ISSN 0970 - 6011

Volume 57, Number 1, 2010

Indian Journal of Fisheries

Indian Council of Agricultural Research

Published by
The Director
Central Marine Fisheries Research Institute
Cochin - 682 018, Kerala, India
www.cmfri.org.in

Indian J. Fish., 57(1) : 45-51, 2010

Amylase and acid protease production by solid state fermentation using *Aspergillus niger* from mangrove swamp

D. BHATNAGAR*, IMELDA-JOSEPH AND R. PAUL RAJ

Central Marine Fisheries Research Institute, Post Box No. 1603
Ernakulam North P. O., Kochi - 682 018, Kerala, India,
**Central Institute of Freshwater Aquaculture, Kausalyaganga,*
Bhubaneswar - 751 002, Orissa, India
e-mail: imeldajoseph@rediffmail.com

ABSTRACT

Production of amylase and acid protease by solid state fermentation employing *Aspergillus niger* strain $S_1$4, a mangrove isolate was evaluated. Wheat bran containing 60% moisture was used for fermentation with two inoculum sizes of $2x10^6$ and $40x10^6$ spores 10 g substrate^{-1}. The maximum amylase activity of 48.13 Ugdfs^{-1} was recorded after an incubation period of 4 days at temperature 30 ^{0}C and pH 4.8 for the inoculum size $40x10^6$ spores 10 g substrate^{-1}. The concentration of reducing sugar showed a significant negative correlation (-0.496, $p<0.05$) with amylase production. The peak acid protease activity was observed on day 6 (54.89 Ugdfs^{-1}) for $2x10^6$ spores 10g substrate^{-1} and on day 4 for $40x10^6$ spores 10g substrate^{-1} under standard assay conditions of 30 ^{0}C and pH 2.7. The concentration of protein in the substrate was significantly ($R=0.778$, $p<0.05$) correlated with the acid protease production.

Keywords: Acid protease, Amylases, *Aspergillus niger*, Mangrove, Solid state fermentation, Wheat bran

Introduction

Solid state fermentation (SSF) is a well established and effective biotechnological tool, which holds tremendous potential for production of a large variety of enzymes. Using number of microorganisms, including bacteria, yeast and fungi, a variety of enzymes can be produced in SSF. Besides, the crude fermented product can also be used directly as an enzyme source (Pandey *et al*., 1999). The *Aspergillus* species produce a large variety of extra cellular enzymes of which amylases and proteases are of significant industrial importance (Pandey *et al*., 2000). Amylases have not only been used in fermentation processes, but also in processed food industry and in the textile and paper industries (Ellaiah *et al.,* 2002; Gigras *et al*., 2002). Proteolytic enzymes account for nearly 60% of the industrial enzyme markets in the world. They have found wide application in several industrial processes such as cheese production, meat tenderization, baking industry, and in many other fields, including textiles and leather industries and as an additive to detergents (Bai *et al*., 1999; Pandey *et al*., 1999). Proteases produced at an industrial scale in SSF systems have been reported to have greater economic feasibility (Mitra *et al.,* 1996).

Mangrove ecosystems are among the most productive natural ecosystems and the biomass released annually from mangrove trees is estimated to be 0.48% of carbon biomass per hectare (Gong and Ong, 1990). Mangrove water bodies contain heavy deposits of particulate organic matter (detritus) derived chiefly from the decomposition of litter. The primary productivity of the mangrove water ranges from 2.0 to 3.6 g C m^3 day^{-1} (Nair and Gopinathan, 1983); the sediment microbial population is about seven fold higher than that of littoral zone, and the suspended particulate matter 20 times greater than that of seawater (Gopinathan and Selvaraj, 1996). The submerged mangrove roots and trunk are surrounded by loose sediment, which attract epifaunal communities including bacteria, fungi, micro-algae and invertebrates (Kathiresan and Bingham, 2001). Assuming their dynamic properties in nutrient recycling, a fungal strain *Aspergillus niger* $S_1$4 inhabiting mangrove soil was used in the present study to evaluate its efficacy for the production of amylase and acid protease under solid state fermentation. In this paper we report the optimum amylase and protease enzyme activity by *A. niger* strain $S_1$4.

Materials and methods

Characterization of fungal isolates

Four fungal strains designated as *Aspergillus niger* $S_1$4, $S_1$5, $S_1$7 and $S_1$9 were isolated from the mangrove soil and the strains were maintained in potato dextrose agar (PDA). Characterization of strains was carried out by observing the growth pattern and colony diameter on czapek yeast extract agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar (G25N) at three different temperatures

(5 ^{0}C, 30 ^{0}C and 37 ^{0}C) and identifying morphological characteristics and the structure of the conidiophores under light microscope under different magnifications (10x, 20x and 100x) (Pitt and Hocking, 1985). Since all the four strains expressed similar characteristics, strain $S_1$4 was randomly selected for the present study.

