

## **Optimization of Alkaline protease production by fungal isolate using solid state fermentation**

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**Abstract :** *The purpose of the present study focuses on production of extracellular alkaline proteases by a local fungal isolate through solid state fermentation. The influence of process parameters such as solid substrates, incubation time, carbon and nitrogen source, inducers, activators and inhibitors pH & temperature were studied. Wheat bran was found to be the best among the three solid substrates. The maximum protease production was seen at 120 h of incubation time. The protease production was increased when the medium was supplemented with Lactose as a carbon source. Among the different organic nitrogen sources investigated Yeast extract was found to produce maximum protease. Milk powder was found as the good inducer and EDTA was found as the protease inhibitor. The optimum pH & temperature was 10 and 40° C respectively.*

**Keywords:** *Alkaline proteases, Milk powder, pH, Solid state fermentation, Solid substrates*

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### **I. Introduction**

Proteolytic enzymes account for nearly 60% of the industrial market and find applications in the number of biotechnological processes and pharmaceutical industry, leather industry, detergent industry etc. Such enzymes may be of commercial significance and hence, exploited to assist protein degradation in various industrial processes [1] [2] [3] [4]. Alkaline proteases hold a great potential for application in the detergent and leather industry due to the increasing trend to develop environment friendly technologies. Recently [5] reviewed production of Proteolytic enzymes in SSF systems. From their viewpoint, proteases produced by SSF have greater economic feasibility. In recent years, there have been increasing attempts to produce different types of proteases (acid, neutral & alkaline) through SSF route, using agro-industrial residues. Alkaline proteases (E.C.3.4.21-24, 99) are defined as those proteases which are active in neutral to alkaline pH range. Alkaline proteases are generally produced by submerged fermentation. In addition solid state fermentation has been exploited to a lesser extent for production of these enzymes [2]. Hence our focus will be on solid state fermentation. Different type of optimization process is carried out to get the maximum enzyme production.

### **II. Materials & Methods**

**2.1 Isolation:** Being a solid-state fermentation we were interested in isolating fungal organism. Samples collected from marshy soil and washing pond were domestic waste water is let out. Soil sample collected were inoculated in potato dextrose broth (PDB), broth was serially diluted and plated on Potato Dextrose Agar (PDA). The plates were then incubated at 30° C for 96 hr, observed fungal isolates were then re-isolated and was maintained on fresh PDA plates, which were used for further screening process.

**2.2 Primary Screening:** The isolates selected for further analysis were subjected to screening for their ability to produce the enzyme of interest. This was done by, selecting an organism which is capable of producing Proteolytic enzyme on sterile PDA containing 2% skim milk as a protein source [6] [7] [8] . Visual observation for zone of clearance was used for detection of enzyme productivity by the isolates [9] [10] [11] [12] . Isolate which showed the maximum zone of clearance was used for further optimization study [13].

### **III. Solid State Fermentation**

The media used for SSF includes; Wheat Bran 6g [14] [15] [16], Soybean meal 2g [2] [17] , Wheat flour 1g [18, 6ml salt solution with 80% total moisture level (6ml Salt solution+2ml inoculum).

### **IV. Media Optimization**

To optimize the cultural conditions for maximum production of protease different parameters were studied, which includes; i) Effect of solid substrates, ii) Effect of incubation time, iii) Effect of carbon sources iv) Effect of nitrogen sources v) Effect of inducers vi) Effect of inhibitors and activators vii) Effect of pH & viii) Effect of temperature.

#### **4.1 Effect of solid substrates**

Solid substrates like Wheat bran, rice bran and rice husk were checked for the maximum production of alkaline protease as they are easily available at low cost. The best substrate which showed promising activity was selected. The selection of substrates for enzyme production in a SSF process depends upon several factors, mainly related to cost and availability of the substrate [19]

#### **4.2 Effect of incubation time**

Incubation time study was carried out to get maximum enzyme production (2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> & 6<sup>th</sup> day)

#### **4.3 Effect of carbon sources**

Various carbon sources were investigated for their effect on enzyme production. The carbon sources tested were fructose, dextrose, sucrose, lactose & maltose. After completion of the incubation period, alkaline protease assay was carried out to determine the best carbon source for optimal enzyme production.

#### **4.4 Effect of nitrogen sources**

Alkaline protease production was investigated using different nitrogen sources like Yeast extract, peptone, beef extract soybean meal, one which gave the maximum enzyme production were used.

#### **4.5 Effect of inducers**

Various inducers were used to enhance enzyme production. Inducers used were highly proteinaceous, which includes Milk powder, Whey powder, & Albumin powder.

#### **4.6 Effect of Activators and Inhibitors**

The effect of various Metal ions and chelators on enzyme production was checked. The compounds which increase the enzyme production, better known as activators, were added, whereas which negatively affected enzyme production was considered as inhibitors. Other inhibitors like EDTA (Ethylenediamine-tetraacetic acid), AgNO<sub>3</sub> & SDS (Sodium Dodecyl sulphate) were also checked.

