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OPTIMIZATION OF CULTURE CONDITIONS FOR THE PRODUCTION OF PROTEASE BY *Pleurotus eryngii*

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ABSTRACT

Present study focuses to optimize the protease enzyme production by edible mushrooms. For the specified goal to achieve the growth of *Pleurotus eryngii* cultured on potato dextrose agar medium incubating at temperature 24ºC, pH 5.6 for 7 days. The maintained culture was processed for the production on fermentation media, *i.e.*, glucose 20 g/L, peptone 10 g/L, corn steep 3.0 g/L and ammonium nitrate 1.0 g/L on pH 6.5. The best time for maximum production was observed to be four days from incubation. After selecting the optimum time for the enzyme production, the optimum nutrient contents were checked. Sucrose (0.5%) and casein (1%) were found to be the best carbon and nitrogen sources, respectively. The maximum protease production was achieved at pH 6.5 and the maximum enzyme yield was found at 22ºC,

Key words: *Pleurotus eryngii*, Protease, Edible mushroom, Culture conditions.

INTRODUCTION

Proteases or the proteolytic enzymes are also known as peptide hydrolyses proteinase and peptidases [Rawlings et al., 2010]. Protease enzymes split the bond among polypeptides and proteins stepwise into smaller peptides and amino acids [Gonzalez-Rabade et al., 2011; Mitchell et al., 2007]. The proteases belong to hydrolases class-3 and subclass 3.4, according to Enzyme Commission of International Union of Biochemistry [Beg et al., 2003]. `

Proteases are classified on the basis of three most important aspects; chemical nature of the catalytic site, type of chemical reaction catalyzed, and evolutionary correlation with indication to structure. The specific peptide bonds are cleaved in protein fragment on the basis of source, Protease enzymes are subdivided into two fore-most classes' exopeptidases and endopeptidases and constitute a large group [Rawlings et al., 2010; Fan and Wu, 2005; Haki and Raksit, 2003]. Endopeptidases act on the internal region of polypeptides while exopeptidases operate on the external region (N or C terminus) of the polypeptide chain [Palma et al., 2002]

On earth proteases are essential constituents of all forms of life including both prokaryotes and eukaryotes. By establish method; in less time proteases can be produced in huge quantity through fermentation for regular supply using microorganisms and providing two third share of commercial protease production in the world [Kummar and Takagi, 1999]. Proteases are also among the bulk of industrial enzymes and proteases from various sources such as plant, animal, and microbes constitute around 60% of the total worldwide enzyme sales [Merheb-Dini et al., 2009: Kunamneni et al., 2003].

At the moment proteases are extremely major commercial enzymes since they have numerous applications in different industries such as pharmaceutical, food, leather and detergent industries [Doran, 2002]. These enzymes are also reported to have a significant role in development and manifestation of dreadful diseases such as AIDS and cancer [Yadwad, et al., 1996]. Protease use to improve blood circulation, to prevent abnormal blood clotting, to reduce pain and inflammation associated with Phlebitis, to alleviate the pain, inflammation, and discomfort of varicose veins; to minimize muscle pain that occurs after exercise, to minimize the inflammation and pain associated with Osteoarthritis and Rheumatoid Arthritis, to alleviate the symptoms of Sinusitis and to alleviate Edema [Patel et al., 2005, Laxman, et al., 2005]. Commercially produced proteases are also used in various processes such as tenderization, dairy processing, milk clotting, cheese making and brewing [Gonzalez-Rabode et al., 2011]. Both crude as well as pure preparations are used in various industries, for example, crude protease are used in bulk quantities in leather and detergent industries while pure proteases preparations are utilized in pharmaceutical industries.

In enzyme market Proteases are on first position and the estimated value is US$ 3 billion [Leary et al, 2009]. Extracellular proteases are important for the hydrolysis of proteins in the cell free environment and enable the cell to absorb and utilize hydrolytic products [Kalisz, 1988]. At the same moment these extracellular proteases have also been commercially exploited to support protein degradation in various industrial processes [Kumar, et al., 1999, Outtrup, et al., 1990].

The aim of this work was to utilize microorganisms for the production of protease enzyme due to versatile applications in various industrial processes

MATERIALS AND METHODS

Isolation of Mushroom:n this study, mushroom strains *Pleurotus eryngii* was purchased from Edible Fungi Institute, Shanghai Academy of Agricultural Sciences, Shanghai, China. Stock cultures of these fungi were maintained on potato dextrose agar slants at 4°C.

