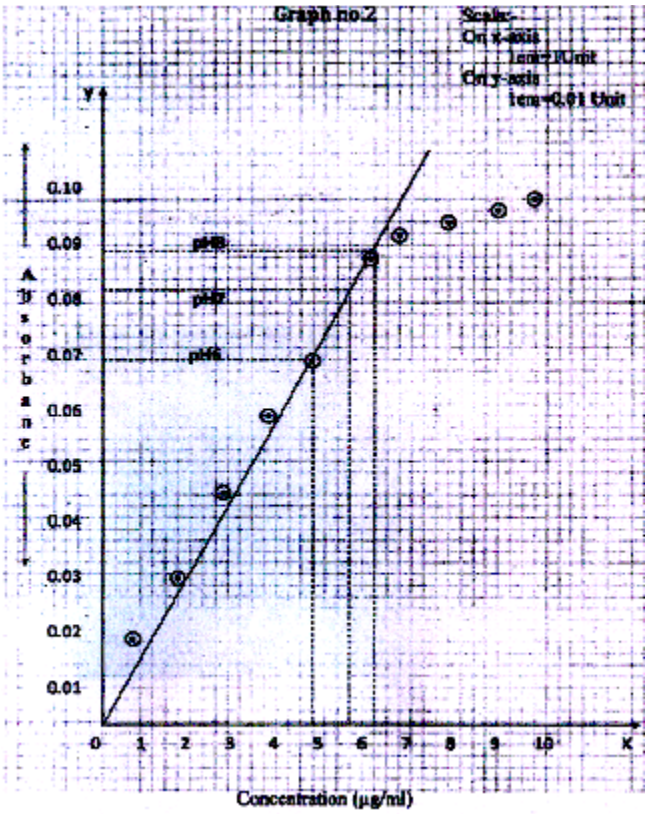
From the successive graphs it has been concluded that the enzyme activity of amylase obtained from bacteria increases as the pH raises from 6 to 8.while the enzymatic activity of amylase obtained from fungus decrease with the increase in pH from 6 to 8. So, the enzyme activity of amylase seems to be best at pH 6 in case of fungus while in case of bacteria it seems to be best at pH 7.

Effect of pH Variation : The effect of varied pH on the amylase activity of the crude extract is presented in the graph no.6 & 7. A narrow pH range (4.0, 5.0, 6.0, 7.0, & 8.0) was chosen for this study, because it has been reported that amylase act well within range (Tindall, 1996). The result shows that amylase activity of bacteria increases rapidly with rise in pH until it reaches its optimum at about pH 7 beyond this the enzymatic activity slows down as shown in graph no.6. while the amylase activity of fungus increases progressively from 4 to 6, then very rapidly its enzymatic activity begins to slow down as shown in graph no.7. This is a basic property of all enzymes and is probably due to concomitant alteration in the conformation of the enzyme protein caused by changes in pH of its environment.

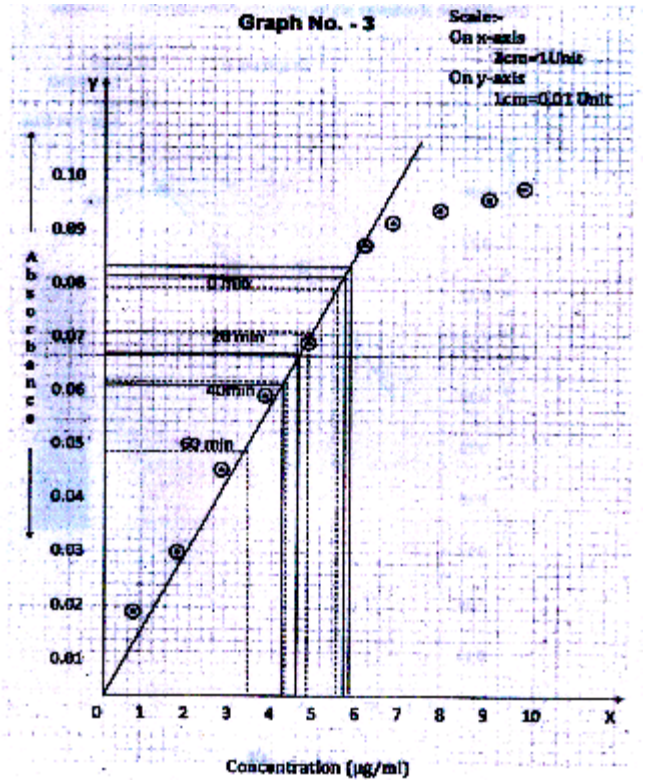
Effect of Time variation : The result of the hydrolytic ability of the crude extract from potato was monitored over 60 min period at 20 min intervals is presented in graph 5. This result shows that the concentration of the substrate decreased with time reducing from about 6.1 μg/ml to 4.0 μg/ml in 60 min in case of fungus. On the other hand in case of bacteria the concentration of substrate reduced from 6.0μg/ml to 3.0μg/ml. This shows that the crude extract in case of bacteria is better in comparison to that of fungus as it is more proficient in reducing the substrate concentration showing high enzymatic activity in given time than in case of fungus, instead of allowing the potato to waste, they could be harnessed for amylase production. This information is important for industrialists, who may be looking for a cheap source of the enzyme.