#### **4.7 Effect of pH**

The effect of pH was studied ranging from pH 6.0 - pH 12 to find out the optimum pH, at which the enzyme is highly active. It was noted that, enzyme activity was in the increasing order from pH 6.0 to pH 10 and showed the maximum activity at pH 10 and activity declined marginally at pH 11 and pH 12.

#### **4.8 Effect of temperature**

The enzyme activity was studied at different temperatures from 20<sup>o</sup> C - 80<sup>o</sup> C. The study determined which temperature helped to attain higher enzyme activity. The maximum activity was observed at 40<sup>o</sup> C and there was a slight drop in activity at 50<sup>o</sup> C and activity dropped drastically at 60<sup>o</sup> C, 70<sup>o</sup> C & 80<sup>o</sup> C.

### **V. Results And Discussion**

#### **5.1 Effect of solid substrates**

Different substrates used to get maximum enzyme yield, like wheat bran, rice husk & rice bran were studied, of the three solid substrates wheat bran gave good results as compared to rice bran and rice husk. (Fig. 1) [20] also got good results using Wheat Bran as the solid substrate. [21] also reported wheat bran as the good substrate.

#### **5.2 Effect of Incubation time**

Incubation time study was carried out by analyzing enzyme activity at 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> & 6<sup>th</sup> day to determine the optimum enzyme production. Maximum enzyme production was seen on 5<sup>th</sup> Day (i.e. 120h) (Fig. 2).

#### **5.3 Effect of carbon sources**

Different carbon sources were investigated to get the maximum enzyme production. Alkaline protease production was enhanced with 0.5% lactose as a carbon source. Other carbon sources failed to give promising results as compared to lactose (Fig.3). Increased yield of alkaline protease production were reported by several other researchers using different sugars such as Fructose, Dextrose, Sucrose, Lactose, Maltose. [22]

#### **5.4 Effect of Nitrogen sources**

Different nitrogen sources were screened, to check the maximum alkaline protease production and it was found that yeast extract and soyabean meal showed promising enzyme activity of all other nitrogen sources (Fig.4).

Similar observation was reported by [23]

#### **5.5 Effect of Inducers**

Highly proteinaceous inducers like Milk powder, Whey powder and Albumen powder were used to enhance the protease production, milk powder was found to be the excellent inducer (Fig. 5)

#### **5.6 Effect of Activators & Inhibitors**

Various Metal ions and chelators were used to check the effect on enzyme production. Ca<sup>2+</sup> enhanced the enzyme activity while EDTA acted as an inhibitor

[24] also reported protease inhibition using 1mM EDTA concentration.

#### **5.7 Effect of pH & Temperature**

The effect of pH & temperature was studied ranging from pH 6.0- pH 12 & 20<sup>o</sup>C - 80<sup>o</sup>C respectively to find out the optimum pH & temperature, at which the enzyme is highly active. It was noted that, enzyme activity was in the increasing order from pH 6.0 to pH 10 and showed the maximum activity at pH 10 (265 u/ml) and activity

declined marginally at pH 11 and pH 12 as shown in (Fig. 6). As shown in (Fig. 7), It was found that enzyme activity increased according to the temperature, showed maximum activity at 40°C (268 u/ml), there was decrease in activity seen at 50°C, drastic drop in enzyme activity was seen at 60°C 70°C & 80°C. Effect of pH on Proteolytic activity was studied by [25] and it was noted that there was a drastic drop in activity at pH 11 and pH 12 instead of being isolated from alkaline habitat and 55°C as the optimum temperature. [26] also noted drop in enzyme activity as the pH increases. [7] noted 140 u/ml of the activity at pH 9 and there was drastic drop in the activity at pH more than 9. Our enzyme is more alkali tolerant, as there is a marginal drop in the activity even at pH 11 and pH 12. Protease activity at 40°C was also reported by [27]. Fungal proteases are usually thermo labile and show reduced activities at high temperatures [27]