Dry biomass and final pH determination: The culture broth was filtered through pre-weighed Whatman No. 1 filter paper and the culture filtrate was used as the source of protease determination. The filter paper containing biomass was washed with distilled water and dried at 105- 110°C in an oven and its dry weight was estimated. The final pH of the culture broth was determined using pH meter (WPA Scientific Instrument).

Effect of cultivation time: The incubation for optimum growth was carried out as various cultivation times period ranging as 48, 96, 144, 192 and 240 at 24±2°C.

Effect of carbon and nitrogen sources on the protease production: Different sugars such as starch, sucrose, fructose, and galactose were replaced with glucose, and incorporated with mineral medium to check their effect on the production of protease activity. Like carbon sources while different nitrogen source such as casein, ammonium nitrate, potassium nitrate, ammonium chloride and urea were replaced with 1% peptone.

Effect of initial pH on the biosynthesis of protease: The effect of pH on protease production was observed by adjusting different pH of culture media ranging from 4.5 to 10.5. The ambient pH was maintained with 0.1 N HCl or NaOH prior to sterilization.

Inoculum Preparation: The inoculums medium was composed of (g/L): glucose 20.0, peptone 10.0, corn steep 3.0, NH_4NO_3 1.0, the medium was adjusted to 6.5 pH. 250 ml Erlenmeyer flasks containing 50 ml of synthetic medium incubate in orbital shaking incubator with mushroom culture at 24±2°C. After four days of incubation mycelial pellets of *Pleurotus eryngii* were harvested and homogenized with a laboratory blender.

Protease production medium: Fermentation mineral medium containing 2% glucose was taken in 250 ml conical flask and the initial pH of the medium was maintained at 6.5. Flasks were autoclaved at 1.5 kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature and inoculated with 1.0 ml of mycelia homogenate of *Pleurotus eryngii*. The culture was incubated in an orbital cooled shaking incubator (Gallen Kamp) at 24±2°C. The culture broth was separated from mycelium after an interval of 24 hours incubation period through Whatman No. 1 filter paper.

Effect of initial temperature on the biosynthesis of protease: The effect of the temperature on protease production was checked by incubating culture flasks at various temperatures ranging from 10–34°C.

Assay of protease activity: Protease activity was determined by spectrophotometer method as reported by Penner and Ashton [1967]. Culture broth 0.5 ml, substrate (1% Soluble casein) 0.5 ml and sodium phosphate buffer 1.0 ml of pH 7.6 were added and incubated at 35°C for one hour. After incubation, 2.0 ml of each sample was taken and 2.0 ml of 15% TCA was added and centrifuged for 5 min at 4000 rpm. To 1.0 ml aliquot, 4.0 ml 0.5 N NaOH and 1 ml Folin-phenol reagent (1:1) were added and then final volume was made up to 10 ml by adding 4.0 ml double distilled water. The absorbance was read at 625 nm.

One unit of protease activity was defined as the amount of protease required to catalyze the liberation of 1 µg of tyrosine under the assay conditions. Protein content from the culture broth was determined by Lowry et al., [1951] method.

RESULTS AND DISCUSSION

In present study, glucose 2% was used as a carbon source in the mineral medium for the growth of mushroom and for the production of protease when incubated at various time periods in batch

wise submerged culture condition. After every 24 hours interval, fermented medium was collected and protease activity was determined. The protease production increased with incubation time and maximum production (0.546 unit /ml) was noted after 96 hours beyond this lower production was detected (Figure-1) when grown on 2% glucose mineral medium. In early stage of growth the pH of culture medium was increased from the initial pH towards the alkaline side. The growth of mushroom increased up to 96 hours whereas concentration of total sugar, reducing sugar and protein decreased with increase of incubation period. Result of this study was related with result of Ravikumar et al., (2012); Hames-Kocabas and Uzel (2007) and Naidu and Devi, (2005).