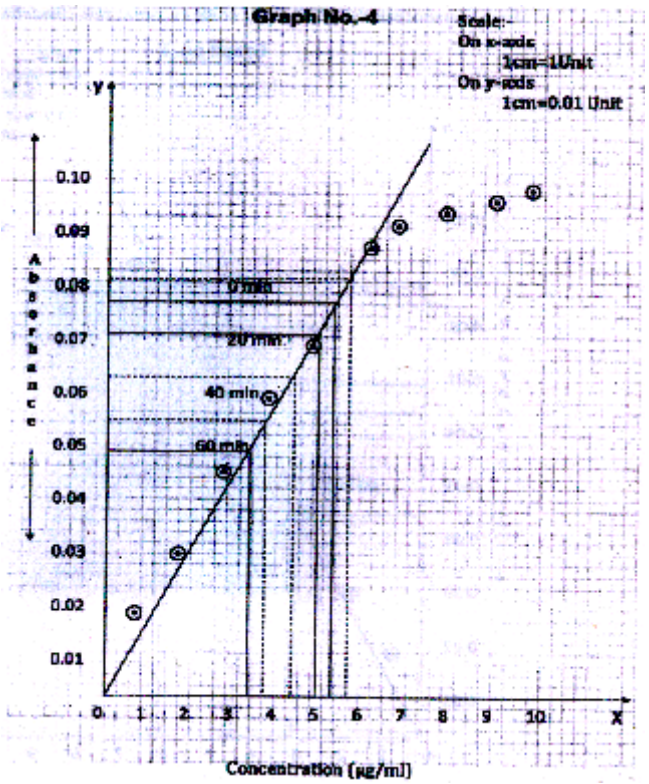
Determination of enzyme activity at different pH (Bacteria)



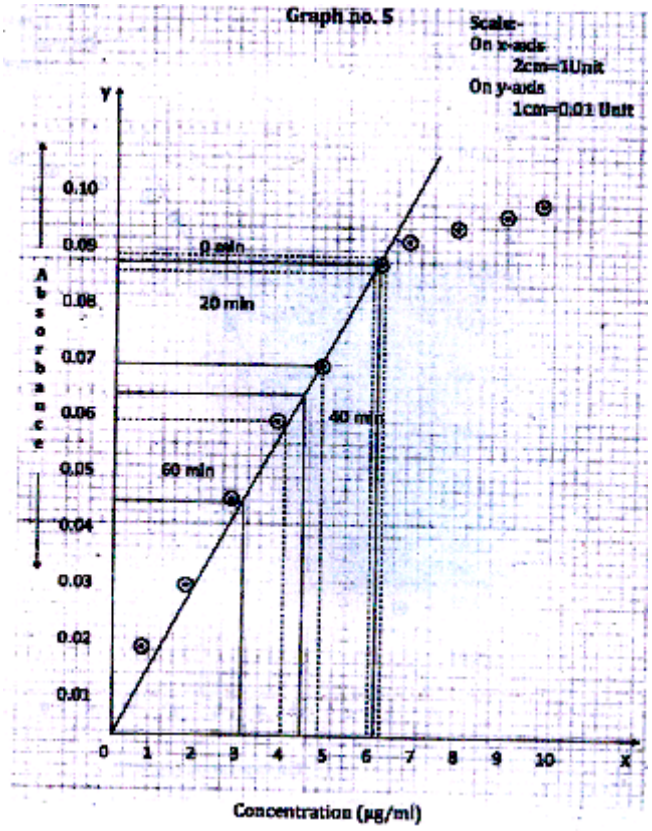
Determination of enzyme activity at different pH (Fungus)



