

Alkaline protease production by solid state fermentation of mustard oil cake

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Abstract—Mustard oil cake being rich in protein offer potential benefits when utilized as substrate for fermentative production of enzymes. In the present study impact of diluents on the production of protease by *A.terreus* grown on mustard oil cake was done. Supplementation of D₃ diluent increase the enzyme production significantly (110 U/g of dry solids). Enzyme production was maximum when mustard oil cakes inoculated with *A.terreus* was incubated at 40°C temperature & 9.5pH. Maximum protease production was obtained after 96 h of incubation period.

Keywords—Alkaline protease, *A.terreus*, Mustard Oil cake, SSF.

I. INTRODUCTION

Protease is one of the most important commercial enzymes, and is used in food processing, detergents, dairy industry and leather making [1]. Although proteases are found ubiquitously in plants, animals and microorganisms, the later are the preferred sources of proteases and dominated the commercial application. Fungi exhibit a wide variety of proteases than bacteria. Furthermore fungi are generally regarded as safe strains and they produce extracellular enzymes, which are easier to be recovered from fermentation broth [2]. The microbial proteases of *Aspergillus* species, in particular, have been studied in detail since they are known for their capacity to secrete high levels of enzymes in their growth environment.

Oil cakes are by-products obtained after oil extraction from the seeds. There are several reports describing production of various enzymes using oil cakes as substrate in solid state fermentation (SSF). Oil cakes are ideally suited nutrient support in SSF rendering both carbon and nitrogen sources, and reported to be good substrate for enzyme production using fungal speices [3]. Sandhya et al., [2] and Sumantha et al., [4] have reported protease production using oil cakes as nutrient source.

Mustard oil cake (MOC) has protein content of about 38.5% and fiber content of 3.5% [5]. Amino acid profile of MOC shows them to be rich in leucine, phenylalanine, arginine and glycine [6].

This work reports on the use of mustard oil cakes for producing proteases of *Aspergillus terreus* by solid-state fermentation.

II. MATERIAL AND METHODS

2.1 Microorganism and maintenance of culture -

Aspergillus terreus JBPSI- 2 used in this study was isolated from soil samples in Jabalpur area and was identified on the basis of molecular analysis by Agharkar Research Institute, Pune (MH). The fungus was grown and maintained on potato dextrose agar (PDA) slants at 28±2°C with fortnightly transfer to fresh medium.

2.2 Substrate -

Mustard oil cake used in this study was obtained from local market in Jabalpur, M.P. India.

2.3 Inoculum preparation

The inoculum was prepared by dispersing the spores from a week old fungal slant culture in 0.1% Tween- 80 solution with a sterile inoculum loop.

2.4 Solid state fermentation

Five grams of mustard oil cake was taken in a 250 ml Erlenmeyer flask moistened with 5 ml of diluent, sterilized at 121°C for 15 min, cooled inoculated with 1 ml of fungal spore suspension and incubated at 28±2°C for 96 h unless otherwise specified . All experiments were carried out in two sets. The results shown are average value ± SD.

2.5 Impact of diluents on protease production following eight diluents (pH adjusted to 8.5) were used to moistened the substrate [7]

D₁: (% W/V) Peptone, 1.0 dextrose 4.0

D₂: (% W/V) NaNO₃, 2.0 ; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.05; CaCl₂.2H₂O, 0.05; ZnSO₄.7H₂O, 0.01

D₃: (%W/V) Glucose, 7.0 ; Peptone, 2.0 ; KH₂PO₄, 0.4; MgSO₄. 7H₂O, 0.05; CaCl₂.2H₂O, 0.05; ZnSO₄. H₂O, 0.01

D₄: (% W/V) Yeast extract, 1.0; Glucose, 3.3; Peptone, 1.0; CaCO₃, 1.0

D₅: (% W/V) Glucose, 1.0; Peptone, 1.0 ; Beef extract, 1.5 ; NaCl, 0.3

D₆: (% W/V) Glucose, 2.0; Peptone, 1.0 ; Beef extract, 1.0; NaCl, 0.3; CaCl₂, 0.1 ; Na₂CO₃, 0.7

D₇: (% W/V) (NH₄)₂ SO₄, 1.4; Urea, 3.0 KH₂PO₄, 2.0; MgSO₄.7H₂O, 3.0; CaCl₂, 3.0

D₈: Distilled Water.

