ABSTRACT
Amylases are enzymes which hydrolyze the starch molecules into polymers composed of glucose units. Amylases are among the most important enzymes and are of great significance for biotechnology. They can be obtained from several sources, such as plants, animals and microorganisms. Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile paper, detergent, and pharmaceutical industries. The effect of temperature on amylase activity A. flavus H4 was studied by varying the temperature from 20°C to 40°C. It is clear from the results that a temperature of 28°C was found to be best suitable for amylase activity and maximum activity found was 8.7U/ml, it was observed that at 20°C enzyme activities were low 4.5 U/ml and showed a gradual increase with the increase in temperature to 28°C. Further increase in temperature resulted in decrease in production of amylase that at 42°C enzyme activities were 4.5U/ml. The effect of pH on amylase activity of A. flavus was studied by varying pH from 4.5 to 7.5, Under experimental conditions, a maximum enzyme activity was produced at pH 5.5, 7.1U/ml and the lowest at pH 7.5, 5.2U/ml. The cultures were incubated for 2 - 8 days, The enzyme was extracted and the specific activity of the amylase produced at different days of incubation was 4.1 U/ml in 2 days of incubation and The specific activity was 7.2 U/ml for the enzyme at 6 days of incubation, while The specific activity was 6.8 U/ml for the enzyme at 8 days of incubation.

KEYWORDS: enzyme, A. flavus, pH, Soil.

INTRODUCTION
Amylases are enzymes which hydrolyze the starch molecules into polymers composed of glucose units. Amylases are among the most important enzymes and are of great significance for biotechnology. They can be obtained from several sources, such as plants, animals and microorganisms.
microorganisms. The amylases of microorganisms have a broad spectrum of industrial applications as they are more stable than when prepared with plant and animal amylases, amylase in industry amylase has been derived from several fungi, yeasts and bacteria. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors.

Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile paper, detergent, and pharmaceutical industries. Fungal sources are confined to terrestrial isolates, mostly Aspergillus and Penicillium. The Aspergillus species produce a large variety of extracellular enzymes and amylases are the ones with most significant industrial importance. Filamentous fungi, such as Aspergillus oryzae and Aspergillus, produce considerable quantities of enzymes that are used extensively in the industry. Aspergillus has important hydrolytic capacities in amylase production and, due to its tolerance of acidity pH it allows the avoidance of bacterial contamination. yield of enzyme has always been a problem in the commercial production of amylases. Moreover, thermal stability is a desirable feature for economic viability of enzymatic processes, Therefore, the present work was undertaken to screen various Aspergillus isolates for α amylase production, optimization of fermentation conditions for maximum yield and to stabilize the enzyme in liquid state us in various additives.

MATERIALS AND METHODS

Isolation the Fungi from Soil
Fungal colonies were isolated form soil samples by serial dilution method, 50µl of soil samples diluted up to five dilutions were spread on respective solidified Potato Dextrose Agar plates. The inoculated Petri plates were incubated at 28°C for 2 days. 12 Aspergillus flavus different isolates differentiated on the basis of physical characteristics obtained after incubation were named as H1,H2, H3……….. and H12. The isolates were further inoculated on sterile PDA plates by point inoculation and incubated at 28°C for 2 days in order to obtain pure fungal plates.

Screening of Fungal Isolates for Amylase Production
12 Aspergillus flavus isolates were screened for amylase production efficiency in starch agar media comprising the following in gm/1 L, yeast extract 1.5, peptone 0.5, sodium chloride 1.5, starch 10, agar 15 pH 5. All the isolates were streaked centrally on sterile solidified starch agar plates, a blank without inoculation was also maintained for comparison. Plates
were incubated at 28°C for 2 days after that all the plates along with blank were flooded with iodine and observed for zone of hydrolysis, after that we measure the zone of hydrolysis (mm).

**Enzyme Production**

Enzyme production was done by submerged fermentation, *A. flavus* was inoculated in potato dextrose broth and incubated at room temperature for 4 days. The medium was then centrifuged at 1000 rpm for 5 min and the supernatant was collected for separating extracellular enzyme amylase. The protein content and enzyme activity were recorded.