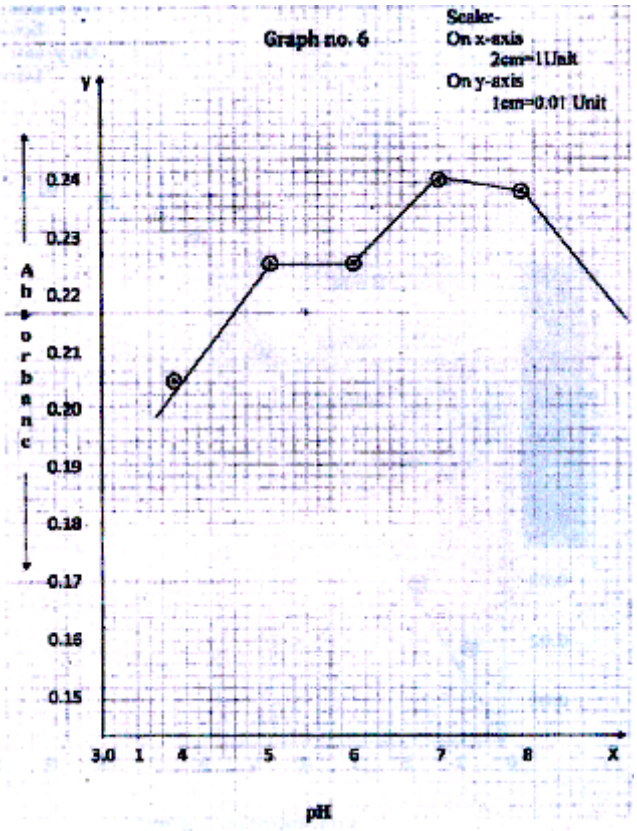
Time course of starch hydrolysis by crude extract at pH6 (Bacteria and Fungus)



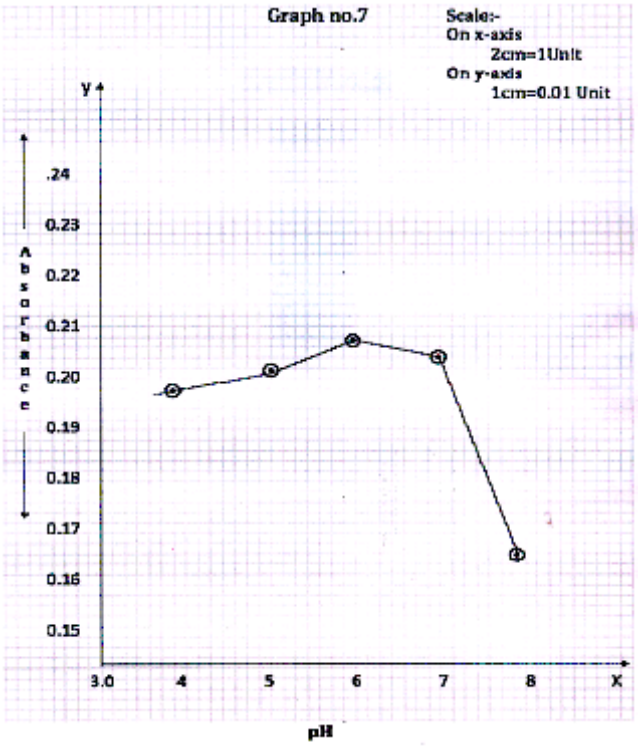
Time course of starch hydrolysis by crude extract at pH7 (Bacteria and Fungus)



Time course of starch hydrolysis by crude extract at pH8 (Bacteria and Fungus)



Determination of optimum pH for maximum enzymatic activity (Bacteria)



Determination of optimum pH for maximum enzymatic activity (Fungus)

Conclusion :

This study has demonstrated that bacteria and fungus are efficient in amylase production. In addition to its ability to hydrolyze gelatinized starch, the crude extract exhibited the pattern of variation in enzyme activity at different pH values and different incubation time common to typical enzymes. The studies show that bacterial amylase shows its maximum activity at pH 7 and fungal amylase at pH 6. The effect of incubation time is also shown that is, the bacterial amylase is more efficient in comparison to the fungal amylase as it is able to reduce the substrate concentration to a greater extent showing more enhanced enzymatic activity. The use of amylolytic microorganisms in the production of enzyme on large scale has

obvious advantages, which include the fast rate of multiplication, diversity of enzyme present and possibility of genetic manipulation. The economics of enzyme production using inexpensive raw materials (mashed maize, agricultural waste, kitchen waste etc) can make an industrial enzyme process competitive. Thus, the potato could be converted to wealth by developing a standard method of producing amylase and other useful enzymes that may be present in it. This would provide income and employment opportunities.

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