Inoculum

The inoculum was obtained from 7 days old culture of *A. niger* strain $S_1$4 on PDA slants. Spore suspension was prepared by pouring 10 ml of 0.1 % sterile Tween 80 (Polysorbate 80) by shaking and scraping using a sterile loop. Spore concentration was determined using Haemocytometer.

Solid state fermentation

Wheat bran (10 g), taken in 500 ml conical flasks, was moistened with czapek dox (Aikat and Bhattacharya, 2001) to make the moisture level to 60% and autoclaved for 20 min at 121 ^{0}C (The composition of czapek dox was (g l^{-1}) : $NaNO_3$ - 2.5; KH_2PO_4 - 1.0; $MgSO_4$. $7H_2O$ - 0.5; KCl- 0.5). Two different spore suspensions, $2x10^6$ (Kaur *et al*., 2003) and $40x10^7$ (Pandey *et al.,* 1995) spores ml^{-1} were inoculated on the substrate under aseptic conditions and incubated at 30 ± 1 ^{0}C for a period of 8 days. All flasks were kept under stationary condition at an initial pH 6.4 - 6.5, with occasional shaking for optimization of SSF.

Extraction of enzymes

After every 24 h interval, the fermented substrate was mixed thoroughly with sterile distilled water @ 5 ml g^{-1} and left for soaking at 4 ^{0}C for 12 h (Aikat and Bhattacharya, 2001). The soaked medium was stirred for 1 min using a magnetic stirrer and the extract was squeezed through a muslin cloth (60 µm). After filtering through Whatman No.1 filter paper, the volume of filtrate was noted and the clear extract was used for enzyme assay.

Amylase assay

Amylase activity was measured by incubating 2 ml of starch solution (1%) (Merck, India) prepared with 0.2M acetate buffer at pH 4.8 (0.02M acetic acid and 0.02M of sodium acetate) in a test tube and 1 ml of adequately diluted enzyme solution with the same buffer for 5 min (Bernfeld, 1955). Amylase units are expressed as milligrams of maltose released per minute by the total amount of enzyme extract from one gram of dried fermented substrate (gdfs) under standard assay conditions (Pandey *et al*., 1995, Uguru *et al*., 1997). Maltose was used as the standard. Amylase activity at pH 6.9 was carried out by the same method using 0.2 M phosphate buffer (pH 6.9). Specific activity was expressed as Units mg $protein^{-1}$ (U mg $protein^{-1}$). The soluble protein concentration in enzyme solution was determined by Lowry's method (Lowry *et al*., 1951).

Estimation of reducing sugars

Reducing sugars in the crude enzyme extract was determined by taking 1 ml of diluted crude enzyme solution with 2 ml of distilled water followed by addition of 2 ml of DNSA reagent. Immediately the test tubes were dipped in a boiling water bath for 5 min, followed by cooling and the OD was measured at 540 nm (Miller, 1959). The amount of maltose released by 1 g dried fermented substrate was calculated and expressed as: mg maltose $gdfs^{-1}$.

Assay of acid protease

Standard procedure was followed for acid protease assay (Ichishima, 1970). The principle involved is measurement of the tyrosine concentration by Lowry's method after the enzyme substrate reaction with casein as substrate (Lowry *et al*., 1951). One milliliter of 2% milk casein solution (Himedia, Mumbai) prepared with 0.1 M sodium acetate buffer at pH 2.7 was digested for 10 min at 30 ^{0}C by 1 ml of dilute acid protease solution prepared by using the same buffer at pH 2.7. The reaction was terminated by addition of 2 ml of 0.4 M tricarboxylic acid (TCA) and filtered (Whatman No.1). The tyrosine and tryptophan equivalents were determined by the blue colour given by the folin- ciocalteaue reagent in alkaline solution. The color was compared against, standards for protein estimation by Lowry *et al.* (1951). Protease units are expressed as micrograms of tyrosine released in one minute by the total amount of enzyme extracted from one gram of dried fermented substrate (gdfs), under standard assay conditions (Pandey *et al*., 1995; Yang and Lin, 1998).