2.6 Optimization of process parameters -

Optimization of growth conditions and enzyme production was done using different parameters such as incubation time, pH and temperature.

2.6.1 Incubation time -

The effect of incubation period on enzyme production (pH 8.5; temperature 28±2⁰C) was investigated by checking the enzyme activity on 0, 24 h, 48h, 72h, 96h, 120h, 144h and 168 h of incubation.

2.6.2 pH -

The effect of pH on enzyme production was investigated by SSF in mustard oil cake by adjusting the pH of D₃ diluent as the maximum enzyme production was obtained when this diluent was used as moistening agent. pH of the diluent was adjusted to 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5 and 11.0 using 1 M NaOH. The substrates inoculated with *A. terreus* were then incubated for 96 h at 28±2⁰C as the maximum enzyme production was at the 96 h of incubation.

2.6.3 Temperature -

The favourable temperature for maximum protease production was studied with a range of 10⁰C to 65⁰C. Substrate was moistened with D₃ diluent (pH-9.5) and incubated for 96h as the maximum enzyme production was obtained under these conditions.

2.7 Enzyme extraction -

A known quantity (5g) of the fermented material was mixed with 10ml of Tween 80 (0.1%) solution in distilled water and homogenized by mixing in a rotary shakes at 150 rpm for 1h. The solids were removed by filtration and filtrate were centrifuged at 8000 rpm for 15 mins at 4⁰C. The resultant supernatant was used for analytical studies.

2.8 Assay for protease activity -

Protease activity was assayed by the method of Anson [8].

III. RESULTS AND DISCUSSION

3.1 Impact of various diluents on protease production -

Impact of various diluents on the production of protease by *A.terreus* grown on mustard oil cake as a substrate was done. In the present study the maximum enzyme production of 110U/g of dry solids was observed when the substrate was moistened with D₃ diluent while minimum protease production was (42U/g of dry solids) obtained when moistened with D₈ diluent i.e. distilled water. The ratio of substrate to diluent was kept 1:1. When substrate was moistened with glucose, peptone, CaCl₂, MgSO₄, KH₂PO₄ (D₃ diluent components), enzyme synthesis was maximum. It shows that organic nutrients (glucose, peptone) and inorganic nutrients (CaCl₂, ZnSO₄, MgSO₄) both stimulate the production of enzyme from *A. terreus*. Potassium dihydrogen phosphate in this diluent act as a constituent of the extraction buffer and may enhanced protease recovery from MOC.

3.2 Optimization of process parameters -

Any fermentation process is governed by a large number of physical, chemical and biological factors [4]. Fermentation time, temperature and pH are the parameters that impact largely on enzyme production.

3.2.1 Incubation time -

The time of incubation has an important role in cheaper commercial production of enzymes. Fig:1 shows that *A. terreus* gave the maximum yield of protease at 96 h of incubation in a SSF process with MOC as the substrate. The subsequent decrease in enzymes activity with increasing fermentation time could possibly be due to cessation of production by SSF [9].

3.2.2pH -

The influence of initial pH on production of protease by *A.terreus* was determined in the pH range of 7-11. Protease synthesis increased with increase of initial pH of the substrate and reached maximum at pH 9.5; whereas below and above this level of pH yield was low (Fig:2). The pH dependent changes in the amount of enzyme production might have been due to pH dependent control of protease gene expression. The results clearly indicate the alkalophilic nature of the fungus. Similar studies on the influence of pH on protease production have been studied in different *Aspergillus* sp. In case of *A.terreus* the optimum pH was reported to be between 5.5 and 9.5 [10].

3.2.3 Incubation temperature -

SSF is more sensitive to temperature variation than broth culture, not only does the temperature regulates the synthesis of the enzyme but possibly the secretion of the enzyme by changing the properties of the cell wall [11]. The maximum protease activity was recorded at 40°C. Increase in temperature resulted in considerable loss of activity, and poor or no growth. *Aspergillus niger* has been reported to produce maximum proteases at 40°C, which is similar to that of our result [12]. An optimum temperature of 40°C for *A.terreus* has also been reported [13] which is similar to our study. Inactivation at higher temperatures may be attributed to incorrect conformation due to hydrolysis of the peptide chain, disruption of amino acids or aggregation.