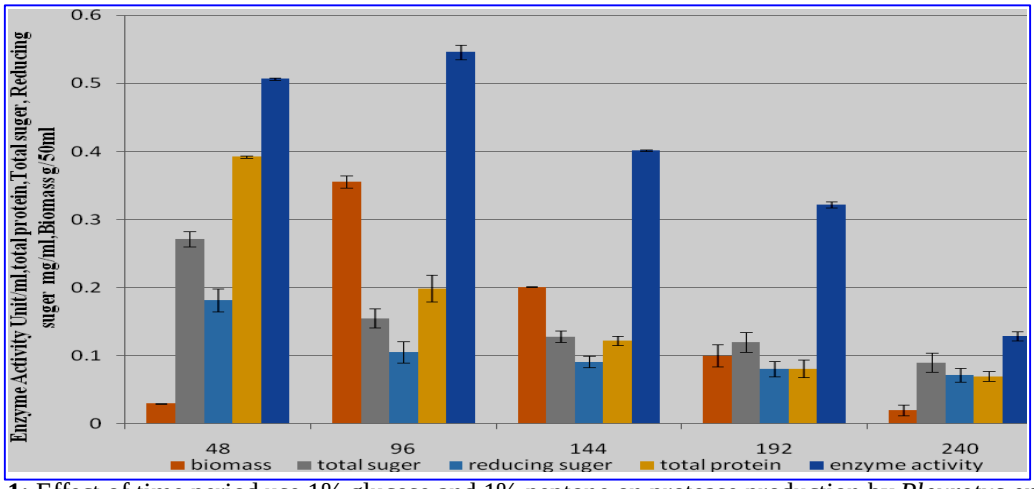

Figure-1: Effect of time period use 1% glucose and 1% peptone on protease production by *Pleurotus eryngii* when incubate at 24°C with initial pH was 6.5.

Effect of carbon source on biosynthesis of protease: In this study two percentage 0.5% and 1% were used of different carbon sources such as starch, sucrose, fructose and galactose (instead of glucose was used during fermentation period) were supplemented with mineral medium for the growth *Pleurotus eryngii* of and production of protease (Figures -2 and 3). Mushroom secreted maximum amount of protease in 0.5% sucrose integrated with mineral medium when grown at 24±2ºC at 96 hours with pH 6.5 in comparison to other sugars. No correlation was found between biomass, total protein enzyme activity.

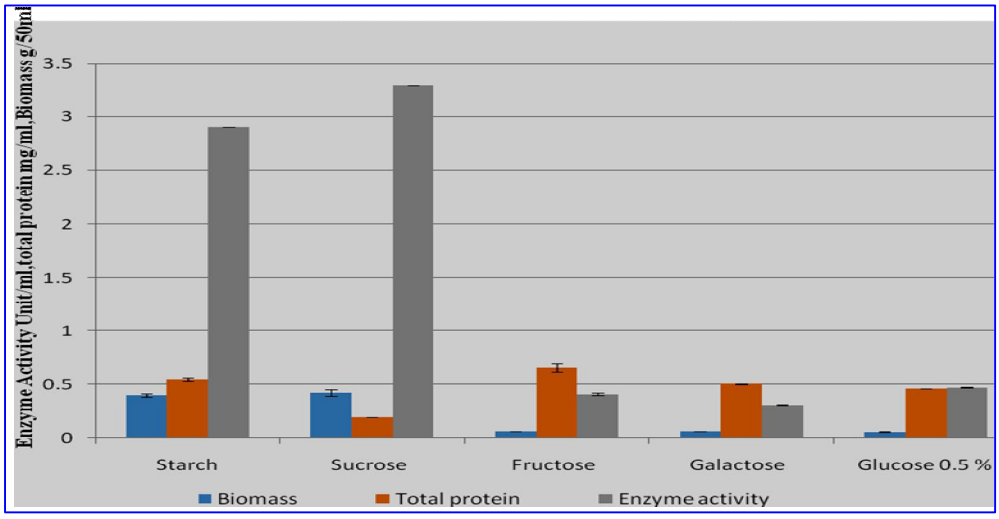

Figure-2: Effect of 0.5% different carbon sources on protease production by *Pleurotus eryngii* when incubate at 24°C and initial pH was 6.5.