Free hydrolyzed peptides

To 1ml of diluted crude enzyme extract, 1ml of distilled water and 2 ml of 0.4M TCA were added to precipitate the unhydrolysed peptides and proteins. The mixture was filtered through Whatman No. 1 filter paper and the extract was subjected to protein estimation by Lowry's method and the result was compared with the BSA standard. The amount of hydrolyzed peptide was calculated per ml, and then was converted to mg g^{-1} of dried fermented mass. Protein content was estimated by the Lowry's method taking bovine serum albumin (BSA, Himedia, Mumbai) as a standard (Lowry *et al*., 1951).

Statistical analyses

The production of amylase and proteases by the selected strain of *A. niger* was analyzed by two-way rested ANOVA. Pearson's correlation analysis was done to estimate the R value and its significance ($p<0.05$).

Results and discussion

The four fungal isolates allowed to grow on petridishes with CYA, MEA and G25N medium were characterized by their plane and velutinous growth pattern with

sub-surface mycelia whitish in colour, surmounted by a layer of closely packed, radiating black conidiophores of 2-3 mm high. Reverse side of the petridishes was yellow and colonies were of 30-60 mm diameter in 4-6 days of culture. Colonies on MEA were comparatively smaller. At 5 ^{0}C no growth was observed. Conidia were spherical (4-5 mm) and black with rough wall, which are the characteristic features of *A. niger*.

On microscopic observations at different magnifications (10 x, 20 x, 40 x and 100 x), it was observed that conidiophores, borne from the surface hyphae of 1.0 - 3.0 mm were smooth and they bore spherical vesicles (50-75 µm). Conidia were spherical (4-5 µm), black with rough wall. Based on these distinguishing characteristics all the four isolates were positively identified as *A. niger.* A random selection of strain $S_1$4 was thus made for enzyme production.

The pH of the enzyme extract varied with the duration of fermentation (Table 1). The initial pH was 6.31± 0.01 which got reduced to 3.64± 0.04 on day three and again increased up to 5.79± 0.02 on day 7 with 2 x 10^6 spores 10 g substrate^{-1}. With 40x10^6 spore10g substrate^{-1}, the initial pH was 6.35 ± 0.04, which got drastically reduced to 3.81±0.03 on day two and thereafter increased to 6.19 on day 7. The significant ($p<0.05$) reduction in pH observed during the initial phase of fermentation may be due to czapek dox (pH of 5.0), which was used as the moistening agent as reported by Verma *et al*. (2001) or may be due to the production of organic acids by fungi during fermentation (Sarangbin and Watanapokasin, 1999; Singh *et al.,* 1999; Aalbaek *et al*., 2002; Clausen, 2003). The steady increase in crude extract volume observed during the course of fermentation might be due to the continuous break down of carbohydrates from the substrate to liberate carbon dioxide and water (Table 1).

Amylase activity, specific activity and protein content

Amylase assay was carried out at an acidic pH of 4.8 since it has been reported that amylase activity of *A. niger* is better analyzed at acidic pH than at alkaline or neutral pH (Bernfield, 1955; Uguru *et al*., 1997; Kaur *et al*., 2003). The amylase activity, specific activity and protein content using 2x10^6 spore 10 g substrate^{-1} and 40 x 10^6 spores 10 g substrate^{-1} during different course of fermentation for *A. niger* $S_1$4 are shown in Fig. 1 and 2. Higher inoculum size had significant influence ($p<0.05$) on enzyme and specific activities especially in obtaining higher peak value on an early phase of fermentation (48.13 U gdfs^{-1} on day 4) compared to lower inoculum size (45.29 U gdfs^{-1} on day 6). Earlier workers reported peak glucoamylase activity on day 4 by *A. niger* in SSF (Pandey *et al*., 1995; Selvakumar *et al*., 1996). Uguru *et al*. (1997) have reported

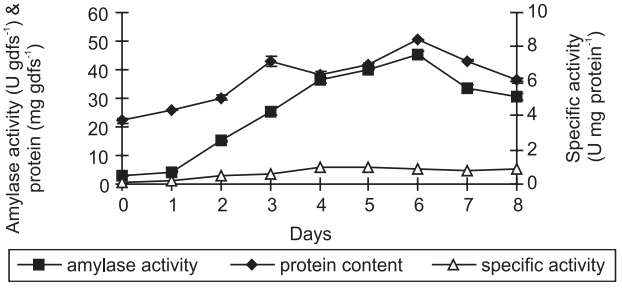

Fig. 1. Amylase activity, specific activity and protein contents at pH 4.8 by using 2x10^6 spores