**Extraction of Crude Enzyme**

Crude enzyme was extracted by adding 100ml of 100mM Tris buffer agitating the flask in shaker at 180 rpm for one hour, the mixture was filtered through cheese cloth and centrifuged at 1000 rpm for 5 min. The supernatant was collected and treated as crude enzyme.

**Protein Estimation in Crude Enzyme**

Protein in crude enzyme was determined by Lowry’s method\textsuperscript{[10]} of protein estimation in which enzyme was reacted with the Lowry’s reagents and the absorbance obtained was compared with a standard graph plotted by reacting a standard protein with known concentrations with the Lowry’s reagents and plotting a graph between concentration of standard protein and absorbance at 660nm.

**Enzyme assay in Crude Enzyme**

Enzyme assay was carried out by DNS method of\textsuperscript{[11]}, 0.5ml enzyme was reacted with 0.5ml of substrate (1% starch in 100mM Tris buffer) under standard reaction conditions, the reaction was stopped by adding 3,5-Dinitrosalicylic acid (DNS) reagent, amount of maltose released was determined by comparing the absorbance reading of the test enzyme at 540 nm with the standard graph plotted by reacting the known concentration of maltose ranging from 0.05mg/ml to 0.5mg/ml.

**Effect of different condition on production of amylase**

1-**Effect of temperature**

The effect of temperature on amylase production was investigated by incubating the fermentation medium at (20°C, 28°C, 35°C, 40°C) at pH 7 for 7 days.
2-Effect of pH
The effect of pH on amylase production was investigated by incubating the fermentation medium at different pH, the pH of basal salt solutions to 4.5, 5.5, 6.5, 7.5, then incubated for 7 days at 25°C.

3-Effect of incubation time
The effect of incubation time on amylase production was investigated by incubating the fermentation medium at different time 2, 4, 6, 8 days of at pH 7 and at 25°C.

RESULTS AND DISCUSSION
Screening of A. flavus isolates for Amylase Production
12 A. flavus isolates differentiated on the basis of colony morphology were obtained after spreading, and were named tentatively as H1, H2, H3……and 128. All the isolates were subcultures by point inoculation and used for further studies All the isolates were subjected to screening procedure and after completion of incubation period plates were flooded with iodine solution and observed for zone of hydrolysis. The results of the same can be seen in Table 1. A. flavus H4 was found to be the best amylase producer and hydrolysis zone of amylase produced by this strain was 7.5 mm in solid state fermentation. So, this potential strain was selected for further optimization of culture conditions. The results of this investigation showed that A. flavus grew on a medium containing starch as sole carbon source producing amylase, capable of degrading glucosidic bonds of starch, hydrolytically Cultural conditions have an influence on enzyme production. Similar values of enzyme production, for Aspergillus isolates JGI24 and GCB – 34 have been reported.

Table 1: Screening for Amylase Production

<table>
<thead>
<tr>
<th>Ser.</th>
<th>Region name</th>
<th>Hydrolysis zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baghdad H1</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>Hilla H2</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>Mysan H3</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>Karbala H4</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>Najaf H5</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>Basra H6</td>
<td>6.4</td>
</tr>
<tr>
<td>7</td>
<td>Dhuk H7</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>Arbeel H8</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>Kut H9</td>
<td>6.1</td>
</tr>
<tr>
<td>10</td>
<td>Naseria H10</td>
<td>7.3</td>
</tr>
<tr>
<td>11</td>
<td>Dayalla H11</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>Dywania H12</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Effect of Different Condition on amylase activity

1- Effect of temperature

*A. flavus* inoculated at different temperature seen from the table 2, that 28°C is the temperature at which maximum production of amylase. Incubation temperature not only influences the growth of microorganisms but also their biological activities. The effect of temperature on amylase activity *A. flavus* H4 was studied by varying the temperature from 20°C to 40°C and the results have been depicted in the table 2. It is clear from the results that a temperature of 28°C was found to be best suitable for amylase activity and maximum activity found was 8.7 U/ml, it was observed that at 20°C enzyme activities were low 4.5 U/ml and showed a gradual increase with the increase in temperature to 28°C. Further increase in temperature resulted in decrease in production of amylase that at 42°C enzyme activities were 4.5 U/ml.