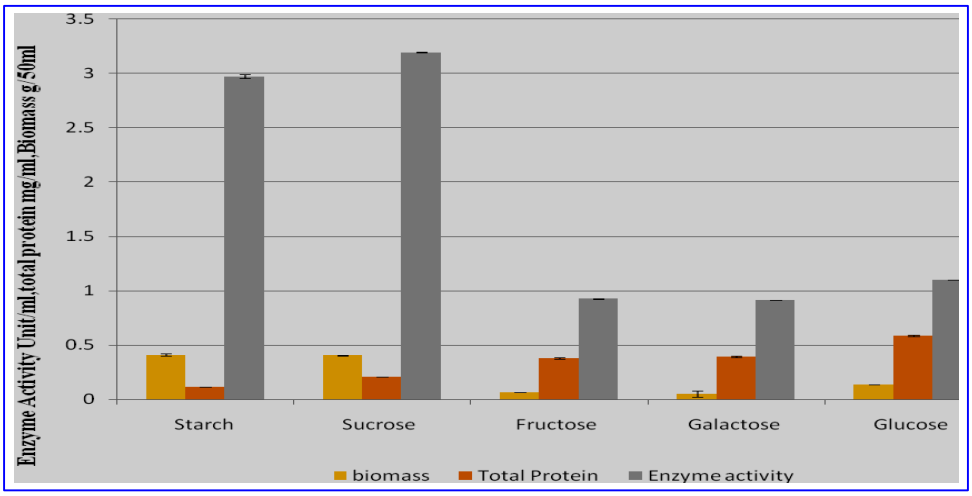

Figure- 3: Effect of 1%different carbon sources on protease production by *Pleurotus eryngii* when incubate at 24°C and initial pH was 6.5.

Effect of nitrogen source on the production of protease: Effect of 1% nitrogen sources such as casein, ammonium chloride, ammonium nitrate, potassium nitrate and urea were used in culture medium instead of peptone, which was used in the fermentation medium. From the result as presented in (Figure-4), it was noted that higher production rate of protease was observed when mushroom was grown on mineral medium contain 0.5% sucrose and 1% casein, incubated at 24±2ºC and pH was adjusted at 6.5 for 96. Mycelia mass and protein contents are also checked but no any resemblance was found. Similar result shown by Femi-Ola et al., [2014].

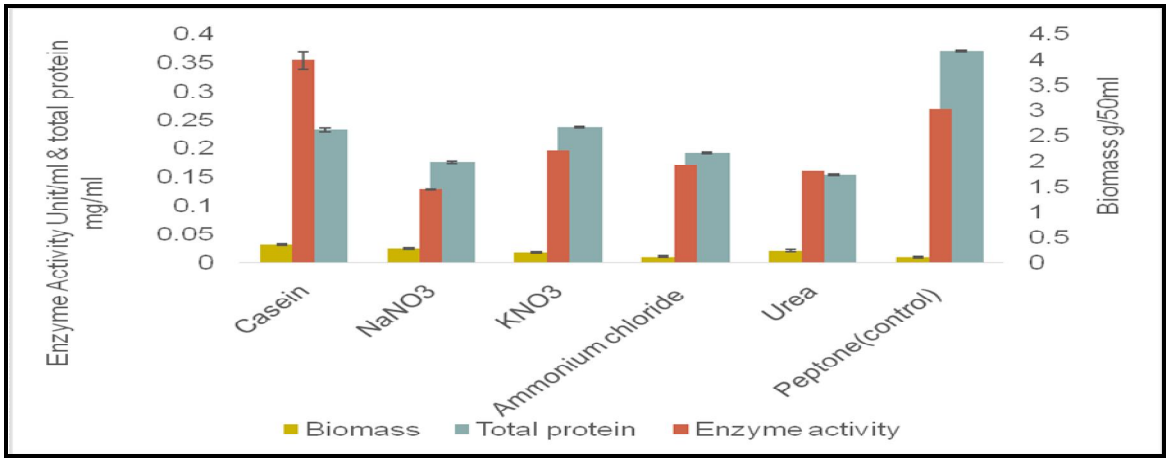

Figure-4: Effect of 1% different nitrogen sources on protease production by *Pleurotus eryngii* when incubate at 24°C and initial pH was 6.5.

Effect of pH on biosynthesis of Protease: According to results represented in Figure-5, the production of protease increased up to pH 6.5 and then enzyme secretion fall with the elevation of pH values towards alkaline side. It is suggested that *Pleurotus eryngii* is slightly acidic mushroom which secrete higher amount of protease at around pH 6.5.Biomass values and protein were also determined from culture broth but no resemblance was found between them. In general high amount of protease activity was synthesized by *Pleurotus eryngii* when grown in weak acidic pH range, present study nearly correlated with the result of Vasantha and Subramanian [2012] and they reported maximum secretion of enzyme at pH 7.0.