VI. CONCLUSION

In present study, the protease production capability of a native isolate of *A.terreus* JBPSI-2 from soil was exploited by SSF of mustard oil cake which is cheap and commonly available. The different parameters such as impact of various diluents, incubation time, pH and temperature were optimized. From the results it could be inferred that alkaline protease produced through solid state fermentation of the mustard oil cake by *A.terreus* JBPSI-2 could possibly find useful application in detergent industry.

V. ACKNOWLEDGMENT

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Table – 1: Impact of Various diluents on protease production by *Aspergillus terreus* in SSF using MOC as a substrate.

Diluents	Protease activity (U/g) \pm SD
D ₁	42 \pm 8
D ₂	64 \pm 6
D ₃	110 \pm 9
D ₄	77 \pm 7
D ₅	66 \pm 5
D ₆	47 \pm 8
D ₇	73 \pm 6
D ₈	42 \pm 5

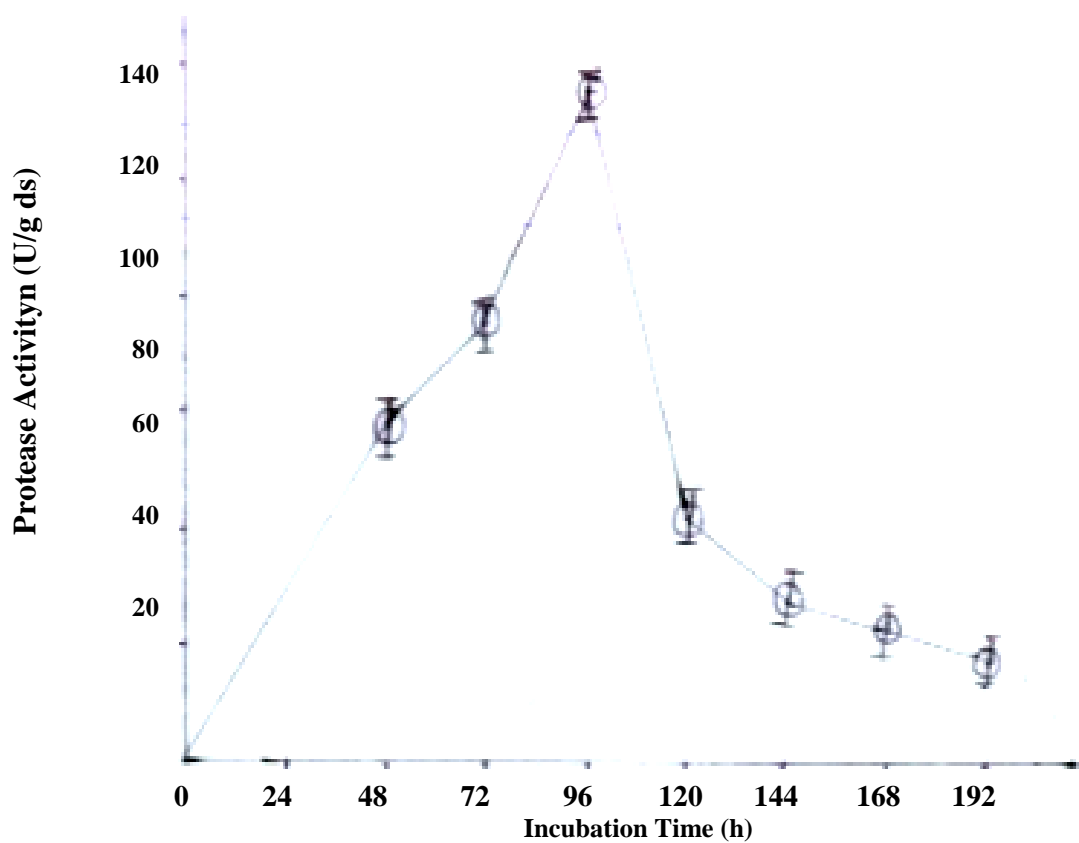


Figure-1: Protease activity by *Aspergillus terreus* in SSF using MOC as a substrate at different time of incubation.