## VI. Figures & Tables

Fig. 1

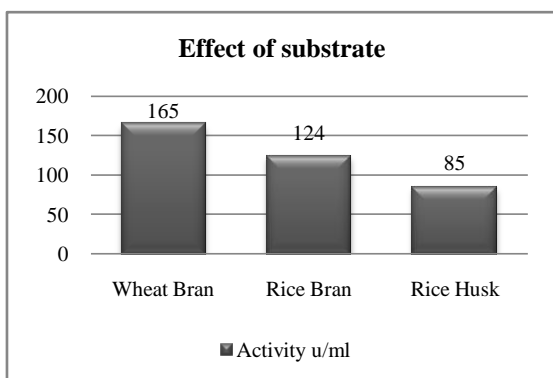


Fig. 2

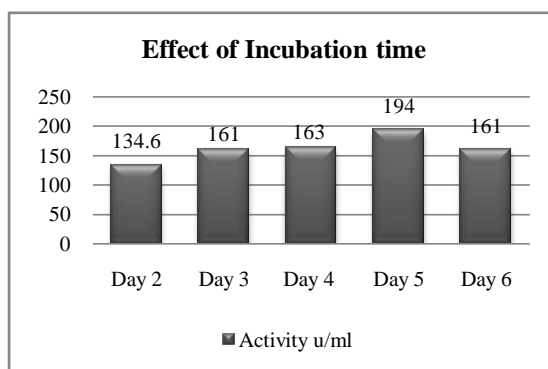


Fig.3

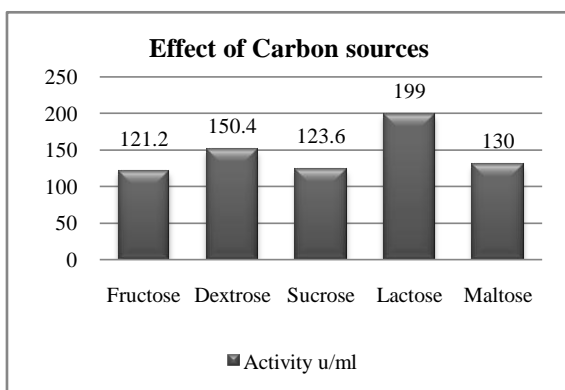


Fig.4

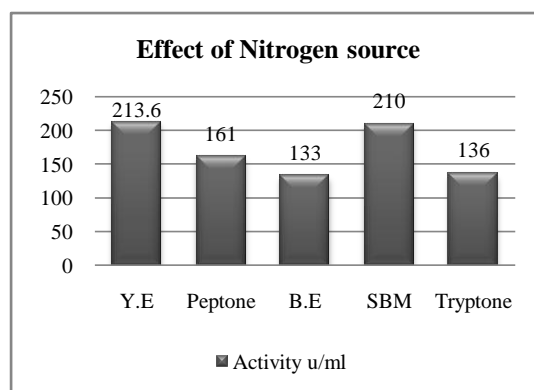


Fig. 5

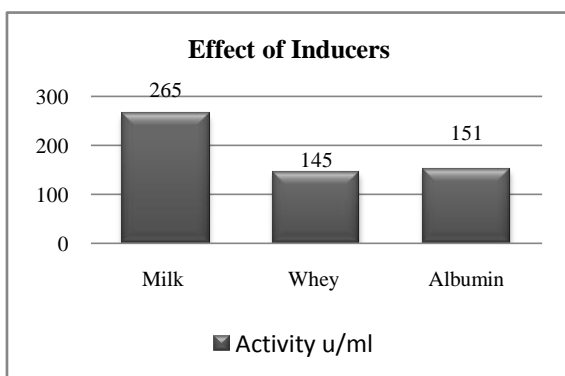
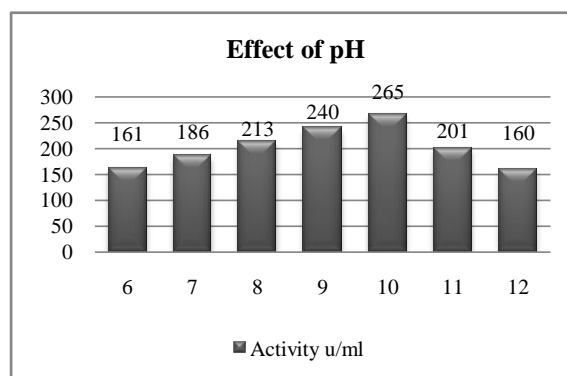
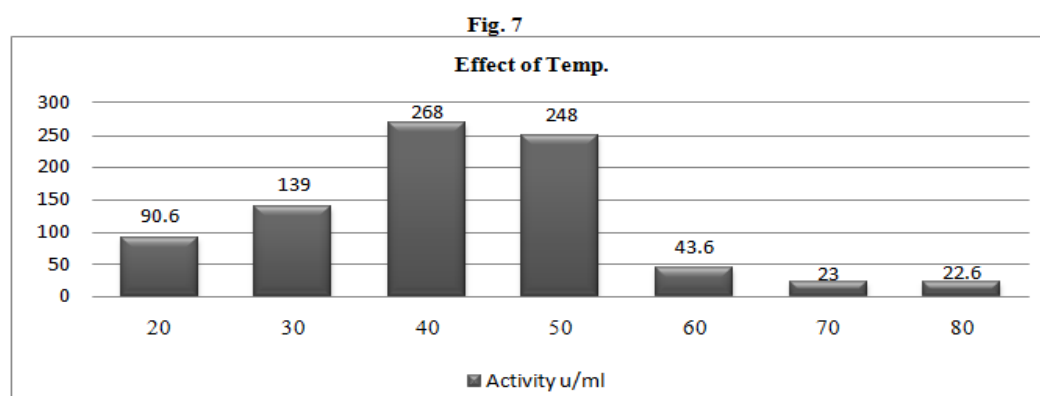


Fig. 6





## VII. Conclusion

Optimization of Alkaline protease production using solid state fermentation was carried out successfully. Wheat bran, proved to be a valuable substrate showing the maximum enzyme production. The organism is capable of producing a highly alkaline enzyme and thus can have a number of commercial applications.

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