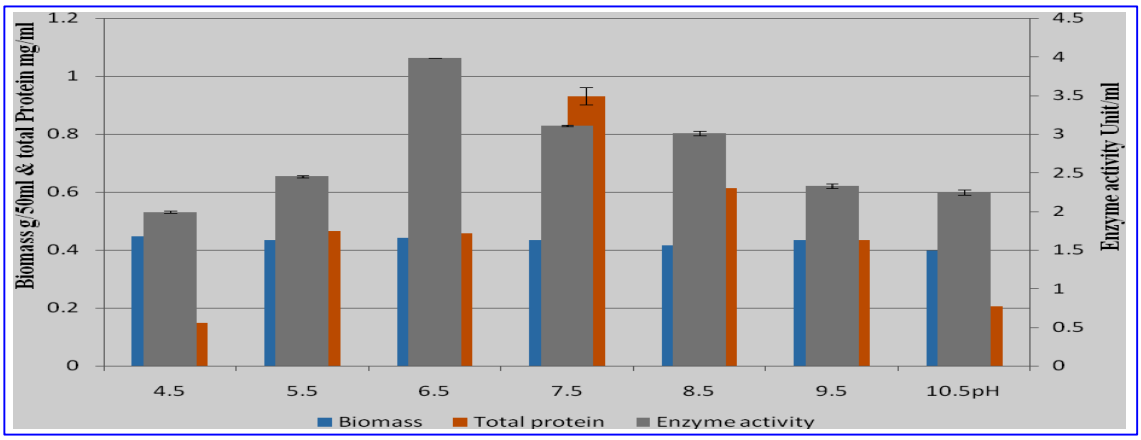

Figure-5: Effect of different of pH on protease production by *Pleurotus eryngii* when incubate at 24°C and initial pH was 6.5.

Effect of temperature on biosynthesis of protease: Mushroom inoculated culture medium was incubated at various temperatures ranging from 10–34°C. It was noted that *Pleurotus eryngii* secreted maximum yield of protease when grown on 22°C and then declined with the increase of incubation temperature as summarized in Figure–6. With the increase of temperature production decreased may be due to inactivation of enzyme at higher temperature [Sinha et al., 2013; Sinha and khare, 2013] observed highest activity of enzyme at 25°C.

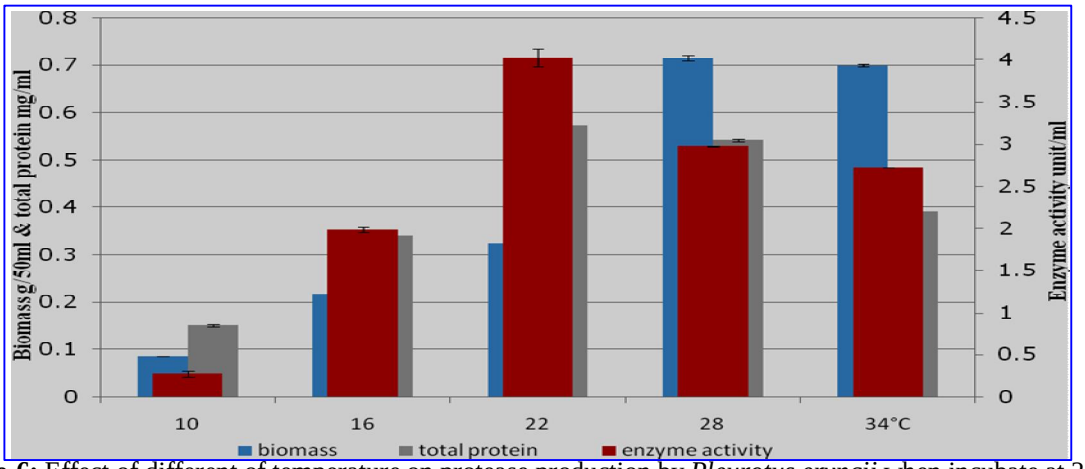

Figure-6: Effect of different of temperature on protease production by *Pleurotus eryngii* when incubate at 24°C and initial pH was 6.5.

Conclusion: The present results suggest that *Pleurotus eryngii* grown best and secreted maximum amount of protease in mineral medium containing 0.5% sucrose and 1% casein when incubated at 22°C for 96 hours with initial pH 6.5.

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