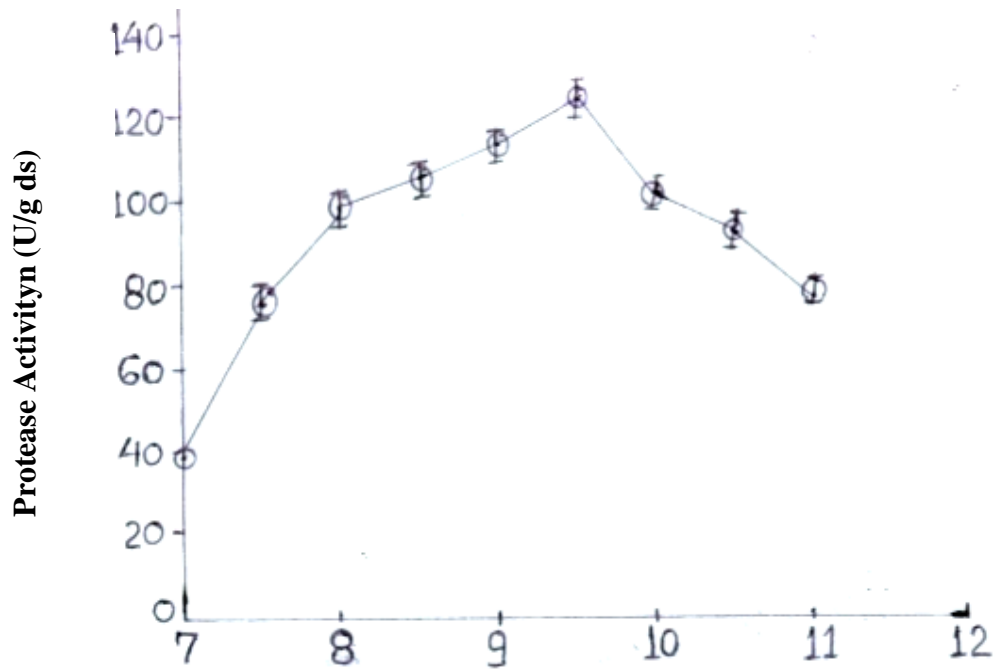


Figure- 2: Protease activity by *Aspergillus terreus* in SSF using MOC as a substrate at different pH

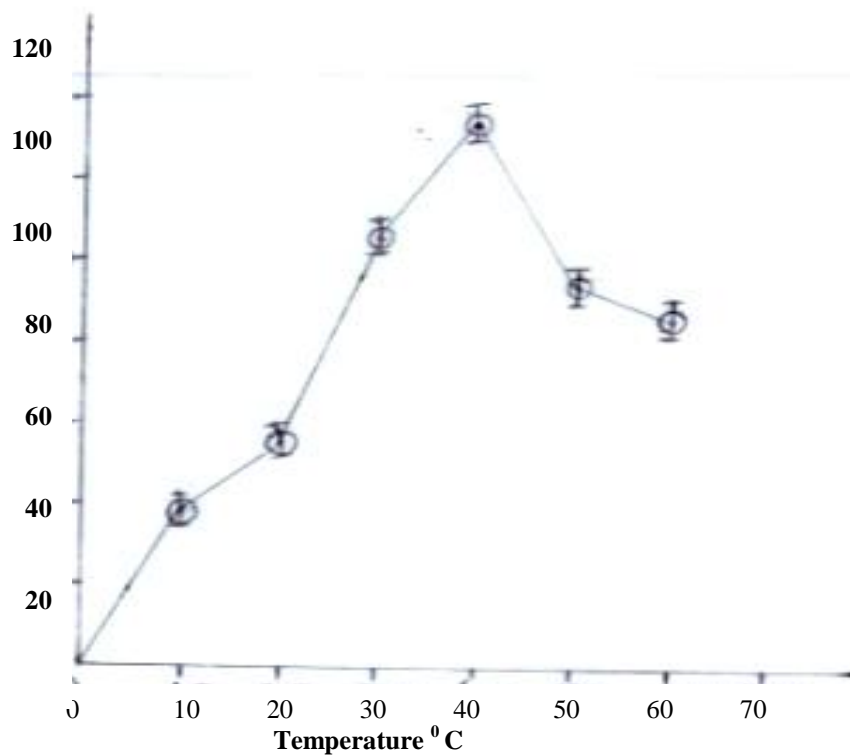


Figure- 3: Protease activity by *Aspergillus terreus* in SSF using MOC as a substrate at different temperatures.