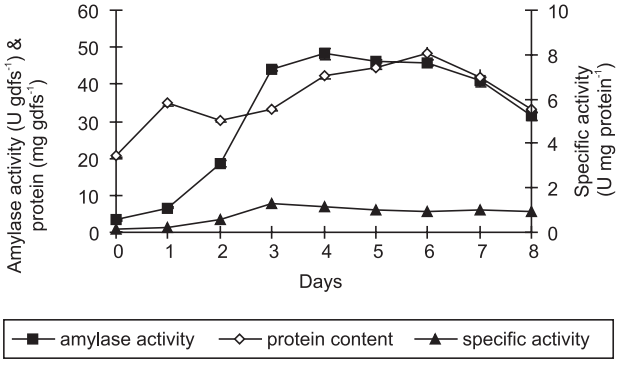

Fig. 2. Amylase activities, specific activity and protein contents at pH 4.8 by using 40x10^6 spores

Table 1. Changes in pH and volume of enzyme extract during SSF using 2x10^6 and 40x10^6 spores

Days	pH		Crude extract volume	
	2x10^6 spores 10 g^{-1}	40x10^6 spores10 g^{-1}	2x10^6 spores 10 g^{-1}	40x10^6 spores10 g^{-1}
0	6.31±0.01	6.35±0.04	86.67±0.43	86.65±0.65
1	5.98±0.02	4.920.02	90.28±0.28	93.11±0.11
2	4.71±0.01	3.810.03	90.81±0.31	94.1±0.6
3	3.64±0.04	4.690.04	95.31±0.31	94.2±0.2
4	5.07±0.08	4.840.02	96.66±0.56	99±1.0
5	5.43±0.05	5.970.03	94.94±0.44	95.88±0.1
6	5.65±0.06	6.020.02	95.61±0.59	95.53±0.53
7	5.79±0.02	6.190.01	95±0.7	95.37±0.37
8	5.76±0.04	6.190.01	91.72±0.69	93.48±0.48

peak amylase activity on day 4 (16 U mg^{-1} 104 U mg^{-1}) in mineral salt medium containing starch or yam peel as carbon source at 70 ^{0}C and pH 5.5, by a thermophilic strain of *A. niger* (where one unit is equivalent to the amount of amylase, which is able to liberate one mg of reducing sugar per min. under standard assay conditions). The stagnancy in amylase activity from day 4 to day 6 may be due to the persistence of stationary growth phase of the fungus till that day (Sani *et al.,* 1992; Pandey *et al*., 1994; 1995). The peak values of amylase activity were significantly different ($p<0.05$) among treatments. Duration also had significant ($p<0.05$) effect on the amylase activity. Significance ($p<0.05$) in amylase activity was observed within and between inoculum sizes. A comprehensive expression of amylase activity and specific activity using both the inoculum sizes is shown in Fig. 3. The occurrence of peak amylase activity in the later phase on day 6 with subsequent reduction in activity on day 7 and on day 8 by using 2 x 10^6 spores 10 g $substrate^{-1}$ may be either due to the cessation of fungal growth or nutrient unavailability, and overcrowding or other unknown factors (Sani *et al.,* 1992; Uguru *et al*., 1997). The presence of some sort of catabolite repressors produced by the fungus in the substrate can inhibit enzyme production as reported by Knapp and Howell (1980) focusing on cellulases, but applicable to all enzymes. Inoculum size, pH and duration of fermentation showed significant ($p<0.05$) effect on amylase production in the present study, as the highest amylase activity was achieved in stationary phase, *i.e.,* between 4 and 6 days of the culture irrespective of the inoculum size

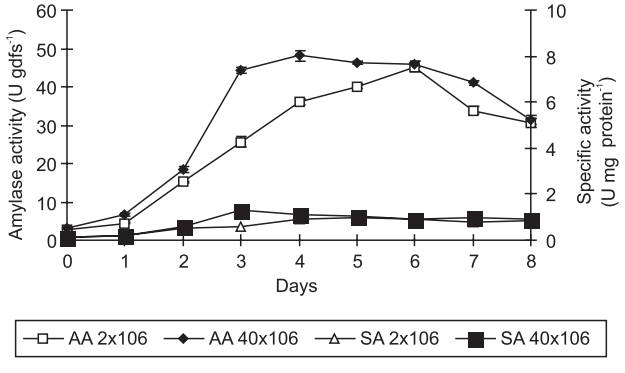

Fig. 3. Comparison of amylase activity and specific activity at pH 4.8 by using 2x10^6 and 40 x 10^6 spores