**Table 2: Effect of Temperature**

<table>
<thead>
<tr>
<th>Ser.</th>
<th>Temperature (°C)</th>
<th>Specific activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>4.5</td>
</tr>
</tbody>
</table>

The influence of temperature on amylase activity of the crude enzyme showed that enzyme activity increased progressively with increase in temperature from 20°C reaching a maximum at 28, Above 35 C there was a reduction in the amylase activity, It is reported that best enzyme production in *A. flavus* at room temperature both in SmF and SSF and reported 30°C to be the best for enzyme production by *Penicillium fellutanum*[^14], However the optimum temperature for enzyme production was reported as 30C in many literatures.[^15][^16]

Temperature changes had an effect on amylase activity produced by *A. niger*. Optimum activity was at 35°C after which there was a decline in activity The rate of enzyme catalyzed reactions increase with temperature. This occurs only within the temperature range at which an enzyme is stable and retains full activity.[^17]

2- Effect of pH

*A. flavus* H4 isolate when inoculated at different pH seen from the table 3 below that pH 5.0 is the pH at which maximum production of enzyme was seen. The effect of pH on amylase activity of *A. flavus* was studied by varying pH from 4.5 to 7.5, The results are depicted in
table 3, which indicate that with increase in pH value from 3 to 5, the activities of amylase enzyme reached to the maximum followed by a gradual decrease thereafter.

It is clear that pH of 5.5 was found to be best for amylase activity and maximum activity recorded was 7.5 U/ml, amylase activity. Among the physical parameters, the pH of medium plays an important role by inducing in enzyme secretion. According to[3] The synthesis of extra-cellular α-amylase is affected by the pH,

**Table 3: Effect of pH**

<table>
<thead>
<tr>
<th>Ser.</th>
<th>pH</th>
<th>Specific activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>7.1</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Under experimental conditions, a maximum enzyme activity was produced at pH 5.5, 7.1U/ml and the lowest at pH 7.5, 5.2U/ml. Our findings are comparable to previous reports[16,18] with *Aspergillus* spp. at pH varying between 5 and 6. In contrast[19] reported pH 3.5 and pH 4.0 to be the best for the production of α-amylase by *B. amylolique faciens* and *A. awamori*.

Low and high pH values can also causes considerable de naturation and hence inactivation of the enzyme.[17], It is reported that amylase production is high at pH 5[15], and Amylase production in *A. ochraceus* and *A. niger* UO 1 was optimum at pH 5. [20] Maximum amylolytic activity of thermophilic fungi *Aspergillus fumigates* isolated from soil was observed pH 5 in mineral.[21]

3- Effect of incubation pored

*A. flavus* H4 isolate when inoculated at different incubation pored it can be seen from the table 4, Incubation period plays an important role in enzyme production. The effect of incubation period was evaluated by checking enzyme activities.

**Table 4: Effect of incubation pored**

<table>
<thead>
<tr>
<th>Ser.</th>
<th>incubation pored</th>
<th>(Specific activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6.1</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>6.8</td>
</tr>
</tbody>
</table>
The cultures were incubated for 2 - 8 days, The enzyme was extracted and the specific activity of the amylase produced at different days of incubation was 4.1 U/ml in 2 days of incubation and The specific activity was 7.2 U/ml for the enzyme at 6 days of incubation, while The specific activity was 6.8 U/ml for the enzyme at 8 days of incubation. The incubation period varies with production of enzyme and Short incubation period offers potential for inexpensive production of enzyme.[22] Similar results were reported earlier that amylase activity was produced after two days of cultivation.[23] The maximum amylase activity was recorded during the period of fungus autolysis and reported the maximum production of amylase enzyme at five days of incubation period[15], A. niger amylase increased with increase in days of incubation, α-amylase was recorded as 450 U/mg after 7 days of submerged fermentation.[24]

REFERENCES


16. Alva, S; Anupama, J; Salva, J; Chiu, Y. Y; Vyshali, P; Shruthi, M; Ogeetha, B.S; Bhavya., D; Purvi, J; Ruchi, K; Kumudini, B.S. and Varalakshmi, K.N. Production and characterization of fungal amylase enzyme isolated from Aspergillus sp. JGI 12 in solid state culture African journal of technology, 2007; 6: 576–581.