The concentration of reducing sugars in the fermented substrate for both the inoculum sizes is shown in Fig. 4. The highest concentration of reducing sugars was obtained on day 2 (188.69± 6.29 mg $gdfs^{-1}$) and day one (129.68± 2.69 mg $gdfs^{-1}$) for inoculum sizes of 2 x 10^6 and 40 x 10^6 spores 10 g $substrate^{-1}$ respectively. This may be due to hydrolysis of carbohydrates by the fungal enzymes immediately after spore germination. The decrease in the reducing sugar level on the third day in the crude enzyme extract may be due to the advent of stationary phase or end of the logarithmic phase when no mycelial growth occurs. The reducing sugar level further reduced significantly ($p<0.05$) on day 4, and from day 5 till day 8, the value remained almost stable (33- 34 mg $gdfs^{-1}$). This may be ascribed to the maximum utilization of the reducing sugars for fungal metabolic activities. Reduction in soluble sugar during time course of microbial growth was reported by Pandey *et al.* (1995). Amylase activity was negatively correlated (R= -0.496) with reducing sugar content in the extract ($p<0.05$).

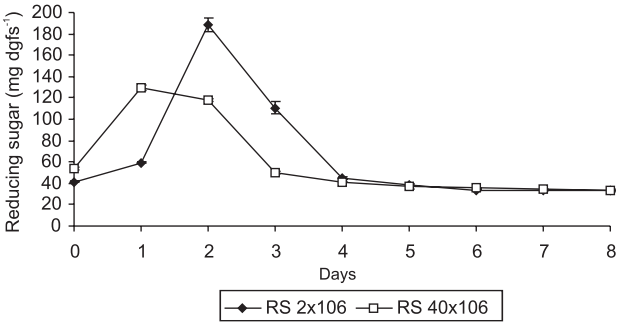

Fig. 4. Concentration of reducing sugar in crude extracts at pH 4.8

Acid protease activity, specific activity and protein content

Acid protease activity was analysed in the crude enzyme extract at pH 2.7 on all 8 days for both the inoculum sizes (2x10^6 and 40x10^6 spores 10 g $substrate^{-1}$)

Protease activity was expressed from day one onwards. The initial activity was very negligible but the peak was observed on day 6 (54.89±1.56 Ugdfs^{-1} and 49.50 ± 2.08 U $gdfs^{-1}$) using inoculum sizes 2 x 10^6 and 40 x 10^6 spores 10 g $substrate^{-1}$ respectively. Protein content in the crude extract showed variation with duration of fermentation and the maximum value was recorded on day 6 (50.34± 0.43 mg $gdfs^{-1}$, 48.29±1.62 mg $gdfs^{-1}$ for 2 x 10^6 and 40 x 10^6 spores 10 g $substrate^{-1}$ respectively) (Fig. 5 and 6). Protein concentration in the crude extract was significantly

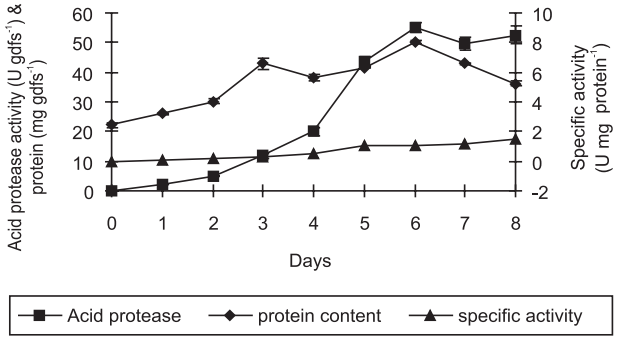

Fig. 5. Acid protease activity, protein content in the crude extract and specific activity using 2x10^6 spores

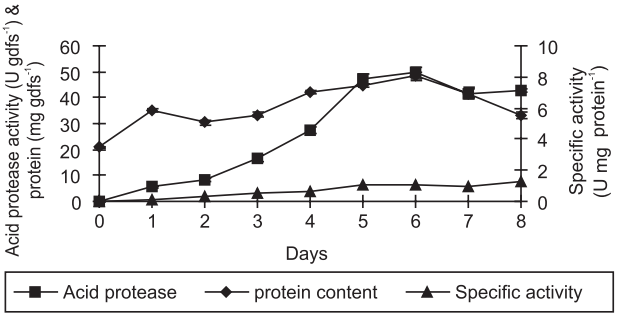

Fig. 6. Acid protease activity, protein content in the crude extract and specific activity using $40x10^6$ spores

($p<0.05$) correlated with acid protease production. The acid protease activity and protein concentration were positively correlated (R= 0.778).

The comparison between the activities for inoculum sizes 2 x 10^6 and for 40 x 10^6 spores 10 g substrate^{-1} is represented in the Fig. 7. Inoculum size did not show significant effect in attaining the peak value. In both cases, the peak value was obtained on day 6. But during early phase of fermentation, it had considerable effect on acid protease production. Inoculum size was not significant ($p>0.05$) in production of acid protease, whereas, duration of fermentation was significant ($p<0.05$).

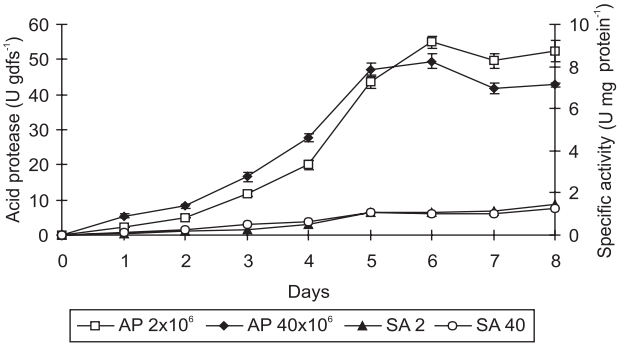

Fig. 7. Comparison of acid protease and specific activity using $2x10^6$ and $40x10^6$ spores

Concentration of hydrolyzed peptides in the crude enzyme extract

The changes in the concentration of hydrolyzed peptides in the crude enzyme extract are represented in Fig. 8. The level of free hydrolyzed peptides in the crude extract had shown different trends for both the inoculum sizes. While the initial concentration was 16-17 mg gdfs^{-1} in both the cases, for inoculum size $2x10^6$ spores 10 g substrate^{-1}, the optimum value was 38.57 mg gdfs^{-1} on day 3, which further remained more or less constant between 28 to 20 mg gdfs^{-1} till day 8.

For inoculum size of $40x10^6$ spore 10 g substrate^{-1}, the highest value of free hydrolyzed peptides was obtained on day one (32.92 mg gdfs^{-1}) and afterwards it was

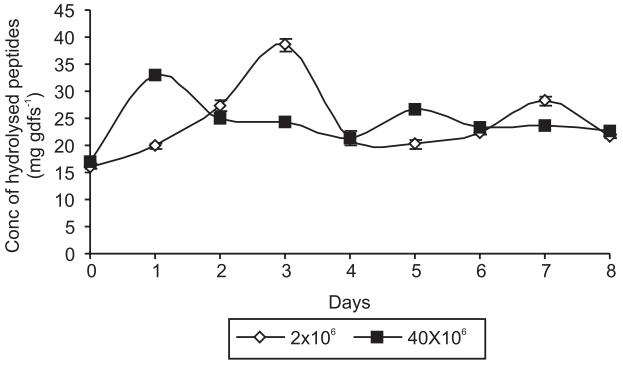

Fig. 8. Comparison of hydrolyzed peptides concentration in the crude extract by using inoculum size @ $2x10^6$ and $40x10^6$ spores

fluctuating between 25 and 22 mg gdfs^{-1} till day 8. Inoculum size did not significantly affect free hydrolyzed peptide concentration. Within both the inoculum sizes, duration of fermentation had a significant ($p<0.05$) effect on the availability of free hydrolyzed peptides.

Protease activity

Estimation of protease production by *A. niger* strain from mangrove swamp was carried out at pH 2.7, since fungal proteases are stable and active at highly acidic conditions (Ichishima, 1970). The maximum protease activity was recorded on day 6 for $2x10^6$ (54.89 Ugdfs^{-1}) and $40x10^6$ spores (49.50 U gdfs^{-1}) respectively. Thus, the peak activity was obtained in the late stationary phase, which is in conformity with the findings of Yang and Lin (1998), who have reported maximum activity of acid protease on day 8 using thin stillage from a rice spirit distillery by *A. niger* in batch fermentation. In the present investigation, the production of enzyme started on day one itself, with the values of 2.34U and 5.5U gdfs^{-1} for $2x10^6$ and $40x10^6$ spores respectively. On day 5, the enzyme activity almost doubled to that on day 4. Yang and Lin (1998) have reported maximum acid protease activity (125U ml^{-1}) on day 6 with shortening of the lag phase by providing surface aeration during fermentation. The highest protease activity obtained on day 6 in the present study may be due to the peak activity at the stationary phase of the fungal growth. It has also been observed that the initial inoculum size does not have any significant effect on the peak activity, though up to day 5 the acid protease activity was slightly higher ($p>0.05$) in the inoculum size of 40 x 10^6 spores. In a study with *Mucor hiemalis*, it had been reported that most of the proteolytic activity to be cell surface bound and the enzyme could be released by elution with sodium chloride or by growing the organism at high salt concentration suggesting that the enzyme was probably bound to the cells by weak ionic bonds (Knapp and Howell, 1980). Also, it had been reported that protease production by *A. niger* was not parallel with mycelial growth but associated with mixed growth (Yang and Lin, 1998).

In the beginning of fermentation, the concentration of hydrolyzed peptides in the crude enzyme extract was almost same (16.06-16.85 mg $gdfs^{-1}$). By using 2 x 10^6 spores, the peak value was obtained on day 3 (38.57 mg $gdfs^{-1}$). The decline in protein concentration from 43.01 mg $gdfs^{-1}$ to 38.24 mg $gdfs^{-1}$ and the peptide value (21.24 mg $gdfs^{-1}$) on day 4, may be attributed to the onset of stationary phase or the end of the log phase wherein the fungi might have used up a greater amount of peptides for their cell growth as reported by Yang and Lin (1998). The increase in the concentration of peptides in the beginning of fermentation may be due to the action of protease, which possibly hydrolyzed the protein in the substrate. The highest protein concentration on day 6 (50.34 mg $gdfs^{-1}$) as compared to that in the control (22.20 mg $gdfs^{-1}$) indicates the subsequent accumulation of a variety of enzymes by *A. niger* (Pandey *et al*., 1999) and free hydrolyzed peptides, which are proteinaceous in nature. After day 6, protein concentration in the crude enzyme extract showed a slow decline possibly due to the synthesis of fungal protein or chitin by utilizing the available simpler peptides, which would not have diffused into the extract. The pattern of total protein recovery was almost similar to the protease recovery and hence the specific activity did not show much variation (Aikat and Bhattacharya, 2000).

The high concentration of hydrolyzed peptides in the crude extracts on day one (32.92 mg $gdfs^{-1}$) while using 40x10^6 spores may be due to the higher inoculum size and concomitant enzyme production. Further drop in hydrolyzed peptides on the second day to 25 mg $gdfs^{-1}$ could be due to the utilization of the free peptides by the fungi. The same reason may be attributed to the decrease in protein concentration from 34.92 mg $gdfs^{-1}$ to 30.42 mg $gdfs^{-1}$ on day 2, while the peak protein concentration recorded on day 6, may be associated with mixed growth of fungi (Yang and Lin, 1998).

Acknowledgements

The first author is acknowledging the Indian Council of Agricultural Research (ICAR), New Delhi, for the financial assistance. The authors are grateful to the Director, Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala, India, for providing the facilities to carry out this work

References

Aalbæk, T., Reeslev, M., Jensen, B. and Eriksen, S. H. 2002. Acid protease and formation of multiple forms of glucoamylase in batch and continuous cultures of *Aspergillus niger. Enzyme Microb. Technol.,* 30: 410-415.

Aikat, K., and Bhattacharya, B. C. 2000. Protease extraction in solid state fermentation of wheat bran by a local strain of *Rhizopus oryzae* and growth studies by the soft gel technique. *Process Biochem.,* 35: 907-914.

Aikat, K. and Bhattacharya, B. C. 2001. Optimization of some parameters of solid-state fermentation of wheat bran for protease production by a local strain of *Rhizopus oryzae*. *Acta Biotechnol.*, 20(2): 149-159.

Bai, H., Ge, S. J. and Zhang, L. X. 1999. Total hydrolysis of food proteins by the combined use of soluble and immobilized protease. *Intl. J. Food Sci. Technol.*, 34: 95–99.

Bernfeld, P. 1955. Amylases: α and β, Enzymes of carbohydrate metabolism. In: *Methods in Enzymology,* Vol. I, p. 149-158.

Clausen, C. 2003. Improving the two-step remediation process for CCA- treated wood: Part I. Evaluating oxalic acid extraction, *Waste Management,* 23(4): 293-296.

Ellaiah, P., Adinarayana, K., Bhavani, Y., Padmaja, P. and Srinivasulu, B. 2002. Optimization of process parameters for glucoamylase production under solid state fermentation by a new isolated *Aspergillus* species. *Process Biochem.*, 38: 615–620.

Gigras, P., Sahai, V., and Gupta, R. 2002. Statistical media optimization and production of ITS α -amylase from *Aspergillus oryzae* in a bioreactor. *Curr. Microbiol.,* 45: 203–208.

Gong, W. K. and Ong, J. E. 1990. Plant biomass and nutrient flux in a managed mangrove forest in Malaysia. *Estuar. Coast. Shelf Sci.,* 31: 519-530.

Gopinathan, C. P. and Selvaraj, G. S. D. 1996. The Mangroves - importance, conservation and management. In: Menon, N. G. and Pillai, C. S. G. (Eds.) *Marine biodiversity conservation and management*, CMFRI, Cochin, India, p. 4-15.

Ichishima, E. 1970. Purification and mode of assay for acid proteinase of *Aspergillus saitoi*. In: *Methods in Enzymology*, Vol., XIX , p. 397-406.

Kathiresan, K. and Bingham, B. L. 2001. Biology of mangroves and mangrove ecosystems. *Adv. Mar. Biol.,* 40: 81-251.

Kaur, P. K., Grewal, H. S. and Kocher, G. S. 2003. Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. *Indian J. Microbiol.,* 43(2): 143-145.

Knapp, J. S., and Howell, J. A. 1980. Solid state fermentation. In: Wiseman, A. (Ed.). *Topics in enzyme and fermentation biotechnology,* Vol. 4, Ellis Horwood Limited, p. 85-143.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein estimation with the Folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Miller, G. L. 1959. Use of dinitorosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.,* 31: 426-428.

Mitra. P., Chakraborty. R. and Chandra, A. L. 1996. Production of proteolytic enzymes by solid state fermentation. *J. Sci. Indus. Res.,* 55: 439-442.

Nair, P. V. R. and Gopinathan, C. P. 1983. Primary production in coastal water in mariculture potential of Andaman and Nicobar Islands - an indicative survey, *Bull. Cent. Mar. Fish. Res. Inst.,* 34 (4): 29-32.

Pandey, A., Ashakumary, L., Selvakumar, P. and Vijayalakshmi, K. S. 1995. Effect of yeast extract on glucoamylase synthesis by *Aspergillus niger* in solid-state fermentation. *Indian J. Microbiol.,* 35(4): 335-338.

Pandey A., Selvakumar, P. and Ashakumary, L. 1994. Glucoamylase production by *Aspergillus niger* on rice bran is improved by addition of nitrogen sources. *World J. Microbiol. Biotechnol.,* 10: 348-349.

Pandey, A., Selvakumar, P., Soccol, C. R. and Nigam, P. 1999. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.,* 77(1): 149-162

Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D. and Mohan, R. 2000. Advances in microbial amylases. *Biotechnol. Appl. Biochem.,* 31: 135–152.

Pitt, J. I. and Hocking, A. D. 1985. *Fungi and Food Spoilage.* Academic Press Australia, 409pp.

Sani, A., Awe, F. A., and Akinyanju, J. A. 1992. Amylase synthesis in *Aspergillus flavus* and *Aspergillus niger* on cassava peel. *Indian J. Microbiol.,* 10: 55-59.

Sarangbin, S. and Watanapokasinb, Y. 1999. Yam bean starch: a novel substrate for citric acid production by the protease-negative mutant strain of *Aspergillus niger*. *Carbohydrate Polymers,* 38: 219–224.

Selvakumar, P., Ashakumary, L. and Pandey, A. 1996. Microbial synthesis of starch saccharifying enzyme in solid cultures, *J. Sci. Indus. Res., 55*: 443-449.

Singh, O. V., Pereira, B. M. J. and Singh, R. P. 1999. Isolation and characterization of a potent fungal strain *Aspergillus niger* ORS-4 for gluconic acid production, *J. Sci. Indus. Res.,* 58: 594-600.

Uguru, G. C., Akinyauju, J. A. and Sani, A.1997. The use of yam peel for growth of locally isolated *Aspergillus niger* and amylase production. *Enzyme Microb. Technol.,* 21:46-51.

Verma, R., Krishnendu, S., Pandey, A. K., and Rajak, R. C. 2001. Solid state fermentation to produce alkaline protease by *Aspergillus fumigatus* B149. *Indian J. Microbiol.,* 41:111-114.

Yang, F. C. and Lin, I. H. 1998. Production of acid protease using thin stillage from a rice –spirit distillery by *Aspergillus niger. Enzyme Microb. Technol.,* 23(6): 397- 402.

Date of Receipt : 07/08/2007
Date of Acceptance : 01/03/2010

INDIAN JOURNAL OF FISHERIES
Volume 57 Number 1 (2010)

CONTENTS

Indexed/Abstracted in: Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Fish and Fisheries Worldwide (NISC), Science Citation Index Expanded, SCOPUS and Zoological Records